Université de Liège
Faculté de Médecine Vétérinaire
Département de Gestion Vétérinaire des Ressources Animales
Service d'Écologie de la Santé et des Productions Animales

Caractérisation écologique, phytotechnique et zootechnique de ressources alimentaires locales pour les caprins dans les élevages du Nord du Maroc

Ecological, phytotechnical, and zootechnical characterization of local feed resources for goats in farms in Northern Morocco

Soumaya BOUKROUH

Thèse présentée en vue de l'obtention du grade de
Docteur en Sciences Vétérinaires

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Abréviations

9t-C18:1	Elaidic acid
a*	Indice du rouge
ADF	Acid detergent fibers
ADG	Average daily gain
ADL	Acid detergent lignin
AG	Acide gras
AOAC	Association of Official Analytical Chemists
ARA	Arachidonic acid
ARES	Académie de Recherche et d'Enseignement Supérieur
AI	Atherogenicity index
b*	Indice du jaune
BIO1	Average annual temperature
BIO2	Average diurnal variation
BIO3	Isothermality
BIO4	Temperature seasonality
BIO5	Maximum temperature of the warmest month
BIO6	Minimum temperature of the coldest month
BIO7	Average temperature of the wettest quarter
BIO8	Average temperature of the wettest quarter
BIO9	Average temperature of the driest quarter
BIO10	Average temperature of the warmest quarter
BIO11	Average temperature of the coldest quarter
BIO12	Annual precipitation
BIO13	Precipitation of wettest month
BIO14	Precipitation of driest month
BIO15	Precipitation seasonality
BIO16	Precipitation of wettest quarter
BIO17	Precipitation of driest quarter
BIO18	Precipitation of warmest quarter
BIO19	Precipitation of coldest quarter
BV	Bitter vetch
C4:0	Butyric acid
C6:0	Caproic acid
C8:0	Caprylic acid
C10:0	Capric acid
C12:0	Lauric acid
C14:0	Myristic acid
C14:1	Myristoleic acid
C15:0	Pentadecanoic acid
C15:1	Pentadecenoic acid
C16:0	Palmitic acid
C16:1	Palmitoleic acid
C18:0 :	Stearic acid
C18:1n-9	Oleic acid
C18:2n-6	Linoleic acid
C18:3n-3	α-linolenic acid
C18:3n-6	γ-linolenic acid
C20:0	Arachidic acid
C20:1	Eicosenoic acid
C20:2	Eicosadienoic acid
C20:3n-3	Eicosatrinoic acid

C21:0	Heneicosanoic acid
C22:1n-9	Erucic acid
C22:2	Docosadienoic acid
C22:6n-3	Docosahexaenoic acid
CIE	Commission International de l'Éclairage
CCW	Cold carcass weight
CL	Carcass length
CF	Crude fibers
CI	Compactness index
Co	Control
CoSilt	Coarse silt
CRRAT	Centre Régional de la Recherche Agronomique de Tanger
CT	Condensed tannins
DayF	Days to flowering
DayM	Days to maturity
DFA	Desirable fatty acids;
DHA	Docosahexaenoic acid
DMI	Dry matter intake
DMY	Dry matter yield
DP	Dressing percentage
DPPH	2,2-diphenyl-1-picrylhydrazy radical-scavenging activity
E	Ecotype
EC	Electrical conductivity
ECM	Energy corrected milk
EE	Ether extract
EF	Days to end of flowering
EPA	Eicosapentaenoic acid
FA	Fatty acid
FAME	Fatty acid methyl Ester
FBA	First bud appearance
FCM	Fat corrected milk
FCR	Feed conversion ratio
FDUR	Flowering duration
FF	Days to full flowering
FMU	Forage unit for milk production
FMY	Fresh matter yield
FnSilt	Fine silt
FRAP	Ferric reducing ability of plasma
GAE	Allic acid equivalent
GAM	Genetic advance as a percentage of the mean
GCV	Genotypic coefficient of variation
GDD	Growing degree days
GFP	Grains filling period
GIT	Gastrointestinal tract
GLM	Generalized linear model
GPL	grains per plant
GPOD	grains per pod
GRY	grain yield
H^2	Broad-sense heritability
HCW	Hot carcass weight
Hi	Harvest index
HPI	health-promoting index
HT	Hydrolyzable tannins
Hum.	Humidity
INRA	Institut National de Recherche Agronomique

IntN	Internodes number
IVEDMD	Enzymatic dry matter digestibility
IVEOMD	Enzymatic organic matter digestibility
IVTD	In vitro true digestibility
IU	International unit
K	Exchangeable potassium
L*	Luminosité
LA	Linoleic acid
LCFA	Long-chain fatty acids
LD	*Longissimus dorsi*
LDL	Low density lipoprotein cholesterol
LL	leaves length
LLN	leaflets number per leaf
LLPB	Length of the longest plagiotropic branch
ALA	Linolenic acid
LN	leaves number;
LOA	Length of orthotropic axis
LSR	Leaf to stem ratio
LWi	Leaf width
MCFA	Medium-chain fatty acids
ME	Metabolizable energy
MI	Muscle index
MUFA	Monounsaturated fatty acids
NDE	Number of days to emergence
NDF	Neutral detergent fibers
NE_{milk}	Net energy of milk
NFE	Nitrogen free extract
NIP	Number of inflorescences per plant
NLP	Number of leaves per plant
NPB	Number of plagiotropic branches
NPB	Number of primary branches
NRC	Nutrient requirement council
NTB	Number of total branches
NTP	Non-tannic phenols
NVI	Nutritive value index
P	Available phosphorus
PH	Plant height
PanL	panicle length
PanW	Panicle weigh
PDIN	Digestible proteins in the intestines allowed by proteins
PDIE	Digestible proteins in the intestines allowed by energy
PCV	Phenotypic coefficient of variation
PedL	peduncle length
TP	Total phenols
pLeav	Percentage of leaves
PODL	pods length
PODN	pods number per plant
PODS	Days to pod setting
pPan	Percentage of panicle
pStem	Percentage of stems
PUFA	Polyunsaturated fatty acids
RuProBal	Rumen protein balance
SBW	Slaughter body weight
SCFA	Short-chain fatty acids
SD	Stem diameter

SEM	Standard error of the mean
SF	Days to start of flowering
SFA	Saturated fatty acids
SM	*Semimembranosus*
SRG	Sorgho
SWP	Seed weight per plant
TAE	Tannic acid equivalent
TL	Thigh length
TI	Thrombogenic index
TT	Total tannins
TT	Thigh thickness
TSW	Thousand seed weight
UF	Unité fourragère
UFA	Unsaturated fatty acids
VLCPUFA	Very long chain fatty acid
WHC	Water holding capacity

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A tous ceux qui me sont chers.

Résumé - Abstract

Résumé

Au Maroc, les deux tiers du cheptel caprin se trouvent dans les zones de montagne. Ces élevages contribuent jusqu'à 68% des revenus des éleveurs. Au Nord du Maroc, la race Beni Arouss, représente aux alentours de 8% du cheptel régional caprin ; c'est la seule race reconnue de cette région. Les caprins sont présents dans deux systèmes d'élevages différents, semi-extensif et extensif, et sont conduits sur des parcours forestiers caractérisés par une variabilité saisonnière de l'offre fourragère et un déséquilibre alimentaire qui ont pour conséquences une faible productivité des élevages. La recherche de nouvelles ressources alternatives et locales s'avère indispensable. Les légumineuses fourragères spontanées des pâturages naturels sont des éléments importants dans l'alimentation des ruminants, entre autres, grâce à leur teneur élevée en protéines. L'espèce *Hedysarum flexuosum* (Sulla flexuosa (L.) Medik.) est une légumineuse spontanée à la région du Nord du Maroc. En revanche, la légumineuse *Vicia ervilia* (orobe) et la céréale *Sorghum bicolor* (L.) Moench (sorgho) sont déjà cultivées par les éleveurs dans cette région ; elles sont exploitées principalement pour leurs grains. Dans la région Méditerranéenne, ces plantes apparaissent prometteuses quant à leur adaptabilité aux environnements marginaux et secs ainsi que leur faible exigence en intrants. Pour augmenter leur diffusion dans la région, il serait intéressant d'étudier leur valeur nutritive ainsi que leurs effets sur les performances animales et la qualité des produits animaux.

L'objectif de ce travail a été d'évaluer la caractérisation écologique, agro-morphologique et bromatologique des écotypes locaux (i) du Sulla flexuosa, (ii) du sorgho, (iii) et d'orobe, ainsi que (iv) la caractérisation des effets de l'incorporation du foin du Sulla flexuosa en replacement de la luzerne dans la ration de la chèvre de la race locale Beni Arouss sur la production et la qualité du lait et, (v) des effets des grains de l'orobe et du sorgho en remplacement respectivement de la féverole et de l'orge dans la ration, sur les performances et sur la qualité de la carcasse et de la viande de chevreaux Beni Arouss.

Pour réaliser cette caractérisation, 21 écotypes de Sulla flexuosa et de sorgho et 17 écotypes d'orobe ont été collectés dans la région du Nord du Maroc. Le sol a été échantillonné parallèlement et les coordonnées des sites de collectes ont été enregistrées. Les essais ont été conduits en blocs aléatoires complets durant les années 2018/2019 et 2019/2020. Le suivi des différents stades phénologiques a été réalisé pour les trois plantes. Pour le Sulla flexuosa qui est valorisée comme fourrage, la caractérisation morphologique, agronomique et bromatologique a été réalisée aux différents stades phénologiques. Pour l'orobe et le sorgho, la caractérisation agro-morphologique et bromatologique a été réalisée majoritairement au stade de la maturité physiologique. La caractérisation bromatologique a concerné l'évaluation de la matière sèche et minérale, l'extrait éthéré, les protéines, les fibres, la digestibilité, les phénols et tannins et l'activité antioxydante (pour l'orobe et le sorgho). L'évaluation du rendement et de ses composantes ainsi que les différents paramètres végétatifs ont concerné la caractérisation agro-

morphologique. L'analyse en composantes principales et l'analyse des cartes thermiques ont permis de distinguer quatre groupes d'écotypes différents pour le Sulla flexuosa et cinq groupes d'écotypes distincts pour l'orobe et pour le sorgho. Pour le Sulla flexuosa, l'écotype E1 appartenant au groupe 1 était intéressant quant à son double usage (productivité, valeur nutritive et paramètres de reproduction élevés). L'écotype E21 du sorgho, appartenant au cinquième groupe, était un candidat à la sélection prometteur par son rendement élevé en grain combiné avec une énergie métabolisable élevée. Concernant l'orobe, le deuxième groupe était le candidat le plus prometteur pour développer des variétés à haut rendement et nutritives dans les régions méditerranéennes. Pour l'orobe, les écotypes avaient présenté des rendements en grains intéressants (1 T/ha) par rapport à des écotypes de cette espèce dans la région Méditerranéenne mais qui varient en fonction des années. La teneur en protéines des grains a été relativement plus faible par rapport aux écotypes de la région méditerranéenne (22,9% MS). Le sorgho a présenté cependant, des rendements en grains intéressants.

Dans la perspective de la mise en culture et de l'utilisation du Sulla flexuosa en nutrition animale, une étude a été menée sur des chèvres en lactation pour évaluer l'effet de son incorporation dans la ration sur la production et la qualité du lait. Trente chèvres de race Beni Arouss ont été réparties en trois lots et du foin de Sulla flexuosa a été introduit à deux niveaux, soit 35 ou 70 % (SF35 ou SF70 respectivement), sur la base de la MS ; il a remplacé partiellement ou totalement le foin de luzerne de la ration témoin. Pendant trois mois, la production de lait a été échantillonnée. L'incorporation de Sulla flexuosa n'a pas affecté la production de lait ou sa composition physico-chimique. Cependant, la composition en acides gras du lait variait en fonction du pourcentage d'incorporation de Sulla flexuosa. Le régime SF70 était associé à une augmentation des proportions dans le lait de C18:1n-9, C18:2n-6, C18:3n-3 et C22:6n-3 et du total des acides gras monoinsaturés, polyinsaturés et n-3. En conséquence, les indices athérogènes et thrombogènes ont été améliorés. En outre, une meilleure capacité antioxydante a été observée dans le SF70.

Pour évaluer l'effet de l'incorporation des grains d'orobe et de sorgho sur les caractéristiques de la carcasse et la qualité de la viande des chevreaux, une étude a été menée sur 24 chevreaux de race Beni Arouss. Les animaux ont été répartis en trois groupes dont le groupe témoin qui a reçu un régime conventionnel à base de foin d'avoine, d'orge et de fèverole. Dans le premier groupe testé, la féverole a été remplacée par l'orobe, et dans le second, l'orge par le sorgho. À la fin de l'essai, les animaux ont été abattus et les caractéristiques de la carcasse et la qualité de la viande des muscles *longissimus dorsi* et *semimembranosus* ont été déterminées. Le régime a affecté le gain quotidien moyen et l'ingestion, mais pas le poids vif à l'abattage, le poids de la carcasse chaude et le pourcentage d'habillage (15,0 kg, 6,8 kg et 44,6 %, respectivement). Les régimes influençaient plusieurs paramètres mais pas de la même manière sur chaque muscle. En ce qui concerne la qualité de la viande, l'orobe a diminué la teneur en protéines des deux muscles.

Ces trois plantes peuvent donc être utilisées comme ressources alternatives dans les rations des chèvres. Les écotypes les plus intéressants seront diffusés et tous les écotypes seront conservés dans la banque de graines de l'INRA afin de maintenir la biodiversité végétale et d'améliorer la production caprine dans le Nord du Maroc.

Summary

In Morocco, two-thirds of the goat herds are located in mountainous areas. This breeding contributes up to 68% of the income of livestock farmers. In Northern Morocco, the Beni Arouss breed represents around 8% of the goats in this region; it is the only pure breed officially recognized in this region. They are raised in two different livestock systems, semi-extensive and extensive, and are conducted on forest rangelands characterized by seasonal variability in forage availability and nutritional imbalance, resulting in low productivity of the herds. Investigating new alternative resources, especially local ones, is essential. Spontaneous forage legumes from natural pastures are important in ruminant feeding due to their high protein content. The species *Hedysarum flexuosum* (Sulla flexuosa (L.) Medik.) is a spontaneous legume in the Northern region of Morocco. On the other hand, the legume *Vicia ervilia* (bitter vetch) and the cereal *Sorghum bicolor* (L.) Moench (sorghum) are already cultivated by farmers in this region; they are used mainly as grains. In the Mediterranean region, these plants appear promising in their adaptability to marginal and arid environments and low input requirements. To increase their spread in the region, it could be interesting to study their nutritional value and their effects on animal performance and the quality of animal products.

The objective of this work was to evaluate the ecological, agro-morphological, and bromatological characterization of local ecotypes of (i) Sulla flexuosa, (ii) sorghum, (iii) and bitter vetch, (iv) to evaluate the effect of incorporating Sulla flexuosa hay instead of alfalfa hay in goat diet on the production and quality of goat milk and (v) to evaluate the effect of incorporating bitter vetch and sorghum grains instead of fava bean and barley grains, respectively, on the production and quality of local chevon.

In order to carry out these characterizations, 21 ecotypes of Sulla flexuosa and sorghum and 17 ecotypes of bitter vetch were collected in the Northern region of Morocco. The soil was parallelly sampled, and the coordinates of the collection sites were recorded. The trials were conducted in complete randomized blocks during the years 2018/2019 and 2019/2020. The monitoring of the different phenological stages was carried out for the three plants. For Sulla flexuosa, used as fodder, the morphological, agronomic, and bromatological characterization was carried out at different phenological stages. For bitter vetch and sorghum, agro-morphological and bromatological characterization was carried out mainly at maturity. The bromatological characterization concerned the evaluation of the dry matter, mineral, ether extract, protein, fiber, phenols and tannins contents, digestibility, and antioxidant activity (for bitter vetch and sorghum). The evaluation of yield, its components, and the different vegetative parameters concerned the agro-morphological characterization. The principal component analysis and the heatmap analysis made it possible to distinguish four different ecotype groups for Sulla flexuosa and five distinct ecotype groups for bitter

vetch and sorghum. For Sulla flexuosa, ecotype E1 belonging to group 1 was promising with its double use (high productivity, nutritional value, and reproductive parameters). The ecotype E21 of sorghum, belonging to the fifth group, was a promising selection candidate due to its high grain yield combined with high metabolizable energy. Regarding the bitter vetch, the second group was the most promising candidate for developing high-yielding and nutritious varieties in Mediterranean regions. For bitter vetch, the ecotypes presented interesting grain yield (1 T/ha) but varied depending on the year. The protein content of the grains was low compared to ecotypes from the Mediterranean region (22.9% MS). However, sorghum presented interesting grain yield.

Regarding the cultivation and use of Sulla flexuosa in animal nutrition, a study was conducted on lactating goats to evaluate the effect of its incorporation into the goats' diet on milk production and quality. Thirty Beni Arouss breed goats were divided into three groups, and Sulla flexuosa hay was introduced at two levels, either 35 or 70% (SF70), on a DM basis; it partially or completely replaced the alfalfa hay in the control diet. For three months, milk production was sampled. Incorporating Sulla flexuosa did not affect milk production or its physicochemical composition. However, the milk fatty acid content varied depending on the percentage of Sulla flexuosa incorporation. The SF70 diet was associated with an increase in the proportions of C18:1n-9, C18:2n-6, C18:3n-3, and C22:6n-3 and total monounsaturated, polyunsaturated, and n-3 fatty acids in milk. As a result, atherogenic and thrombogenic indices were improved. Moreover, a better antioxidant capacity was observed in SF70.

To evaluate the effect of incorporating bitter vetch and sorghum grains on goat kids' carcass characteristics and meat quality, a study was conducted on 24 Beni Arouss breed goat kids. The animals were divided into three groups, with the control group receiving a conventional diet based on oat, barley, and bean hay. In the first tested group, fava bean grains were replaced by bitter vetch grains, and in the second, barley was replaced by sorghum. At the end of the trial, the animals were slaughtered, and the carcass characteristics and meat quality of the *longissimus dorsi* and *semimembranosus* muscles were determined. The diet affected the average daily gain and ingestion but not the live weight at slaughter, hot carcass weight, and dressing percentage (15.0 kg, 6.8 kg, and 44.6%, respectively). The diets influenced several parameters but not in the same way for each muscle. Regarding meat quality, bitter vetch decreased the protein content of both muscles.

These three plants can be used in the diets of goats. The most interesting ecotypes will be disseminated, and all ecotypes will be conserved in the seed bank of INRA to maintain plant biodiversity and improve goat production in Northern Morocco.

Préambule général

Préambule général

Ce travail de thèse se présente sous la forme d'une compilation d'articles. Le document est réparti en neuf chapitres et contient cinq articles scientifiques.

Le premier chapitre est une introduction générale qui présente le contexte général du travail, une revue de la littérature décrivant l'élevage caprin au Maroc généralement et dans le Nord plus spécifiquement, ainsi que les systèmes d'élevage pratiqués dans cette région, la dégradation des parcours forestiers, et l'utilisation des ressources alimentaires alternatives locales dans l'alimentation des ruminants. Ce chapitre permet de mieux comprendre les objectifs de ce travail de thèse.

Le deuxième chapitre présente les objectifs généraux de ce travail.

Le troisième chapitre est constitué de la première étude qui présente les résultats de l'évaluation de la caractérisation agro-morphologique et phénologique, de la composition chimique et de la digestibilité des écotypes locaux de Sulla flexuosa (L.) Medik. (*Hedysarum flexuosum L.*) selon plusieurs stades phénologiques. Ce travail est soumis à la revue *Scientific reports*.

Le quatrième chapitre rapporte les résultats de l'évaluation de la caractérisation agro-morphologique et phénologique, de la composition chimique et de la digestibilité des grains et de la paille des écotypes locaux de *Sorghum bicolor (L.) Moench*. Ces résultats sont soumis à la revue *Scientific reports*.

Le cinquième chapitre rapporte les résultats de l'évaluation de la caractérisation agro-morphologique et phénologique, de la composition chimique et de la digestibilité des grains et de la paille des écotypes locaux de l'orobe (*Vicia ervilia*). Ce travail sera soumis prochainement.

Le sixième chapitre porte sur l'évaluation de l'effet de l'incorporation du foin de Sulla flexuosa sur la production et la qualité du lait des chèvres locales allaitantes de race Beni Arouss. Ce travail a été publié dans la revue *Animals*.

Le septième chapitre étudie l'effet de l'introduction des grains d'orobe ou de sorgho dans la ration des chevreaux à l'engraissement sur la qualité de la carcasse et de la viande. Cet essai sera soumis prochainement.

Le huitième chapitre présente la discussion générale, intégrant et mettant en valeur les résultats obtenus dans le cadre de cette thèse.

Le dernier chapitre donne les conclusions et les perspectives proposées à la lumière des acquis de ce travail de thèse.

Introduction

Chapitre 1. Introduction

1. L'élevage caprin au Maroc

Au Maroc, l'élevage de chèvres est l'une des activités économiques les plus anciennes des communautés montagneuses où les autres espèces de bétail sont difficiles à élever. Par ailleurs, les chèvres sont une source importante de protéines à travers la production de lait et de viande, contribuant à la fois à la sécurité alimentaire et financière des ménages (Godber *et al*., 2020). Contrairement à la tendance observée dans la plupart des pays en développement où les populations caprines ont connu des augmentations significatives, la population caprine marocaine a révélé une diminution d'environ un quart de ses effectifs au cours des cinq dernières décennies. En 2021, le cheptel caprin comptait 6,2 millions de têtes alors qu'en 1971, il y en avait 8,2 millions (FAOSTAT, 2021). Les raisons de cette tendance incluent la sédentarisation accrue des nomades pastoraux, la diminution de la disponibilité de la main-d'œuvre familiale due à la scolarisation des enfants et à la migration urbaines des jeunes, les sécheresses sévères et la diminution des ressources fourragères sur les parcours. Cependant, le Maroc occupe toujours la troisième position dans la région méditerranéenne quant à la taille de son cheptel caprin (FAOSTAT, 2021).

En 2021, la production nationale de viande caprine a été estimée à 31 mille tonnes pour 2,4 millions d'animaux abattus. Le poids moyen d'une carcasse a été de 12,8 kg ; cela représentait 6,3% de la production totale de viande rouge au Maroc (FAOSTAT, 2021). La production laitière caprine était de 47 mille tonnes pour 1,7 millions de chèvres traites, ce qui donnait une production annuelle moyenne de 28,3 kg de lait par animal (FAOSTAT, 2021). La répartition géographique des caprins dans le pays indique que les troupeaux sont majoritairement répartis en zone de montagne : le Haut Atlas (40%), le Rif (25%) et le Moyen Atlas (20%) (Benlekhal, 2005).

Au Nord du Maroc, c'est-à-dire, la région de Tanger – Tétouan – El Hoceima (Figure 1), l'effectif des caprins est de 470 mille têtes soit, 7,6% du troupeau caprin national et 28,9% du cheptel régional des ruminants (MAPMDREF, 2023). La prédominance des caprins est due à leur adaptation à la topographie montagneuse et à la végétation forestière existante. Le revenu annuel total provenant des ventes de produits laitiers et de chèvres se situe aux alentours de 77,8€ par chèvre dans les systèmes de production mixte (Godber *et al*., 2020). Sous les efforts étatiques de développement de l'élevage caprin dans cette région, en plus de l'élevage caprin extensif traditionnel dominant à vocation de viande, un système de production mixte (lait et viande) a émergé grâce à l'intensification des modes de production (Chentouf *et al*., 2011). Les ressources génétiques caprines du Maroc sont représentées pas des populations autochtones non standardisées. Seule la race caprine locale Beni Arouss (Figure 2) est reconnue par le Ministère de l'Agriculture, de la Pêche maritime, du Développement rural, et des Eaux et Forêts.

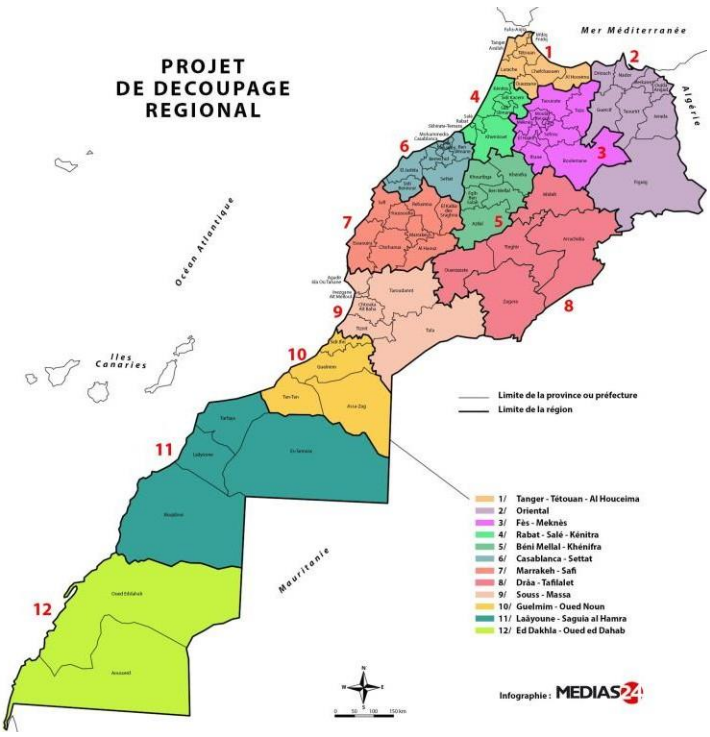

Figure 1 : Carte du Maroc.

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Figure 2 : Chèvre de race Beni Arouss.

2. Systèmes d’élevage caprin au Nord du Maroc

2.1. Système d’élevage caprin extensif à production de viande

Au Nord du Maroc, la topographie montagneuse, la végétation forestière existante, et l'adaptation des caprins aux environnements difficiles expliquent la conduite des caprins majoritairement dans des systèmes d'élevage extensifs sylvopastoraux et agrosylvopastoraux (figure 3) (Chebli *et al*., 2021a). Ces troupeaux de caprins sont des populations locales rustiques bien adaptées à leur milieu, appartenant à une majorité de petits éleveurs, peu organisés et peu encadrés (Hilal *et al*. 2013). Les troupeaux de chèvres en élevage à viande ont une taille moyenne de 26 animaux et sont conduits dans des exploitations agricoles de petite taille avec une surface moyenne de 4,6 ha (Chentouf *et al*., 2011). Le nombre d'animaux fluctue au fil des années, en fonction des périodes de sécheresse (et de la diminution des ressources fourragères) et de la motivation des éleveurs (Chebli *et al*., 2021a).

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Figure 3 : Élevage extensif.

Sur les parcours, les espèces arbustives et arborées constituent les principales ressources alimentaires des animaux. La rentabilité économique de ces élevages caprins réside dans la gratuité de ces ressources (Godber et *al.* 2016). Cependant, celles-ci sont irrégulières. En effet, le pâturage est pratiqué toute l'année, avec une disponibilité fourragère maximale enregistrée au printemps. Durant l'hiver, l'accès aux parcours est difficile à cause des périodes de fortes pluies (Chebli *et al*., 2020). Cependant, bien qu'elles soient très diversifiées, les zones sylvopastorales se caractérisent par un niveau de production faible. Cette situation s'explique par une dégradation alarmante (Figure 4) due à la combinaison de plusieurs facteurs tels que l'urbanisation, les feux de forêt, l'augmentation des surfaces cultivées, le surpâturage… (Chebli *et al*., 2018).

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Figure 4 : Parcours sylvopastoral dégradé.

Les espèces végétales pâturées par les chèvres conduites dans ces systèmes sont sujettes à des variations temporelles de la composition chimique, de la digestibilité et de l'énergie métabolisable (Chebli *et al*., 2021a), ces variations pouvant être dues aux facteurs environnementaux (longue période sèche et réduction des précipitations) et aux stades phénologiques des plantes (Chebli *et al*., 2021a). Ces différents éléments induisent une faible marge brute des chèvres conduites dans ces conditions (12 à 37 €) et la valeur des ventes est estimée à 67,3% des revenus dans les élevages à production de viande (Chentouf *et al*., 2011 et 2015).

Dans l'objectif de valoriser la viande caprine locale, des initiatives pour la création d'un label « Chevreau du Nord » ont été encouragées (Chentouf *et al.*, 2015). Cependant, les ventes de chevreaux revêtent un caractère saisonnier puisqu'elles coïncident, en général, avec des périodes de forts besoins lors des périodes de l'Aïd el Kebir et du Ramadan, durant lesquelles les prix augmentent significativement. La structure traditionnelle des marchés locaux, faiblement intégrés au marché national, ne permet pas une stabilité des prix en raison d'une forte spéculation et d'un faible pouvoir de négociation des éleveurs en l'absence d'une quelconque organisation formelle ou informelle de la commercialisation (Alami *et al*., 2005).

Pour trouver des solutions, certains éleveurs ont adopté un système semi-extensif pour améliorer la production laitière de leurs chèvres, en améliorant, entre autres, les apports alimentaires, afin d'augmenter la rentabilité de leur exploitation.

2.2. Système d'élevage semi-extensif à production mixte (lait et viande)

Les troupeaux de chèvres en élevage semi-extensif ont en moyenne 51 animaux, et sont conduits dans des exploitations agricoles de petite taille d'une surface moyenne de 7,5 ha (Chentouf *et al*., 2011).

Pour les systèmes d'élevage semi-extensifs à production de lait, les ressources forestières sont pâturées lorsque la météo le permet, et les arbres sont ébranchés lorsque la durée du jour est courte ou la météo est mauvaise, d'octobre à février. L'exploitation agricole ne contribue que faiblement à l'alimentation des caprins. Le déprimage de l'orge est généralement utilisé par les jeunes ou les femelles ayant mis bas précocement durant le mois de décembre. L'utilisation des chaumes en vaine pâture est pratiquée pendant 2 à 3 semaines après la moisson en juin. Contrairement aux systèmes extensifs à production de viande où les caprins ne bénéficient d'aucune supplémentation en aliments concentrés, la production de lait repose sur la supplémentation en concentrés, surtout pour les élevages commerciaux de lait, par rapport aux élevages commerciaux de fromage ou bien aux élevages non-commerciaux (Godber et al., 2016). Cette supplémentation en aliments concentrés (grains d'orge, d'avoine, de féverole…) (figure 5) est estimée à environ 150 kg par chèvre et par an (Chentouf *et al.*, 2007). Dû à la faible production laitière, l'indice de consommation est nettement plus élevé au Nord du Maroc par rapport aux zones Méditerranéennes avoisinantes (Chentouf *et al.*, 2007). La faible production laitière peut être due soit au faible apport en aliment grossier et en fourrages qui entraine un mauvais fonctionnement du rumen avec des effets négatifs sur le niveau de production des animaux, soit au potentiel de production des populations caprines locales (Chentouf *et al.*, 2015).

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Figure 5 : Élevage semi-extensif

Dans les élevages mixtes, la vente du lait représente une part importante du produit brut de l'exploitation soit, 40%. Dans les élevages qui transforment le lait, la vente de fromage représente 60% du produit brut (Chentouf *et al.*, 2011). La vente d'animaux vivants représente 50% des revenus des élevages semi-extensifs sans surface agricole, 52% dans ceux avec une surface agricole et 34% dans les élevages qui transforment le lait (Chentouf *et al.*, 2011). Dû à une insuffisance des infrastructures pour collecter le lait et le valoriser en fromage, le lait produit et non récolté est autoconsommé, vendu ou transformé sur l'exploitation en fromage. En général, les produits de l'élevage caprin sont orientés vers la commercialisation. Cette production (lait et fromage) trouve son débouché dans les souks hebdomadaires ou au bord des routes. La marge brute par chèvre et par an en élevage mixte est de 67 € à 71 €, respectivement pour les élevages sans et avec une surface agricole (Chentouf *et al.*, 2011). La marge brute par chèvre et par an est de 81 € chez les élevages fromagers et de 12 € pour les élevages pour la viande (Chentouf *et al.*, 2011). Dans une étude comparative des systèmes de production caprins semi-extensifs en Andalousie et au Nord du Maroc, la marge nette par litre de lait produit est plus élevée au Nord du Maroc qu'en Andalousie (0,44 € *vs*. 0,30 €). En revanche, la marge nette par chèvre a été plus élevée en Andalousie (113 € *vs*. 58 €). La différence a été attribuée aux faibles niveaux de production en lait et en viande des troupeaux et à la petite taille des troupeaux au Nord du Maroc qui mobilisent une main d'œuvre plus importante (Chentouf *et al*., 2007).

3. Productivité du cheptel caprin au Nord du Maroc

3.1. Production laitière

Au Nord du Maroc, la production laitière vient majoritairement des populations caprines locales. Elles représentent une population non-standardisée, issue d'un métissage entre les animaux locaux et des races caprines espagnoles ou suisses (Alpine). A l'aide de dix-neuf marqueurs microsatellites, El Moutchou *et al*. (2018) avaient montré des liens génétiques proches entre les populations caprines marocaines et les races espagnoles notamment les races Agrupacion de las Mesetas, Payoya et Malagueña. Elles se caractérisent par une bonne adaptation aux rudes conditions climatiques et à la résistance aux parasites et maladies locales. L'intensification du mode de conduite des élevages a permis une amélioration de leur production laitière (122 vs 47 kg/chèvre/an) (Chentouf *et al*., 2007). Le niveau de production laitière de ces chèvres locales est comparable à celui rapporté pour plusieurs races locales Méditerranéennes comme la tunisienne avec 133 kg (Gaddour & Najari, 2009) ou la grecque avec 160 kg (Haenlein, 2007). Cependant la production reste inférieure à celle d'autres races voisines telles que la Murciano-Granadina avec 410 kg (León *et al*., 2012).

La race Beni Arouss est la seule race caprine reconnue officiellement par le ministère de l'Agriculture, de la Pêche Maritime, du Développement rural et des Eaux et Forêts (MAPMDREF) au Nord du Maroc. Il s'agit d'une chèvre mixte. Sa production laitière est de 61 kg/lactation en élevage extensif (El Otmani *et al*., 2013). Des essais de supplémentation par des ressources alternatives n'ont pas réussi à dépasser ses niveaux de production (El Otmani *et al*., 2021a). Sa relativement faible production laitière est due au fait qu'elle n'a pas bénéficié d'un programme d'amélioration génétique (Chentouf *et al*., 2007) ; un programme d'amélioration de la race en pure a été proposé (Chentouf *et al.*, 2015). En conduite extensive la production laitière est destinée en priorité à l'allaitement des chevreaux, mais des traites sont réalisées chez les meilleures laitières. Ce lait est destiné à la fabrication d'un fromage frais vendu dans les marchés traditionnels (Chentouf *et al*., 2011). Cette chèvre est caractérisée par une robe rouge, une poitrine développée, des membres fins, serrés et couverts de poils. Elle a un taux de fertilité moyen (femelles pleines/femelles mises à la reproduction) de 95,5%, un taux de prolificité (chevreaux nés/femelles ayant mis bas) de 128% et un taux de fécondité (chevreaux nés/femelles mises en lutte) de 112%. La production laitière de 120 jours est estimée à 71,7 kg (Hilal *et al*., 2013).

3.2. Croissance des jeunes

La production de viande rouge a atteint 502 mille tonnes en 2021, grâce à l'amélioration de la productivité du cheptel ruminant Marocain (FAO *et al.*, 2021). La consommation moyenne de viande rouge a connu une évolution importante avec près de 17,2 kg/habitant/an en 2019 (MAPMDREF, 2023). Bien que la production de viande soit l'objectif principal des élevages extensifs, la productivité pondérale de la race Beni Arouss est plutôt faible, estimée à environ 2, 3, 4,6 et 9 kg, à 0, 30, 60 et 90 jours d'âge, respectivement. Le gain moyen quotidien entre 30 et 70 jours est de l'ordre de 71-74 g/jour chez les mâles et 59-78 g/jour chez les femelles (Hilal, 2018). Au Nord du Maroc, les éleveurs abattent les animaux à un poids plus élevé et à un âge plus avancé (170 jours) pour un prix moyen unitaire de 65 € (Chentouf *et al*., 2007). Les performances de croissance des chevreaux sont liées à plusieurs facteurs, principalement, les conditions climatiques qui agissent sur l'offre fourragère des parcours forestiers ainsi que sur les performances de production laitière de leur mère (Chebli *et al*., 2021a).

4. Contraintes de l'élevage caprin au Nord du Maroc

Les élevages caprins dans la région du Nord du Maroc sont caractérisés par une diversité génétique des populations caprines locales et une forte adaptation aux conditions du milieu montagneux de la région. En effet. Cette rusticité est un élément à développer et à conserver, au lieu de chercher à créer de nouvelles conditions de production pour des races importées. En outre, l'élevage caprin est souvent ancré dans les traditions locales, avec des pratiques transmises de génération en génération. Ils sont adaptés aux conditions locales, notamment aux reliefs montagneux et aux pâturages naturels de la région, ce qui peut conférer aux produits caprins une qualité spécifique liée au terroir. Bien que la productivité des troupeaux puisse être relativement plus faible, les produits caprins au Nord du Maroc, tels que le lait, le fromage et la viande, sont réputés pour leur qualité. Les méthodes traditionnelles de transformation et de fabrication peuvent contribuer à la saveur unique des produits. En outre, cette saveur pourrait être le résultat de la diversité du couvert végétal sur les parcours, ce qui pourrait déboucher sur une labélisation de la viande du chevreau de montagne s'appuyant sur les vertus de certaines espèces végétales médicinales appétibles existantes dans ces espaces pastoraux (Laroussi et Chentouf, 2016). En général, la viande de chevreau s'impose sur le marché par sa qualité diététique, la qualité de son gras, sa valeur nutritive et son goût apprécié. Dernièrement, la demande en viande caprine augmente dans le milieu urbain. Malgré tous ces atouts, l'élevage caprin au Nord du Maroc fait face à plusieurs défis, notamment une plus faible production de viande et de lait, expliquant le faible revenu des éleveurs (Chentouf, 2014). Ils font face également aux contraintes de la conduite technique des élevages, dont l'absence de la gestion de la reproduction, des plans de réforme, du renouvellement aléatoire des animaux (Chentouf, 2014), et la saisonnalité de l'offre fourragère (Chebli *et al*., 2021a). Au Nord du Maroc, l'alimentation est souvent le coût principal dans les exploitations et représente 72%, 69% et 35% des dépenses totales en exploitations laitières commerciales, laitières non-commerciales et commerciales fromagères, respectivement, malgré des subventions étatiques (Godber *et al*., 2020). L'orientation des élevages caprins vers la production laitière permettrait une amélioration des revenus et du niveau de vie des éleveurs. Cependant l'adoption de ce système de production n'est à la portée que d'une faible proportion d'élevages de la région. En effet, l'enclavement des principales zones de production caprine, l'éloignement des circuits de collecte de lait, la dominance des petites exploitations agricoles, le morcellement des terres et la faible capacité d'investissement des élevages sont autant de raisons expliquant le faible développement du système caprin laitier dans cette région (Chentouf *et al*., 2011). Par ailleurs, même si certaines études indiquent une meilleure rentabilité des élevages qui transforment leur lait par rapport aux élevages qui vendent leur lait, ces élevages sont confrontés à des défis tels que des problèmes liés à la fabrication du fromage et à l'écoulement de la production, qui engendrent d'énormes pertes aux producteurs. La saisonnalité de la production laitière dans l'exploitation agricole pose également un problème de fidélisation des clients dans un marché local qui peut être considéré comme embryonnaire (Chentouf *et al*., 2011). En effet, Hilal *et al*. (2016) a montré que la production laitière pour les chèvres ayant mis bas en hiver-printemps était plus élevée que celle des chèvres ayant mis bas en été-automne (55 vs. 38 litres respectivement). Ils ont attribué cette différence à la disponibilité de l'aliment sur les parcours pendant la période hivernale et printanière.

5. Parcours forestiers

Au Maroc, la superficie forestière est d'environ 5,7 millions d'hectares (FAOSTAT, 2021). Même si les steppes de *Stipa tenacissima*, représentant environ 30% des parcours, les zones sylvopastorales fournissent 1,5 à 2 milliards d'unités fourragères par an, ce qui couvre 17% des besoins alimentaires des ruminants (Chebli *et al*., 2021a). Ces parcours forestiers peuvent atteindre jusqu'à 80% des surfaces forestières dans le Nord du Maroc et le Haut Atlas (centre du Maroc) (Chebli *et al*., 2021a). Huit millions de ruminants, soit environ 32% du cheptel national, broutent toute l'année dans les zones sylvopastorales (FAOSTAT, 2011). Comme tous les écosystèmes forestiers, les forêts marocaines présentent des avantages écologiques et socio-économiques considérables. Elles ont le rôle écologique dans la régulation du régime hydrique, la protection contre l'érosion des sols, la lutte contre la désertification, la conservation de la biodiversité… En plus de sa contribution à la création de richesses, d'emplois et de revenus, la forêt est une composante essentielle des activités touristiques et récréatives. Cependant, la forêt marocaine subit une pression croissante en termes d'occupation humaine, pastorale et foncière dont les effets sont accentués par une absence de règle d'organisation, ce qui impacte négativement la conservation et la régénération des terres sylvopastorales. Par ailleurs, la croissance démographique et la diminution des surfaces de pâturage au profit des activités agricoles ont conduit à un non-respect des pratiques pastorales traditionnelles et communes (Chebli *et al.*, 2021a).

Au Nord du Maroc, les forêts couvrent une superficie de 545 mille hectares dont 476 mille et 69 mille hectares de formations naturelles et artificielles, respectivement (Chebli *et al.*, 2021a). Elles sont dominées par les conifères et les arbres à feuilles caduques, et la strate arbustive est dominée principalement par l'arbousier commun (*Arbustus unedo*), le ciste crépu (*Cistus crispus*), le ciste de Montpellier (*Cistus monspeliensis*), la bruyère arborescente (*Erica arborea*), la lavande stoechade (*Lavandula stoechas*), et le pistachier lentisque (*Pistacia lentiscus*) (Chebli *et al.*, 2021b).

Grâce à sa position géographique privilégiée, le Nord du Maroc est considéré comme l'une des 34 zones du monde caractérisées à la fois par une biodiversité élevée et des niveaux de menace alarmants (Mediani *et al*., 2015). Ce système sylvopastoral est marqué par son histoire, sa nature et ses contraintes. C'est une zone polyvalente caractérisée par une grande diversité de ressources, et qui joue des fonctions stratégiques utiles aux niveaux écologiques, socio-économique et pastoral. Cependant, au cours des 30 dernières années, les zones forestières et sylvopastorales ont subi une nette diminution de la couverture végétale d'environ 25%, en raison de la conversion de la croissance démographique, le surpâturage, l'expansion agricole, la culture du cannabis et la céréaliculture qui nécessitent souvent de nouvelles terres fertiles, au détriment des zones sylvopastorales (Aubert, 2013). Cette diminution est également due aux incendies de forêts et à la récolte du bois qui dépasse 2,5 fois le potentiel de production forestière (SPEF, 2006). Cette surexploitation des forêts et donc, cette dégradation du couvert végétal a augmenté la dégradation des sols et par la suite, le ruissellement, le ravinement, les glissements de terrain des collines environnantes et la sédimentation accélérée dans les barrages (Chebli *et al.*, 2021a).

En outre, la dégradation des parcours entraîne la dominance d'espèces non appétentes, notamment le mouron rouge (*Anagallis arvensis*), l'arum sauvage (*Arisarum vulgare*) et la corroyère (*Coriaria myrtifolia*) au détriment des espèces les plus appétentes, comme l'arbousier commun (*Arbutus unedo*), la calicotome velue (*Calicotome villosa*), le ciste crépu (*Cistus crispus*), le ciste de Montpellier (*Cistus monspeliensis*), le ciste à feuilles de sauge (*Cistus salviifolius*), la bruyère arborescente (*Erica arborea*), la lavande stoechade (*Lavandula stoechas*), le myrte commun (*Myrtus communis*), le filaire àlarges feuilles (*Phillyrea latifolia*), le pistachier lentisque (*Pistacia lentiscus*), la ronce à feuilles d'orme (*Rubus ulmifolius*), le chêne kermès (*Quercus coccifera*), le chêne portugais (*Quercus faginea*), le chêne-liège (*Quercus suber*), et l'olivier sauvage (*Olea europaea* L. subsp. europaea var. sylvestris) (Chebli *et al*., 2023).

Sur ces parcours dégradés, le régime alimentaire des chèvres est en grande partie composé d'arbustes (64–90%) et d'arbres (2–35%), alors que la contribution des herbacées ne dépasse pas 8%

(Chebli *et al*., 2021a). Par ailleurs, la production fourragère est caractérisée par sa variation saisonnière. Ainsi, le calendrier alimentaire des caprins se caractérise par une longue période de soudure pendant l'automne et l'hiver. La disponibilité fourragère sur les parcours induit un bilan énergétique positif seulement pendant la saison verte, au printemps. Les valeurs de la disponibilité dépassent 2 500 kg MS/ha au printemps contre seulement 1700 kg MS/ha en été et en automne (Chebli *et al*., 2021a). Ce déficit énergétique se traduit par une plus faible production laitière qui ne satisfait pas les besoins nutritionnels des chevreaux et qui se traduit par une perte de poids des chèvres, un taux d'avortement plus élevé, un taux de mortalité néonatale plus élevé et des performances de croissance des chevreaux plus faibles (Chebli *et al*., 2022). Cette diminution des ressources a traditionnellement été aussi surmontée par un changement du comportement des animaux. En saison sèche, les chèvres ont tendance à augmenter la vitesse d'ingestion, ainsi que la durée et le nombre de pas parcourus pour augmenter le taux d'ingestion afin de compenser la faible valeur nutritive des plantes (Chebli *et al*., 2021a), et à diminuer le temps de pâturage comme réponse aux conditions stressantes dues à des températures élevées en saison sèche (Chebli *et al*., 2022). Ce changement lié à l'activité physique peut augmenter l'énergie dépensée par les animaux au pâturage, et peut accentuer la diminution de leur performance de production. Ce changement peut également conduire au surpâturage des terres. Par ailleurs, étant donné que les parcours sont collectifs, cela rend leur gestion durable plus difficile (Jouven *et al*., 2010).

6. Production fourragère

Au Maroc, les grandes exploitations laitières s'appuient sur les aliments concentrés, l'ensilage de maïs, et peu de paille. Les petits éleveurs de bovins consomment du fourrage vert, de la paille et peu de concentrés. Les cultures fourragères comprennent des cultures fourragères irriguées, principalement la luzerne, suivie du trèfle et de l'ensilage de maïs, et des cultures fourragères pluviales, notamment l'orge, l'avoine et la vesce mélangée à d'autres céréales (Brandolini *et al*., 2021).

Les céréales, cultivées sur 59 % des terres, principalement en bour peu favorable, sont de loin la culture la plus importante au Maroc. La production de céréales très vulnérables aux aléas climatiques varie fortement d'une année à l'autre (de moins de 2 millions à plus de 10 millions de tonnes par an) et a un impact important sur les performances du secteur agricole et de l'économie marocaine (Harbouze *et al*., 2019). Les fortes variations de la valeur ajoutée du secteur agricole qui témoignent de la dépendance de ce secteur aux conditions climatiques, et notamment à la pluviométrie, se répercutent sur la croissance du PIB. Quant aux légumineuses, elles sont surtout localisées dans les régions à pluviométrie favorable. Elles couvraient en moyenne 3% de la SAU, soit 385 000 hectares (48% de fèves, 19% de pois chiche, 12% de lentilles et 11% de petits pois), sur la période 2011-2015. La production moyenne a été estimée à 2,8 millions de quintaux (soit un rendement de 7,3 quintaux par hectare sur la période 2006-2017) (Harbouze *et al*., 2019).

Au Nord du Maroc, les cultures fourragères n'occupent qu'une superficie d'environ 28 000 ha, soit 7% de la surface agricole utile de la région, et se localisent principalement dans les plaines (Chentouf *et al*., 2015). En zone de montagne, l'absence des cultures fourragères peut être expliquée par l'exiguïté et le morcellement des exploitations agricoles.

7. Changement climatique

La variabilité climatique a toujours constitué une contrainte au développement du secteur agricole au Maroc. Aussi, les scénarios de changements climatiques montrent que le climat du Maroc tendra de plus en plus vers l'aridité et la diminution de la biodiversité comme conséquence de la baisse des précipitations, de l'augmentation de la température, du début précoce de la sécheresse, en plus de l'apparition plus fréquente d'évènements extrêmes (Cramer *et al*., 2018). En effet, les températures moyennes au Maroc devraient augmenter de 2,3 à 2,9 °C et les précipitations diminuer de 13 à 30% d'ici 2050 (Harbouze *et al*., 2019). Ces sécheresses récurrentes ont multiplié l'utilisation de pratiques hors exploitation, telles que l'achat de concentrés, et par conséquent, ont augmenté les frais de production. Dans certaines zones, elles ont épuisé les eaux souterraines, mettant ainsi en danger la survie des troupeaux eux-mêmes (Brandolini et al., 2021).

Dans le cadre de la stratégie « Génération Verte (2020-2030) » comme continuité au « Plan Maroc Vert (2008-2020) », pour atténuer les effets des changements climatiques, l'État Marocain a adopté deux composantes clés liées à l'adaptation aux changements climatiques et à l'atténuation des effets des gaz à effet de serre (MAPMDREF, 2023). Les efforts d'adaptation ont porté principalement sur la maîtrise de l'eau d'irrigation tandis que les efforts d'atténuation se sont portés notamment sur l'extension des plantations pour augmenter le potentiel de séquestration du carbone (MAPMDREF, 2023). En outre, la structure de la valeur ajoutée agricole par filière montre une tendance à la baisse de la part des céréales dans la valeur ajoutée agricole (-11%) entre les périodes 2003-2005 et 2015-2019. Cette baisse a été réalisée principalement au profit de l'arboriculture (+11%) et des viandes blanches (+2%) (MAPMDREF, 2023). Afin de développer une agriculture plus résiliente, la plantation d'espèces plus adaptées à l'aridité du climat est reprise dans les plans à court et à long terme du Maroc (MAPMDREF, 2023). En outre, le recours à l'agriculture de conservation notamment le semis direct sont parmi les piliers de la stratégie « Génération Verte (2020-2030) » pour faire face aux changements climatiques. Le Maroc prévoit le semis direct pour 1,2 millions d'hectares dans l'horizon 2023. Cette technique a montré des résultats positifs quant à la diminution de la dégradation des sols et l'amélioration de leur qualité et de leur fertilité.

Les stratégies d'alimentation alternative aux ressources déjà utilisées sont susceptibles de devenir une nécessité encore plus grande dans tous les systèmes de production caprine, quel que soit leur niveau d'intensité.

8. Utilisation des ressources alternatives dans l'alimentation des ruminants

8.1. Anciennes ressources

Pour éviter une forte abondance d'espèces non appétibles et protéger la sécurité alimentaire à long terme, il est donc impératif de conserver la biodiversité dans la région du Nord du Maroc. L'amélioration des performances économiques des élevages peut se faire moyennant soit, l'augmentation de la taille des troupeaux avec les risques en termes de dégradation des ressources naturelles, soit, l'amélioration des performances individuelles des animaux par le recours à une intensification durable des systèmes d'élevage existants. La première option a été rapportée difficile à mettre en place, à cause des défis majeurs liés à la dégradation des parcours. A l'opposé, une amélioration de la productivité des troupeaux en gardant leur vocation de production de viande en extensif basée sur les ressources pastorales est possible à travers la promotion de techniques de production en adéquation avec les besoins des animaux et la préservation des ressources naturelles. Parmi les options citées dans la littérature, l'introduction de légumineuses fourragères (p. ex. la vesce) et de mélanges céréales/légumineuses dans les systèmes de culture et l'utilisation des résidus de culture (Chentouf *et al*., 2015). Sont cités également, l'utilisation des ressources alternatives et le remplacement de la jachère par des cultures fourragères (Devkota *et al*., 2022). Au Nord du Maroc, ces cultures fourragères notamment l'orge, l'épeautre, le triticale, l'avoine, la vesce, la féverole et le bersim, offrent de grandes options de différenciation de la sole fourragère afin de mieux répondre aux besoins alimentaires des troupeaux tout au long de l'année. Cependant, ce potentiel de production est très peu valorisé ; les cultures fourragères n'occupent qu'une superficie d'environ 28 000 ha, soit 7% de la SAU de la zone, et se localisent principalement dans les plaines (Chentouf *et al*., 2015). Le Tableau 1 présente les ressources fourragères utilisées dans la région du Nord du Maroc. L'avoine est caractérisée par une production de biomasse et de grains élevée, due principalement à un cycle de production plus long lui permettant de profiter d'une quantité maximale de facteurs de production et aussi à la tolérance aux principales maladies redoutables dans la région, particulièrement la rouille brune et l'oïdium (Chentouf *et al*. 2015). Le triticale se caractérise par une bonne adaptation aux sols lourds et sablonneux prédominants et au climat de la région, avec une meilleure tolérance aux maladies cryptogamiques par rapport aux autres graminées telles que l'avoine et l'orge. Cette adaptation explique ses niveaux de production comparables à l'avoine soit en culture pure, pour le foin et le grain, soit en mélange avec la vesce. Il peut être utilisé en vert avant le stade de montaison pendant l'hiver tout comme l'orge ou l'avoine. Mais vu que les variétés actuelles contiennent des teneurs élevées en lignine et pauvres en protéines, le mélange vesce-triticale est une bonne option, surtout que son ensilage a confirmé une amélioration substantielle de la valeur alimentaire (Chentouf *et al*., 2015). Le bersim est la seule légumineuse fourragère qui fournit d'importantes quantités de fourrage de qualité quand la luzerne entre en dormance en hiver. Il est exploité dès la fin décembre jusqu'à fin mai et début juin. Utilisé seulement en vert vu sa grande teneur en eau, ses rendements varient entre 10 et 13 tonnes de MS/ha, avec en moyenne 3 à 4 coupes, la dernière repousse étant généralement réservée à la production de semences (Chentouf *et al*. 2015). A cause de l'extension de l'orobanche, les superficies de fève et de féverole ont diminué, laissant la place au lupin doux qui peut valoriser les différents sols de la région, avec des rendements pouvant atteindre 36 q/ha pour l'espèce *Lupinus albus* sur les sols sablonneux. Cependant, la prospection de variétés avec des teneurs faibles en alcaloïdes s'avère une nécessité pour son incorporation sans danger dans la ration des animaux (Thami Alami *et al*., 2004). En ce qui concerne les vesces, elles sont soit exploitées pour la production de grains comme les protéagineuses, soit utilisées en mélange avec les graminées fourragères. Certaines variétés locales ont montré des teneurs en protéines variant de 205,5 à 215,4 g/kg MS ; elles montrent également une teneur en constituants indigestibles plus faible que le fourrage d'avoine, d'orge et de triticale (264,7 à 272,1 g/kg MS).

Ces ressources sont caractérisées par plusieurs contraintes, notamment la faible productivité ; celles caractérisées par une forte production, notamment l'avoine, sont sujettes à une forte pression d'utilisation, d'où la nécessité de prospecter de nouvelles ressources pour diminuer cette pression.

Tableau 1: Types et disponibilité des ressources alimentaires dans le Nord du Maroc

Espèces	Nom commun	Variétés locales	Utilisation	Atouts/challenges	Références
Céréales					
Hordeum bulgare	Orge	Ambia Beldi	Le fourrage vert du déprimage de l'orge est utilisé pendant une durée de 1 à 2 mois, généralement à partir de décembre ; l'orge fourragère ou l'association orge-bersim fauchés ou pâturés directement par les animaux. L'orge était préférée pour les ânes et les mulets.	Niveau élevé de flux de gènes entre les communes et menaces pour la conservation des variétés traditionnelles d'orge.	(Jensen et al., 2013 ; Zaharieva and Monneveux, 2014; Chentouf et al., 2015).
Triticum monococcum	Épeautre	-	Utilisé comme grains et fourrages, en plus de la valorisation de la paille. Les épillets de petit épeautre sont également donnés à manger aux animaux, seuls ou mélangés avec de l'orge.	La diversité variétale est faible et chaque culture n'est représentée que par une ou deux variétés.	(Zaharieva and Monneveux, 2014)
Triticosecale	Triticale	Juanillo, Borhane et Moumtaz.	En culture pure ou en mélange avec la vesce (vesce-triticale), peut être utilisé dans l'alimentation des élevages caprins en vert, en foin et en grain. La paille de triticale doit être traitée par l'urée pour être au niveau de la paille d'orge ou de blé.	Elle a une bonne adaptation aux sols lourds et sablonneux prédominants ainsi qu'une meilleure tolérance aux maladies cryptogamiques.	(Chentouf *et al.*, 2015)
Avena sativa	Avoine	Ghali, Tissir, Zahri, Nasr Amellal, Hamel	Le fourrage d'avoine ou l'association vesce-avoine sont fauchés ou pâturés directement par les animaux. Souplesse d'utilisation pour le vert, le foin ou le grain.	Production élevée en biomasse et grains. Résistance rouille brune, l'oïdium et le virus nanisant de l'orge.	(Hmimsa and Ater, 2008 ; Chentouf et al., 2015)
Sorghum Bicolor	Sorgho	-	Le sorgho est largement exploité pour ses grains. Il est également exploité sous forme de fourrage quand les conditions climatiques le permettent.	Avec le mais, constituent les seules cultures fourragères de printemps qui peuvent être conduites en été sans irrigation.	(Chentouf et al., 2015)
Légumineuses					
Vicia sativa	Vesce	Marhaba Guich1	En mélange avec l'avoine ou le triticale, soit exploitée pour les grains et la paille.	Ressources génétiques pas encore étudiées.	(Chentouf *et al.*, 2015)
Vicia faba var. equina ou minor	Féverole	-	En mélange avec l'avoine ou le triticale, soit exploitée pour les grains et la paille.	-	(Chentouf *et al.*, 2015)
Trifolium alexandrium	Bersim	-	Fauchés ou pâturés directement par les animaux.	Forte teneur en eau.	(Hmimsa and Ater, 2008)

8.2. Nouvelles ressources alternatives locales

Le Sulla flexuosa, le sorgho et l'orobe pourraient être de bons candidats à une valorisation dans la région, comme aliments alternatifs locaux pour les caprins.

Le Sulla flexuosa L. Medik. (*Hedysarum flexuosum L.*) est une légumineuse spontanée (figure 6) poussant dans certaines régions Méditerranéennes (Nord du Maghreb) en conditions pluviales et froides. Au Nord du Maroc, elle est présente sous forme d'écotypes sauvages couvrant des petites et grandes superficies, selon l'état de dégradation du site. Des études sur le rhizobium du Sulla indiquent que la plante est adaptée au milieu Méditerranéen et pourrait être utile dans d'autres parties du monde, comme l'Australie et la Chine (El Yemlahi *et al*., 2022). Elle a été signalée sur des sols marneux et marneux-calcaires dans des régions avec une pluviométrie moyenne supérieure à 550 mm (Abdelguerfi-Berrekia et *al.*, 1991). Cependant, en raison de son caractère spontané et non cultivé, le Sulla flexuosa (L.) Medik. est classée sur la liste rouge des espèces de l'UICN à haut risque d'extinction (Groom, 1998). Étant donné la disparition de plusieurs écotypes dans des zones où ils étaient observés il y a quelques années, cette raréfaction pourrait être accentuée par l'extension de la culture, la dégradation des sols, le surpâturage ou le changement climatique. En Algérie, les valeurs morphologiques et nutritionnelles du Sulla flexuosa (L.) Medik. frais et séché au soleil ont été évaluées pour l'engraissement des lapins (Zirmi-Zembri et *al.*, 2020 ; Kadi et *al.*, 2011). Au Maroc, Errassi *et al*. (2018a ; 2018b) ont signalé des variations significatives de la digestibilité et des composés phénoliques entre les écotypes de Sulla flexuosa; cette première étude a été réalisée uniquement pour certains paramètres sur quelques écotypes.

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Figure 6 : Plante de Sulla flexuosa

Dans le Nord du Maroc, le sorgho (*Sorghum bicolor* (L.) Moench) (Figure 7) est rapporté comme une culture mineure avec deux variétés principales "hamra" et "bayda" se référant aux couleurs blanches et rouges des grains. Concernant l'utilisation régionale, le sorgho bicolor est considéré comme très connu et exploité par les agriculteurs (Hmimsa & Ater, 2008) Des études antérieures ont également rapporté l'utilisation de ses sous-produits : l'éclaircissage en juin et les résidus entre août et octobre (Chentouf *et al.*, 2015). Le sorgho grain est largement produit et utilisé dans l'alimentation des caprins. Les cultures du sorgho fourrager se limitent à certaines plaines côtières, notamment dans des exploitations d'élevage de bovins laitiers (Chentouf *et al.*, 2015). La fluctuation des rendements selon les conditions climatiques est un des défauts majeurs de cette culture. Les études des ressources génétiques réalisées se sont concentrées sur sa caractérisation phénotypique, sans faire de lien avec la nutrition animale (Bouargalne *et al*., 2022 ; Djè *et al.*, 1999, 2007). Cependant, il pourrait être une bonne alternative pour diminuer la pression sur l'orge qui est également utilisé en nutrition animale.

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Figure 7 : Plante de Sorgho

L'orobe (*Vicia ervilia*) (Figure 8) est signalée comme étant une culture marginalisée et de faible abondance dans la région du Nord du Maroc (Hmimsa & Ater, 2008). Principalement exploitées pour les grains et la paille, les ressources génétiques ont été faiblement étudiées. Les études phénotypiques se sont concentrées sur la variabilité génétique et phénotypique sans caractérisation agronomique des potentialités de rendement. L'étude de la valeur nutritive n'a pas été réalisée pour les écotypes Marocains. Les études édapho-climatiques (relatives à l'influence du sol et du climat) seraient aussi un plus pour expliquer la rareté de la culture d'orobe dans la région du Nord du Maroc.

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Figure 8 : Grains d'orobe

8.3. Utilisation des plantes alternatives dans l'alimentation des ruminants

Le Sulla flexuosa et l'orobe sont utilisés comme sources de protéines (Kadi et al., 2011 ; Larbi, Abd El-Moneim et al., 2011) alors que le sorgho est utilisé en tant que source d'énergie (Hmimsa & Ater, 2008). Des études de l'incorporation du Sulla coronaria dans l'alimentation des ovins ont été menées pour étudier l'effet de cette légumineuse sur la production laitière et de la viande (Bonanno *et al*., 2007, 2011, 2016 ; Cabiddu *et al*., 2009 ; Gannuscio *et al*., 2022 ; Leto *et al*., 2002 ; Ponte *et al*., 2022 ; Priolo *et al*., 2005). L'effet de Sulla flexuosa a été évalué chez le lapin (Kadi *et al*., 2011). A notre connaissance, aucune étude n'a déterminé l'effet des foins de Sulla flexuosa sur les performances de production et la qualité du lait des chèvres. Dans la région Méditerranéenne, les efforts d'incorporation des grains d'orobe ont été initiés à travers son incorporation dans des rations de volaille et d'ovins (Abdullah *et al*., 2010 ; Sadeghi *et al*., 2009). Cependant, au Maroc aucune étude n'a évalué son effet sur la production et la qualité de la viande caprine. Il en va de même pour le sorgho, qui a été rarement étudié chez les ruminants au Maroc.

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Objectifs

Chapitre 2. Objectifs

L'objectif de ce travail est d'améliorer la conduite alimentaire du cheptel caprin au Nord du Maroc par l'utilisation des ressources alimentaires alternatives locales afin de réduire la pression sur les parcours forestiers, d'améliorer la productivité des élevages et le revenu des éleveurs caprins.

D'une manière spécifique, les objectifs sont :

(i) d'évaluer l'écologie des sites de collecte et de réaliser la caractérisation agronomique (rendement), morphologique (hauteur, nombre de feuilles, longueur et largeur de feuilles, …) et bromatologique (valeur nutritive et digestibilité) des écotypes locaux de Sulla flexuosa, sorgho et orobe ;

(ii) d'étudier les effets de l'incorporation du foin de Sulla flexuosa dans la ration de la chèvre locale du Nord du Maroc de race Beni Arouss sur la production laitière et la qualité du lait (composition chimique, profil en acides gras, activité antioxydante);

(iii) de déterminer l'effet des grains de sorgho et d'orobe sur la croissance, les caractéristiques de la carcasse et la qualité (composition chimique, profil en acides gras) de la viande chez les chevreaux de la même race.

En outre, les analyses multivariées devraient permettre de classifier les écotypes des trois plantes étudiées en fonction des caractéristiques recherchées et ainsi, de diffuser et de conserver dans la banque de graines de l'INRA, les écotypes les plus intéressants afin de maintenir la biodiversité au Nord du Maroc.

Section expérimentale

Section expérimentale

Etude 1:

Ecological, morpho-agronomical and nutritional characteristics of Sulla flexuosa (L.) Medik. ecotypes

Chapitre 3. Étude 1: Ecological, morpho-agronomical and nutritional characteristics of Sulla flexuosa (L.) Medik. ecotypes

Préambule

Le Sulla flexuosa (L.) Medik. est une légumineuse fourragère spontanée à la région du Nord du Maroc et, présente sur la liste rouge mondiale des espèces menacées. Elle appartient au genre *Hedysarum* dont l'espèce *Hedysarum coronarium* (L.) Medik. (Sainfoin espagnol ou italien) a été caractérisée par son adaptabilité aux environnements marginaux et secs, sa faible exigence en intrants, sa concentration modérée à élevée en tannins et sa teneur élevée en protéines. A cause des changements climatiques, du coût élevé des aliments concentrés et de leur concurrence avec l'alimentation humaine, la prospection de nouvelles ressources alternatives locales, adaptées aux conditions environnementales a émergé. Les objectifs de cette étude étaient d'abord de caractériser l'habitat naturel des écotypes collectés de Sulla flexuosa (L.) Medik., et de vérifier si la répartition de ces écotypes dans la région du Nord du Maroc était liée aux caractéristiques édapho-climatiques. Cette observation sera utile pour la dissémination des écotypes performants par la suite. La première étape a donc été la collecte de 21 écotypes dans la région du Nord du Maroc et en même temps que des échantillons de sol. Durant deux années (2019 et 2020), les écotypes ont été semés en blocs aléatoires complets avec trois répétitions. Des suivis réguliers ont été menés pour noter les différents stades phénologiques. Aux stades de bourgeonnement, début et pleine floraison, des échantillons ont été réalisés pour déterminer les rendements en matière verte et en matière sèche. Pour les stades début et pleine floraison, cinq plantes ont été choisies pour la caractérisation morphologique. Les échantillons coupés ont servi également pour la caractérisation de la composition chimique et de la digestibilité. Les analyses multivariées (matrice de corrélations, analyses en composantes principale et heatmap) ont servi à classifier les écotypes en plusieurs groupes facilitant le choix en fonction des objectifs pour leur dissémination et leur conservation. Le groupe 1 était le plus intéressant avec sa productivité élevée et sa bonne valeur nutritive. L'écotype E1 appartenant à ce groupe était à double usage avec sa productivité élevée, sa bonne valeur nutritive et ses paramètres de reproduction intéressants. L'écotype E4 constituant le groupe 3 a également été mis en évidence comme ayant une floraison tardive mais une productivité intermédiaire, qui peut être utilisée principalement pour la fenaison car la période de séchage pourrait coïncider avec les dernières pluies dans la région. En conclusion, afin d'obtenir les teneurs en protéines et en matière sèche les plus élevées pour l'exploitation du Sulla flexuosa (L.) Medik. dans l'alimentation animale, la coupe au stade début du de floraison peut être suggérée.

Les hypothèses étaient donc :

- ✓ Les caractéristiques du sol des sites de collecte sont similaires ?
- ✓ Les paramètres agro-morphologiques et bromatologiques sont influencés par :
    - Le stade phénologique ?
    - Les écotypes ?

Section expérimentale

Etude 1 :

Ecological, morpho-agronomical and nutritional characteristics of Sulla flexuosa (L.) Medik. ecotypes.

Scientific reports (Soumis)

S. Boukrouh, A. Noutfia, N. Moula, C. Avril, J. Louvieaux, J.L. Hornick, M. Chentouf, J.F. Cabaraux

Abstract

The present work was part of the assessment of wild genetic plant resources of forage interest in Northern Morocco and aimed to study the agro-morphology and nutritional value of Sulla flexuosa (L.) Medik. (*Hedysarum flexuosum* (L.) Medik.*)* ecotypes. The seeds of twenty-one wild Sulla flexuosa (L.) Medik. ecotypes were collected from 21 sites. The edaphic and climatic characteristics of the collection sites were studied and testified to the remarkable adaptability of Sulla flexuosa (L.) Medik. During two consecutive years, these 21 ecotypes were cultivated in three complete randomized blocks design. Statistical analysis showed substantial variability between the collected ecotypes. Principal component analysis and heatmap analysis allowed to distinguish four groups of ecotypes mainly based on bromatological parameters (fiber content and digestibility), forage production (dry matter yield, number of leaves per plant and total number of branches) and reproduction (number of inflorescences per plant and, weight of thousand seeds and seeds per plant). Furthermore, the present study pointed out the value of ecotype 1, which was dual purpose with its high productivity, nutritive value, and reproductive parameters. Ecotype 4 was also highlighted as having late flowering but intermediate productivity, which can be used mainly for haymaking as the drying period could coincide with the last rainfall in the region.

Introduction

In the southern Mediterranean area, such as Northern Morocco, goat farming significantly contributes to rural household income. These farms are often familial, extensive, driven by traditional knowledge and know-how, and based on the exploitation of free natural resources (Chebli, *et al*., 2021a). The feeding of this extensive or semi-extensive livestock is characterized by a strong pressure on the silvopastoral rangelands and an irregular forage supply that does not meet animal requirements throughout the year (Chebli *et al*., 2018). To sustain forests and improve animal productivity, it is therefore necessary to study new fodder resources to secure forage systems (Chebli *et al*., 2021a; Chebli et al., 2018).

Spontaneous fodder legumes of natural pastures are essential elements in the diet of ruminants, among others, because of their high protein content.

Among the wild legumes found in the Mediterranean region, annual and perennial species of the genus Hedysarum spp. grow on a remarkable range of bioclimatic and soil conditions (Abdelguerfi-Berrekia *et al*., 1991). In addition, due to their moderate to high concentration of condensed tannins, species of the genus Hedysarum spp. exhibit antiparasitic effects in the digestive tract, a decrease in microbial degradation of proteins in the rumen with improved intestinal absorption, and positive environmental impacts by reducing methane production in ruminants (Ramírez-Restrepo & Barry, 2005). The interest in the genus Hedysarum spp. also comes from the fact that some species have good agronomic characteristics and, in particular, excellent adaptability to marginal and dry environments (Annicchiarico *et al*., 2014) low input requirement, and contribution to soil nitrogen enrichment through their ability to fix atmospheric nitrogen by symbiotic association with rhizobia (Sulas *et al*., 2019). They are nutritious and palatable legumes primarily used as a green forage for grazing and hay production, and in some regions of the Mediterranean area, they were also exploited as silage. Sulla (L.) Medik. is also a cover crop that improves soil fertility and reduces erosion (Chisci *et al*., 2001). In addition, its importance also lies in its versatility for its agricultural and non-agricultural uses. Sulla was used for honey production and landscape architecture (Talamucci, 1998).

Among the species of the genus *Hedysarum* spp., *Hedysarum coronarium* (L.) Medik., also called Sulla coronaria (L.) Medik., Italian or Spanish sainfoin, is commonly cultivated in the Mediterranean basin (Annicchiarico *et al*., 2014a; Córdoba *et al*., 2013) and has been widely studied for its nutritional value (Borreani *et al*., 2003) and phenolic compounds (Tibe *et al*., 2011) at different morphological stages and through different conservation methods (Rufino-Moya *et al*., 2019a). In Italy, some varieties of Sulla coronaria (L.) Medik (Grimaldi, Sparacia, Bellante, and S. Homer) were selected and are already cultivated and listed in the Italian national seed register.

In Morocco and Algeria, it is *Hedysarum flexuosum* L., also known as Sulla flexuosa (L.) Medik. which is encountered. This legume is a wild and neglected plant found in the wild natural grasslands in the form of small and isolated populations.

It is reported on marly and marl-limestone substrates in regions with average rainfall above 550 mm (Abdelguerfi-Berrekia *et* al., 1991). However, because of its spontaneous and non-cultivated character, Sulla flexuosa (L.) Medik. is classified on the IUCN's red list of species at high risk of extinction (Groom, 1998). Given the disappearance of several ecotypes in areas where they were once observed some years ago, this rarefaction could be accentuated by the extension of cultivation, soil degradation, overgrazing, or climate change. However, the conservation and use of this local plant resource could increase fodder supply and thus be introduced into the ruminant diet as an alternative to other legumes (Boukrouh *et al*., 2023). In Algeria, morphological and nutritional values of fresh and sun-dried Sulla flexuosa (L.) Medik. were assessed for rabbit fattening (Kadi *et al*., 2011; Zirmi-Zembri & Kadi, 2020). In Morocco, Errassi *et al*., (2018a; 2018b) reported significant variations in digestibility and phenolic compounds between Sulla flexuosa (L.) Medik. ecotypes; this first study was realized only for some parameters on a few ecotypes.

The present work aimed to deeply evaluate the ecological, agro-morphological, and nutritional characteristics of twenty-one Sulla flexuosa (L.) Medik. ecotypes in Northern Morocco. The aim was to enrich INRA Morocco seed bank was to create a seed bank for conservation and choose the more productive and interesting ecotypes for cultivation and animal nutrition.

Results & discussion

Ecological characterization. In Northern Morocco, the wild-collected Sulla flexuosa (L.) Medik. ecotypes were found on sites with an altitude not exceeding 358 m and minimum annual rainfall of 661 mm (Table 2). The physico-chemical characteristics of the soils were variable from one site to another. However, the results showed that these soils were rather dry (5% moisture), basic (pH = 8.4), and poor in organic matter (2%), exchangeable potassium (137 ppm), and available phosphorus (9.6 ppm). The average electrical conductivity of the soil (9.8 mS/m) was below 20 mS/m and corresponded to a non-saline soil, according to Boulding, (1994). The limestone content did not exceed 20%. These results are close to those reported by Abdelguerfi-Berrekia *et al*., (1991) and Zirmi-Zembri & Kadi, (2020) with a minimum rainfall of 550 mm, a maximum altitude of 600 m, a pH ranging from 7.4 to 8.9, an organic matter (OM) content ranging from 0.21 to 2.54%, and a $CaCO_3$ lower than 20%. However, these authors reported rather clayey or sandy soils on the collection sites of wild Sulla flexuosa (L.) Medik. In contrast, El Yemlahi *et al*., (2020) found Sulla flexuosa (L.) Medik. in clay-loam soils. In the present study, the soil contained a minimum of 11% clay, while for sand and silt, the minimum percentages could reach 0%; the soil must apparently contain a minimum of clay for Sulla flexuosa (L.) Medik. growth. A high content of coarse silt (76%) was observed at one collection site. All these results show the high adaptability of Sulla flexuosa (L.) Medik. to grow on very different soils in textural composition.

Table 2: Climatic and physico-chemical characteristics of the soils of the 21 collection sites of wild Sulla flexuosa (L.) Medik. ecotypes and of the experimental site of Sulla flexuosa (L.) Medik. cultivation in Northern Morocco.

	Collection sites							Experimental site	
	Mean	Minimum	Maximum	Standard deviation	1st quartile	Median	3rd quartile	2019	2020
Altitude (m)	120.1	9.0	358.0	85.4	69.0	106.0	143.0	128.5	110.4
Edaphic parameters									
Humidity (%)	5.0	1.6	7.2	1.4	4.2	5.2	5.8	5.2	6.4
pH water	8.4	7.4	8.9	0.3	8.3	8.5	8.6	8.4	8.3
pH KCl	7.7	6.9	8.0	0.3	7.5	7.7	7.7	7.4	7.2
Electrical conductivity C (mS/m)	9.8	6.7	11.8	1.2	8.9	9.8	10.5	31.0	39.0
Organic matter (%)	1.9	0.6	3.7	0.9	1.3	2.0	2.5	2.2	2.6
Limestone ($CaCO_3$, %)	5.1	0.5	20.3	5.5	2.0	3.2	5.3	7.4	3.9
Exchangeable potassium (K, ppm)	136.5	53.8	319.6	70.4	84.5	122.9	169.0	705.3	367.7
Available phosphorus (P, ppm)	9.6	4.1	11.9	1.7	8.8	9.6	10.4	39.7	28.0
Carbon to nitrogen ration (C/N)	11.7	2.6	24.5	5.8	6.6	9.2	15.4	6.4	8.0
Nitrogen (N, %)	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.2	0.2
Clay (%)	30.0	11.0	52.0	13.6	19.7	29.2	41.5	50.0	30.0
Coarse sand (%)	8.2	0.1	40.0	11.1	1.3	2.1	13.2	1.6	2.0
Fine sand (%)	4.4	0.2	13.7	4.4	1.5	2.5	5.6	1.1	0.9
Coarse silt (%)	38.6	0.0	75.6	22.5	18.1	45.2	46.2	35.0	34.0
Fine silt (%)	18.9	0.0	50.0	12.6	10.7	20.0	24.0	10.0	10.0
Bioclimatic parameters									
Average annual temperature (BIO1, °C)	18.1	17.3	18.4	0.3	18.1	18.2	18.2	18.1	18.5
Average diurnal variation (BIO2, °C)	8.8	6.0	10.0	1.1	8.2	9.1	9.7	22.9	20.4
Isothermality (BIO3 = BIO2/BIO7×100, %)	39.1	33.1	41.1	2.1	38.5	39.8	40.6	57.3	51.1
Temperature seasonality (BIO4)	480.1	439.5	548.2	29.0	454.6	478.7	503.3	542.1	520.4
Maximum temperature of the warmest month (BIO5, °C)	29.0	27.9	29.9	0.7	28.4	29.1	29.7	40.7	35.7
Minimum temperature of the coldest month (BIO6, C°)	6.6	4.3	9.8	1.2	6.0	6.4	7.1	0.7	1.6
Temperature Annual Range (BIO7 = BIO5-BIO6, °C)	22.5	18.1	25.2	1.8	21.4	22.7	23.7	40.0	39.9

Average temperature of the wettest quarter (BIO8, °C)	12.8	10.8	14.2	1.0	12.1	12.5	13.9	16.2	14.6
Average temperature of the driest quarter (BIO9, °C)	24.0	23.3	24.5	0.4	23.6	23.9	24.4	21.8	22.33
Average temperature of the warmest quarter (BIO10, °C)	24.3	23.7	24.8	0.4	24.0	24.3	24.6	24.4	25.3
Average temperature of the coldest quarter (BIO11, °C)	12.4	10.8	13.2	0.6	12.1	12.5	12.7	11.8	12.8
Annual precipitation (BIO12, mm)	775.8	661.0	873.0	54.2	750.0	779.0	811.0	563.4	736.8
Precipitation of wettest month (BIO13, mm)	146.6	123.0	167.0	12.9	138.0	143.0	161.0	152.0	232.8
Precipitation of driest month (BIO14, mm)	0.4	0.0	2.0	0.6	0.0	0.0	1.0	0.0	0.0
Precipitation seasonality (BIO15, %)	81.8	74.1	87.3	4.6	76.9	82.8	85.8	117.8	106.3
Precipitation of wettest quarter (BIO16, mm)	398.7	334.0	468.0	36.7	377.0	396.0	429.0	129.8	141.4
Precipitation of driest quarter (BIO17, mm)	24.0	23.3	24.5	0.4	23.6	23.9	24.4	0.1	1.5
Precipitation of warmest quarter (BIO18, mm)	24.3	23.7	24.8	0.4	24.0	24.3	24.6	1.3	4.5
Precipitation of coldest quarter (BIO19, mm)	12.4	10.8	13.2	0.6	12.1	12.5	12.7	44.9	51.6

Agro-morphological characterization. The results revealed that all the agro-morphological parameters of Sulla flexuosa (L.) Medik. were significantly ($p < 0.01$) influenced by the ecotype and phenological stage (Table 3), whereas the year significantly ($p < 0.05$) influenced all the parameters except DMY. The interactions ecotype × year and ecotype × phenological stage showed significant interaction ($p < 0.01$) for all the agronomical and morphological parameters. The interaction year × phenological stage had a highly significant influence ($p < 0.001$) on the number of plagiotropic branches, leaves, and inflorescences per plant and on the length of the longest plagiotropic branch per plant. The interaction ecotype × year × phenological stage was significant ($p < 0.05$) for all parameters except DMY. Several authors reported climatic conditions (Sousa *et al*., 2020) effects on plant development, which could explain the ecotype × year interactions for all the morphological parameters, as the 2020 growing season was 31% wetter than one of 2019.

The number of plagiotropic branches varied greatly between ecotypes, but the average number (9.1) was in the range of values reported for alfalfa (Medicago sativa) (Yun *et al*., 2022), but higher than values reported for Sulla coronaria (L.) Medik. (4.4) (Annicchiarico *et al*., 2014). The same observation was made for the number of total branches, with an average of 165 in the present trial versus 43 for Sulla coronaria (L.) Medik. (Annicchiarico *et al*., 2014). The increase in the plagiotropic number (9.8 vs. 8.5) and the number of total branches (176 vs. 153) in the second year was probably due to the higher water and light availability than in the first year. Indeed, in a wet year, more water is available for plant growth, which can lead to increased vegetative growth and branching. Also, plagiotropic branches, which grow horizontally, could enhance light capture efficiency, and increase the amount of light received by the plant. In a wet year, there could be more diffuse light due to cloud cover, which can also promote branching (Osada & Takeda, 2003).

The average plant height of Sulla flexuosa (L.) Medik. was 65 cm. Values of 40 cm and 60 cm have been reported for Sulla coronaria (L.) Medik. and alfalfa, respectively (Annicchiarico *et al*., 2014; Yun *et al*., 2022). The length of the longest plagiotropic branch per plant was about 107 cm compared to 159 cm observed for Sulla coronaria (L.) Medik. (Gaad, 2010). Thus, this last one tends to grow horizontally compared to Sulla flexuosa (L.) Medik., which would grow more vertically. The average main stem diameter (1.07cm) of the ecotypes in this study was smaller than the average diameter observed for Sulla coronaria (L.) Medik. in Spain (1.9 cm) (Cordoba *et al*., 2013). This smaller stem diameter with a higher height of Sulla flexuosa (L.) Medik. could make it more susceptible to lodging.

The wide variability of morphological parameters (with 12.6 % for stem diameter and 35.9 % for the number of inflorescences per plant) between ecotypes reflected the low level of human intervention for selection. The low variability for phenology (with 0.24% for the start of flowering and 7.91% for the number of days to emergence) was also reported for other *legume* species (Yohannes *et al*., 2020). Concerning yield parameters, Sulla coronaria (L.) Medik. is a biennial fodder while Sulla flexuosa (L.) Medik. is annual (Annicchiarico *et al*., 2014; Zirmi-Zembri & Kadi, 2020). However, the annual observed fresh matter yield (33.0 T/ha) in the present study was close to that reported by Córdoba et al., (2013) (33.5 T/ha) with biennial populations of Sulla coronaria (L.) Medik. in the first year. In contrast, DMY was higher in the present trial (4.8 vs. 3.5 T DM/ha). This difference in DM content could be explained by the lower leaf-to-stem ratio (0.55) due to greater branching in Sulla flexuosa compared to the one of Sulla coronaria (L.) Medik. (0.4 to 3.2) (Abdelguerfi & Abdelguerfi-Laouar, 2004; Annicchiarico *et al*., 2014). Moreover, the Sulla flexuosa (L.) Medik plants should be handled with care as wild species are reported to be less resilient compared to crop progenitors in their response to defoliation (Cunniff *et al*., 2014). This results from selecting traits that allow crops to recover quickly from damage caused by grazing or harvesting.

In this study, all the ecotypes in 2020 were leafier and denser than in 2019 (428 vs. 262 for the number of inflorescences per plant and 176 vs. 153 for the number of total branches). It could be due to higher rainfall during the second growing season (736.8 vs. 563.2 mm). However, only an increase in fresh matter yield and no significant increase in DM yield were observed. This was possibly explained by the increase in leaf-to-stem ratio as DM of leaves is lower compared to the stems.

Concerning seeds yield parameters, total seed weight averaged 5.9 g and appeared in the range of values reported for Sulla coronaria (L.) Medik. (Córdoba *et al*., 2013). Those values are interesting as crop species are reported to have a higher thousand seed weight than wild species due to artificial selection for larger seeds, which can improve competitive ability during the early stages of plant establishment and increase crop yield and growth (Cunniff *et al*., 2014).

The phenology and optimal conditions for each phase of the crop cycle are essential in deciding the most appropriate ecotypes for a particular region. Even under favorable conditions (greenhouse sowing in trays), the number of days between sowing and emergence of Sulla flexuosa (L.) Medik. seeds, calculated in GDD, varied from 134.8 to 230.3°C, with an average of 89.3°C, showing a powerful impact of the ecotype on this parameter. The appearance of the first flower bud (1224.4°C) and the early (1383.1°C), full (1439.7°C), and late (1638.8°C) stages of flowering were also significantly ($p < 0.001$) different between ecotypes.

Table 3: Descriptive statistics and means of agro-morphological traits of 21 cultivated Sulla flexuosa (L.) Medik. ecotypes across phenological stages (B Budding (days), SF start of flowering (days) and FF full flowering (days)) and years (2019 and 2020).

	Mean	SEM	Min.	Max.	CV (%)	Phenological stage (PS)			Year (Y)		Ecotype (E)	Y	PS	E × Y	E × PS	Y × PS	E × Y× PS
						B	SF	FF	2019	2020							
Number of plagiotropic branches	9.1	0.1	4.9	14.9	15.4		9.0	9.3	8.5	9.8	***	***	**	***	**	***	***
Plant height (cm)	65.2	0.8	40.7	107.4	16.6		61.7	68.8	64.0	66.5	***	**	***	***	***	ns	***
Length of orthotropic axis (cm)	60.5	0.7	36.4	98.7	18.1		57.0	64.0	59.8	61.2	***	**	***	***	***	ns	***
Length of the longest plagiotropic branch (cm)	106.8	1.3	66.8	138.3	19.0		99.2	114.4	92.4	121.2	***	***	***	***	***	ns	***
Number of leaves per plant	344.8	8.8	164.0	861.2	24.7		326.9	362.7	261.9	427.6	***	***	***	***	***	***	***
Number of inflorescences per plant	159.8	3.9	79.9	316.6	35.9		143.4	176.1	148.7	170.9	***	***	***	***	***	***	***
Number of total branches	164.5	3.6	70.2	413.6	24.2		159.5	169.4	152.9	176.0	***	***	**	***	***	ns	***
Stem diameter (cm)	1.1	0.0	0.9	1.5	12.6		1.1	1.1	1.1	1.1	***	*	***	***	***	ns	***
Fresh matter yield (T/ha)	33.0	0.6	19.6	50.0	3.8	28.8^{c}	32.9^{b}	37.2^{a}	32.6	33.3	***	***	***	***	***	ns	***
Dry matter yield (T/ha)	4.8	0.1	3.0	7.2	5.8	3.8^{c}	4.8^{b}	5.9^{a}	4.8	4.9	***	ns	***	***	***	ns	ns
Leaf to stem ratio	0.57	0.01	0.39	0.84	6.00	0.69^{a}	0.56^{b}	0.47^{c}	0.55	0.59	***	***	***	***	***	ns	*
Number of days to emergence (GDD, °C)	181.3	3.5	134.8	230.3	6.5						***						
Flower bud appearance (GDD, °C)	1224.4	7.3	1125.4	1336.7	0.9						***						
Start of flowering (GDD, °C)	1383.1	6.5	1252.3	1525.3	0.3						***						
Full flowering (GDD, °C)	1439.7	5.1	1367.1	1547.2	1.2						***						
End of flowering (GDD, °C)	1638.8	9.0	1534.6	1896.7	0.8						***						
Flowering duration (GDD, °C)	253.9	6.2	173.7	371.4	8.0						***						
Thousand seeds weight (g)	5.9	0.1	4.6	6.9	2.8						***						
Seed weight per plant (g)	23.6	0.5	17.1	31.0	4.9						***						

SEM standard error of the mean, *Min.* minimum, *Max.* maximum, *CV* coefficient of variation (%). n.s, *, ** and *** represent non-significant, significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Bromatological characterization. All the bromatological parameters (Table 4) of Sulla flexuosa (L.) Medik. were significantly influenced ($p < 0.001$) by ecotype and phenological stage. Likewise, the year effect had significant ($p < 0.05$) influence on all bromatological parameters except on Ash, IVECPD, and ME. Ecotype × year interaction effect was highly significant ($p < 0.001$) for all parameters, possibly due to the differences in the environmental conditions, especially in the precipitations between years, or in the genetics that affect their response to environmental variation.

The ecotype × phenological stage interaction effect was highly significant ($p < 0.01$) for all parameters except for Ash and ADF. Year × phenological stage interaction effect was significant ($p < 0.05$) for all parameters except for DM, Ash, CF, ADL, IVTD. Finally, the triple interaction was significant ($p < 0.05$) for Fat, ADF, IVTD, Phenols, NTP, CT and HT. It could be explained that for each phenological stage, it was not the same ecotypes that were the more performant. Thus, the accurate phenological stage must be determined for each ecotype to ensure its best results.

Legumes are considered a supplementary high-quality protein-based feed. Bromatological values can vary among species and cultivars, throughout the years and between phenological stages (Borreani *et al*., 2003; Córdoba *et al*., 2013; Ruisi *et al*., 2011). Sulla flexuosa (L.) Medik. showed a high average CP content (19.4% DM) which is close to values reported by Zirmi-Zembri & Kadi, (2019) for Sulla flexuosa (L.) Medik. and values reported by Borreani *et al*., (2003) for Sulla coronaria (L.) Medik. All the ecotypes had CP content higher than 9% which is the minimum level required for adequate microbial synthesis in the rumen (Sampaio *et al*., 2010). Both NDF (47.1 vs. 48.6% DM) and ADF (32.2 vs. 34.5% DM) contents were also close to values observed by Zirmi-Zembri & Kadi, (2016) while for ADL, their value was almost two times higher (14.1 vs. 9.0% DM), respectively.

With the advancement of the phenological stage of the plants (Table 4), a significant ($p < 0.001$) increase in Fat (+22.5%), Ash (+14.0%), CF (+21.8%), NDF (+11.9%), ADF (+7.9%) and ADL (+20.0%) contents as well as an equally significant decrease in NFE (-7.2%), in ME (-19.3%) and in the OM (-9.8%), crude protein (-2,8%), and true (-12.5%) digestibilities were observed. The start of flowering showed lower CF and ADF contents than the other two stages (budding and full flowering). Increasing fresh or dry matter yield usually leads to higher contents of NDF because of increased stem proportion, cell wall thickening, or lower contents of protein and other soluble cell contents (Elgersma & Søegaard, 2018). These changes in plant development occur with the advancing of the phenological stage (Borreani *et al*., 2003). Phenols, NTP, CT, and HT decreased by an average of 37% from the budding to the full flowering stage. The results of the present study concerning CT were lower than the ranges obtained for Sulla coronaria (L.) Medik. (Tibe *et al*., 2011). This decrease in the present trial could be due to the decrease in leaf-to-stem ratio, as condensed tannins are concentrated in leaves for the close species Sulla coronaria (L.) Medik. (Tibe *et al*., 2011).

Table 4 : Descriptive statistics and means of bromatological traits of 21 cultivated Moroccan Sulla flexuosa (L.) Medik. ecotypes across phenological stages (B Budding (days), SF start of flowering (days) and FF full flowering (days)) and years (2019 and 2020).

	Mean	SEM	Min.	Max.	CV (%)	Phenological stage (PS)			Year (Y)		Ecotype (E)	Y	PS	E × Y	E × PS	Y × PS	E × Y× PS
						B	SF	FF	2019	2020							
Dry matter (DM, %)	14.55	0.11	12.48	16.59	5.12	13.27^{c}	14.56^{b}	15.82^{a}	14.80	14.29	***	***	***	***	**	ns	ns
Crude proteins (% DM)	19.43	0.16	17.80	21.52	4.53	21.79^{a}	19.82^{b}	16.99^{c}	19.31	19.76	***	***	***	***	***	***	ns
Crude protein yield (T/ha)	0.93	0.02	0.56	1.51	6.25	0.83^{c}	0.95^{b}	1.00^{a}	0.92	0.93	***	*	***	***	***	*	ns
Fat (% DM)	2.49	0.03	2.04	2.98	6.23	2.22^{c}	2.52^{b}	2.72^{a}	2.46	2.52	***	**	***	***	***	***	***
Ash (% DM)	14.09	0.10	12.22	16.42	4.90	12.99^{c}	14.66^{b}	14.81^{a}	14.17	14.01	***	ns	***	***	ns	ns	ns
Crude fiber (% DM)	24.35	0.35	20.16	32.28	5.65	24.11^{b}	19.58^{c}	29.38^{a}	23.99	24.72	***	***	***	***	***	ns	ns
Neutral detergent fiber (% DM)	47.12	0.21	44.68	52.03	1.80	44.57^{c}	46.92^{b}	49.87^{a}	48.12	46.12	***	***	***	***	***	**	ns
Acid detergent fiber (% DM)	32.20	0.18	29.64	34.76	5.04	32.05^{b}	29.79^{c}	34.59^{a}	32.31	32.10	***	***	***	***	***	***	*
Acid detergent lignin (% DM)	14.12	0.15	11.03	18.00	3.24	12.79^{c}	14.23^{b}	15.35^{a}	14.25	13.99	***	***	***	***	ns	ns	ns
Nitrogen free extract (% DM)	39.54	0.34	31.52	45.41	4.34	38.89^{b}	43.63^{a}	36.10^{c}	40.08	39.00	***	**	***	***	***	ns	ns
In vitro enzymatic crude proteins digestibility (% DM)	43.68	0.15	40.91	49.53	2.25	43.69^{b}	44.87^{a}	42.46^{c}	43.68	43.68	***	ns	***	***	***	*	ns
In vitro enzymatic organic matter digestibility (% DM)	61.36	0.22	58.30	65.02	1.64	64.58^{a}	61.25^{b}	58.25^{c}	61.12	61.60	***	***	***	***	***	*	ns
In vitro true digestibility (% DM)	68.45	0.34	61.85	73.33	1.72	73.07^{a}	68.33^{b}	63.95^{c}	68.22	68.68	***	**	***	***	***	ns	***
Metabolizable energy (KJ/kg DM)	8.88	0.15	8.16	9.72	4.39	9.82^{a}	8.90^{b}	7.92^{c}	8.87	8.89	***	ns	***	***	***	***	ns
Phenols (% DM)	3.62	0.08	1.99	5.43	2.26	4.50^{a}	3.55^{b}	2.82^{c}	3.68	3.57	***	***	***	***	***	***	***
Non-tannic phenols (% DM)	1.72	0.04	0.72	2.97	5.54	2.16^{a}	1.66^{b}	1.34^{c}	1.75	1.70	***	***	***	***	***	***	***
Condensed tannins (% DM)	1.11	0.03	0.68	1.89	5.85	1.37^{a}	1.07^{b}	0.88^{c}	1.16	1.05	***	***	***	***	***	***	***
Hydrolysable tannins (% DM)	0.79	0.06	0.40	1.58	17.24	0.96^{a}	0.82^{b}	0.60^{c}	0.77	0.81	***	**	***	***	***	*	***

SEM standard error of the mean, *Min.* minimum, *Max.* maximum, *CV* coefficient of variation (%). n.s, *, ** and *** represent non-significant, significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

As expected, CP decreased by 22% DM from the budding to the full flowering stage.

This could be due to a growth dilution effect due to the increase in structural carbohydrates with the advancing maturity stage. However, although CP percentage decreased, crude protein yield increased significantly ($P < 0.001$) with the phenological stage and thus, with the increasing yield.

Sulla flexuosa (L.) Medik. ecotypes showed little increase in CP (+2.3%), Fat (+2.4%), and CF (+3.0) contents, and a small decrease in NDF (-4.2%), ADF (-0.7%), and ADL (-1.8%), NFE (-2.7%) contents between the two years. For OM and true digestibilities, slow increases (<1.0%) were found. Phenols, NTP and CT were significantly ($P<0.05$) 3.0%, 2.9% and 9.5 % lower in the second year, respectively. However, HT were 5.2 % higher. The low increase in fibers during the second year could be due to low differences in temperatures between the two years because plant development and fiber accumulation were reported to be negatively related to temperature (Pinnamaneni & Anapalli, 2021). It was stated that higher temperatures of about 1.5 °C in crop season led to 3.5% higher NDF and 21% higher ADL contents. However, in the present study, it was only a 0.4°C increase. The absence of differences between the overall growth, shown by the same yields between the two years, resulted in a weak change in fiber accumulation (Bhattarai *et al*., 2018). The CP Digestibility (43.7%) was close to values obtained for lucerne (46.4% DM) (Antoniewicz & Kosmala, 1995), and was not influenced by the year. The differences observed with the CP digestibility could be explained by the fiber contents and by the inverse relationship between these two parameters. The increased fiber content with the phenological stage also explained the decreased ME. However, ME always ranged in values reported for red clover (Trifolium pratense) and alfalfa in Sweden (Sousa *et al*., 2020).

Correlation matrix. *Correlation between morpho-pheno-agromorphological and edapho-climatic parameters.* The understanding of the correlations between different parameters is crucial because it allows the accomplishment of the indirect selection of the parameters that are inherited quantitatively and influenced by genetic effects (Mazid *et al*., 2013). The Pearson correlation coefficient between pairs of the parameters and the associated probabilities is given in Figure 9.

As expected, some agro-morphological parameters were correlated. The number of days to emergence was negatively correlated to the minimum temperature of the coldest month. This could be an adaptation strategy of ecotypes to cold winter. Annicchiarico *et al*., (2014) found that ecotype adaptation to cold winter was associated with latitude and, more specifically, the extent of cold stress in collecting sites. However, the appearance of the first flower bud was positively correlated to the mean temperature of the wettest quarter, the mean temperature of the coldest quarter, and the precipitation of the driest quarter, which confirms the results of Iannucci et al., (2008) for Mediterranean species, including Sulla coronaria (L.) Medik. that was strongly influenced by increasing temperature in accelerating the development rate and in earliness for time to flowering.

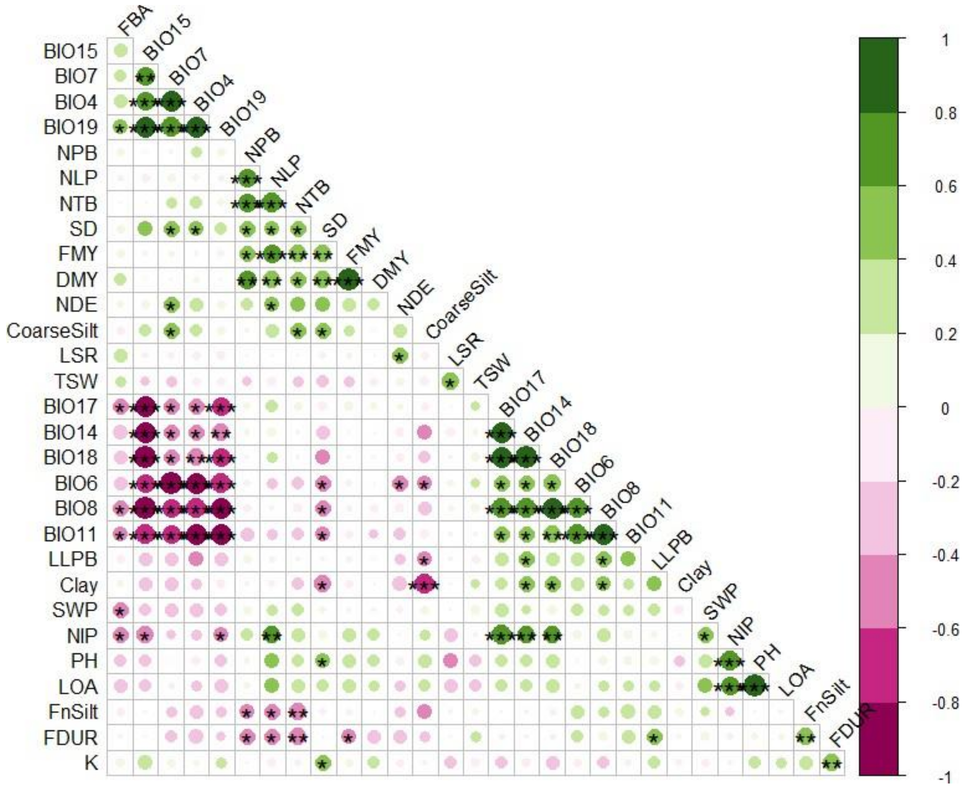

Figure 9: Correlation matrix of agro-morphological and edapho-climatic traits evaluated in 21 Moroccan Sulla flexuosa (L.) Medik. ecotypes.

Positive correlations are displayed in green and negative correlations in purple color. Color intensity and the size of the circle are proportional to the correlation coefficients. In the right side of the correlogram, the legend color shows the correlation coefficients and the corresponding colors. NPB Number of plagiotropic branches, PH plant height (cm), LOA length of orthotropic axis (cm), LLPB length of the longest plagiotropic branch (cm), NLP number of leaves per plant, NIP number of inflorescences per plant, NTB number of total branches, SD stem diameter (cm), FMY fresh matter yield (T/ha), DMY dry matter yield (T/ha), LSR leaf to stem ratio, NDE number of days to emergence, FBA first bud appearance, FDUR flowering duration, SWP seed weight per plant (g), K exchangeable potassium (ppm), FnSilt fine silt (%), CoSilt coarse silt (%), BIO4 temperature seasonality (standard deviation×100), BIO6 minimum temperature of the coldest month (C°), BIO7 average temperature of the wettest quarter (°C), BIO8 average temperature of the wettest quarter (C°), BIO11 average temperature of the coldest quarter (°C), BIO15 precipitation seasonality (coefficient of variation (%), BIO18 precipitation of warmest quarter (mm), BIO19 precipitation of coldest quarter (mm).

The appearance of the first flower bud was also positively correlated to precipitation of the coldest quarter, which confirms the relationship between precipitation and phenology. A plant could adjust its maturity to soil moisture availability to facilitate seedset before the start of the dry summers faced in the Mediterranean region (Pecetti & Piano, 2002). Accordingly, the negative correlation between the number of inflorescences per plant and the first bud appearance exhibits the effect of dry conditions on the reproductive parameters of the plants (Liu *et al*., 2012).

Issolah & Yahiaoui (2008) stated that populations of Sulla coronaria (L.) Medik. with high thousand seeds weight are native to high altitude regions while no significant correlation was reported for Sulla flexuosa (L.) Medik. ecotypes, probably due to low ranges of altitudes of collection sites (9-350 m) compared to Sulla coronaria which can be found at more than 1000 m (Ruisi *et* al., 2011). This study confirms previous studies of annual legumes by Pecetti & Piano, (2002) and Berger *et al*., (2008) that natural selection for flowering time is strongly influenced by eco-geographic variables (precipitations and temperature).

This study showed that ecotypes with a high number of plagiotropic branches had highly branched axes, which is in agreement with a study on Sulla coronaria (L.) Medik. (Gaad *et al*., 1998). In the present study, high total branches were also correlated to dry matter yield, as reported by a previous study (Bhattarai *et al*., 2018). Increasing Sulla flexuosa (L.) Medik. DM yield is an important goal in future breeding. This study showed significant positive correlations between DM yield and the number of plagiotropic branches, the number of total branches, and the number of leaves per plant, which is in agreement with previous studies on other legumes (Bhattarai *et al*., 2018). The present results also indicate that selection for high DM yield of Sulla flexuosa (L.) Medik. can be accomplished by selecting dense and leafy plants. All these characteristics could contribute to increase photosynthetic activity and hence lead to higher DM production. However, the leafy nature might be a disadvantage indry areas as it facilitates rapid water loss through transpiration (Sharkey, 1984). In the case of other legumes, the longer stems length is translated into a higher total DMY (Bhattarai *et al*., 2018). However, in the present study, no significant correlation was observed between those parameters. As mentioned earlier, these results are undoubtedly dependent on the plant genetic background and the environmental and growth conditions. Indeed, in the present study, we revealed that the geographical and bioclimatic origins of Sulla flexuosa (L.) Medik. ecotypes played an important role in plant adaptation due to their correlations with reproductive and phenological parameters.

*Correlation between bromatological and edapho-climatic parameters***.** The second hypothesis was that variation in bromatological parameters was related to the eco-geography of their collection sites (Figure 10). No significant correlation was found between DM yield and whole plant CT content, suggesting that selection must be based on the dry matter yield of ecotypes and the targeted CT content.

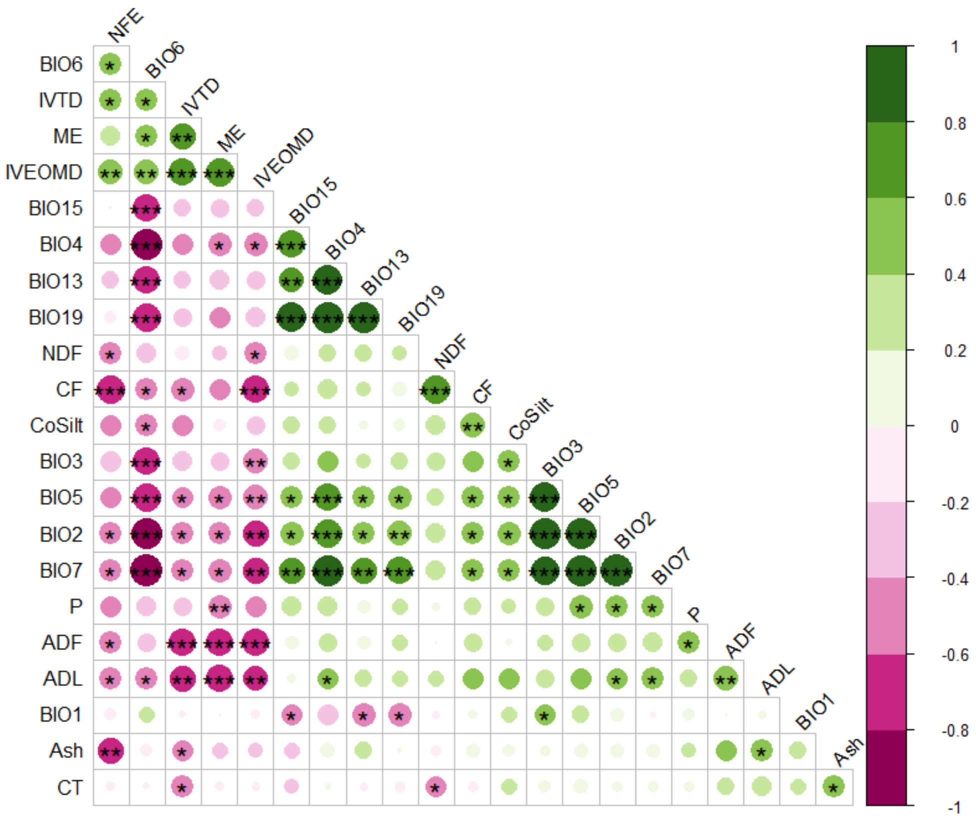

Figure 10: The correlation matrix of bromatological and edapho-climatic traits evaluated in 21 Moroccan Sulla flexuosa (L.) Medik. ecotypes.

Positive correlations are displayed in green and negative correlations in purple color. Color intensity and the size of the circle are proportional to the correlation coefficients. In the right side of the correlogram, the legend color shows the correlation coefficients and the corresponding colors. Ash (% DM), NFE nitrogen free extract (% DM), CF crude fibers (% DM), NDF neutral detergent fibers (% DM), ADF acid detergent fibers (% DM), ADL acid detergent lignin (% DM), IVEOMD enzymatic organic matter digestibility (%), IVTD in vitro true digestibility (%), ME metabolizable energy (kJ/kg DM), CT condensed tannins (% DM), CoSilt coarse silt (%), P available phosphorus (ppm), BIO1 average annual temperature (°C), BIO2 average diurnal variation [monthly average (max temperature - min temperature)](°C), BIO3 isothermality (BIO2 / BIO7 × 100)], BIO4 temperature seasonality (standard deviation * 100), BIO5 maximum temperature of the warmest month (C°), BIO7 average temperature of the wettest quarter (°C), BIO13 precipitation of wettest month (mm), BIO15 precipitation seasonality (coefficient of variation), BIO19 precipitation of coldest quarter (mm).

The leaf-to-stem ratio is considered a positive indicator of forage quality; this is due to its close association with forage digestibility and intake (Borreani *et al.*, 2003). Paradoxically, in this study, there was no significant correlation between the leaf-to-stem ratio and the digestibilities or the different fiber contents. Links between higher temperatures during the growing season and declining nutritive values have been established under controlled conditions (Lee *et al.*, 2017). The negative correlation of ADL and CF with the minimum temperature of the coldest month (BIO6) was similar to results found by Ruisi *et al.*, (2011) for Sulla coronaria (L.) Medik. They reported that populations coming from a higher altitude with higher rainfall and lower temperature were characterized by small leaves and plants and by low total biomass production. In the present study, fresh matter and DM yields were positively correlated to the number of total branches, which were richer in fibers than leaves. The positive correlation of ADL and CF with average diurnal variation (BIO2) and annual temperature range (BIO7) could be due to rising temperatures as forage quality declines with rising temperatures (Lee *et al.*, 2017). The same explanation also goes for NFE as it was positively correlated to OM and true digestibilities, and negatively to BIO2, BIO7, ADL, and CF. High long-term phosphorus fertilization was reported to increase ADF accumulation in alfalfa (Lissbrant *et al*., 2009). In the present study, the positive correlation between ADF and P could be explained by the fact that ecotypes adapted to low P amounts in their collection sites could not use the P present in the experimental field due to fertilization and thus had a lower fiber production. The true digestibility was higher than OM digestibility. This could be due to the methodology where the true digestibility included rumen microbes, which increased digestibility compared to enzymatic digestibility, which used commercial enzymes.

Principal component analysis (PCA). The PCA is an important tool in determining the most important variables contributing to variation (Price *et al*., 2006). The PCA for quantitative parameters (Figure 11) showed that a major part of variation (64.9 %) in Sulla flexuosa (L.) Medik. ecotypes was explained by the first three components. PC1, which was the most important component, explained 33.4 % of the total variation with the positive contribution of CF, ADL, SD, NDF, and DMY and the negative contribution of OM Digestibility, ME, NFE, and thousand seed weight. The PC1 was positively correlated to the contents of different fibers and negatively to digestibility. The PC1 axis was therefore determined by a low nutritional value. The second component axis accounted for 17.9 % of the total variation, and the traits with a positive weight on this component were the seed weight per plant and the number of inflorescences per plant, while the start and the end of flowering had a negative weight. The PC2 axis was therefore determined by plant reproductive parameters and phenology. The edapho-climatic parameters (Table 2) were used as supplementary variables. The annual precipitation (BIO12), the precipitation of the wettest month (BIO13), the precipitation of the wettest quarter (BIO16), and the precipitation of the coldest quarter (BIO19), which are indicative of an abundance of precipitations were at the same direction of phenology parameters (start and end flowering).

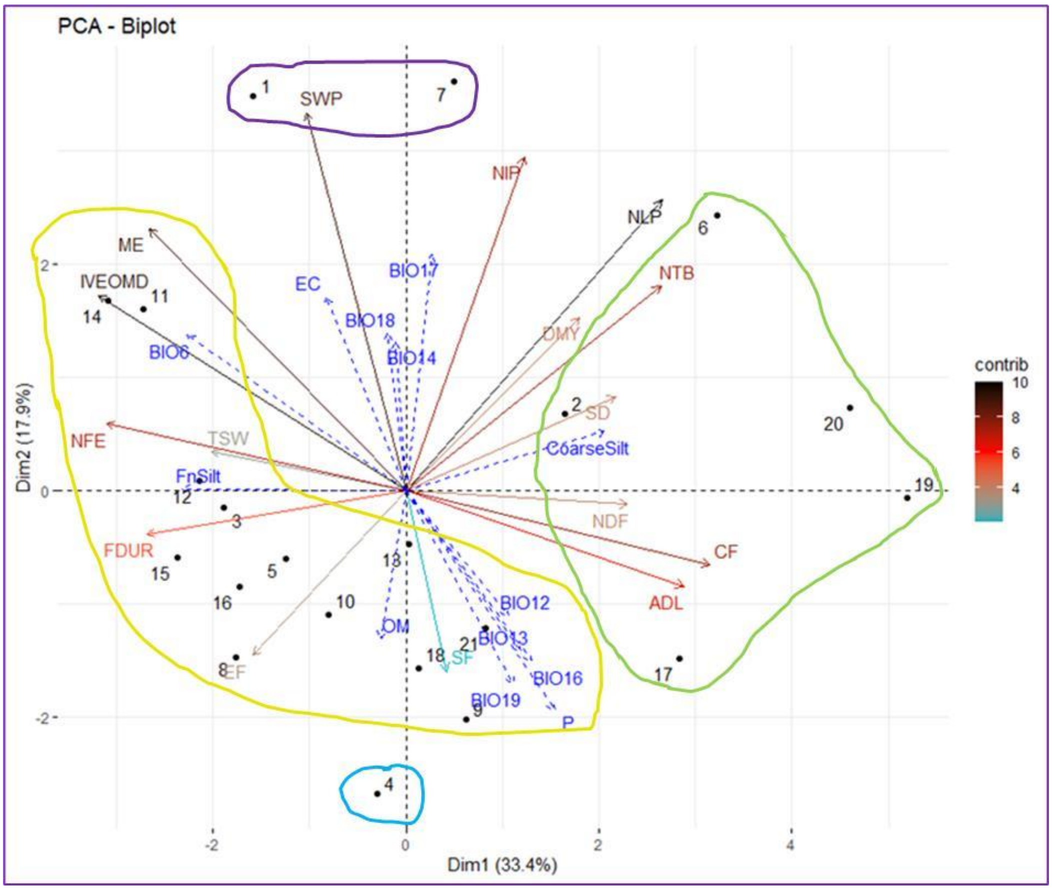

Figure 11 : Graph of the variables and individuals of the principal component analysis.

NLP number of leaves per plant, SWP seeds weight per plant (g), TSW thousand seed weight (g), NIP number of inflorescences per plant, NTB number of total branches, SD stem diameter (cm), DMY dry matter yield (T/ha), CF crude fiber (% DM), NDF, neutral detergent fibers (% DM), ADF, acid detergent fibers (% DM), ADL acid detergent lignin (% DM), IVEOMD enzymatic organic matter digestibility (%), NFE nitrogen free extract (% DM), ME metabolizable energy (kJ/kg DM), Hum. humidity (%), FnSilt fine silt (%), P available phosphorus (ppm), EC electrical conductivity (mS/m), OM organic matter (%), BIO6 min temperature of coldest month (°C), BIO12 annual precipitation (mm), BIO13 precipitation of wettest month (mm), BIO14 precipitation of driest month (mm), BIO16 precipitation of wettest quarter (mm), BIO17 precipitation of driest quarter (mm), BIO18 precipitation of warmest quarter (mm), BIO19 precipitation of coldest quarter (mm). Four clusters were determined by the cluster heatmap analysis and represented via the four coloured circles (green, purple, yellow and blue) on this figure.

In the Mediterranean area, several authors reported late flowering for ecotypes that have sufficient water supply to take advantage of the long season for a high yield and not overlap the harvesting season with rainfall during haymaking (Ruisi *et al*., 2011). However, the precipitation of the driest month (BIO14), the precipitation of the driest quarter (BIO17), and the precipitation of the warmest quarter (BIO18) were in the opposite way of the phenology parameters, showing that also extreme drought leads to late flowering. Annicchiarico *et al*., (2014) also reported low adaptation of Sulla coronaria (L.) Medik. to drought summer compared to winter cold. The minimum temperature of the coldest month (BIO6), which is indicative of extreme coldness, was in the same direction as nutritive value parameters (ME, OM Digestibility, and NFE). This could be due to the sensitivity of plants to cold, which leads to small developed plants with low branches and low fibers and thus with high digestibility (Annicchiarico *et al*., 2014).

Heatmap analysis. To visualize and investigate more detailed differences between the ecotypes based on morphological, agronomic, and bromatological parameters, a heatmap analysis was performed. In contrast to clustering methods, which allow the clustering of ecotypes on groups having similar characteristics, heatmap allows the identification of differences at the cluster's levels. The heatmap analysis showed a couple of dendrograms. The first one (Dendrogram1) structured on the left, an arrangement that corresponds to the Sulla flexuosa (L.) Medik. ecotypes, and the second one, on the top (Dendrogram2), clustered the agro-, morpho-, pheno-, and bromatological parameters that affected the dendrogram 1 distribution. These parameters were classified into five groups of fibers (CF, NDF, and ADL), fodder production (DMY, stem diameter, number of total branches, and number of leaves per plant), phenology (start and end flowering), reproductive parameters (seed weight per plant and number of inflorescences per plant), and nutritive value (OM Digestibility, ME and thousand seed weight, NFE and flowering duration). The heatmap figure (Figure 12) displayed four main groups of ecotypes: the first one above, which corresponds to E1 and E7, E4 was clustered as the third group, E2, E6, E17, E19, and E20 that formed the fourth group and the second group with the rest of the ecotypes.

The first and second groups were characterized by low fiber values, while the third and fourth groups were characterized by the opposite. The second group was characterized by lower yield parameters, contrary to the other groups. The third and fourth groups were opposite according to phenology. The first and second groups were intermediate. The fourth group was characterized by low nutritive value. Except for the first group, the other groups had low reproductive parameters. In the present collection, extreme ecotypes are found. For example, in some studies, DMY was positively correlated to NDF and ADF (Bhattarai *et al*., 2018). However, the E1 was the best ecotype because it had the highest productivity and nutritive value (low NDF and CF, high DMY, ME, and OM digestibility), with a high stem diameter, which means that it is also protected from lodging (Gawłowska *et al*., 2021). This ecotype was of dual purpose because it also showed high reproductive parameters, which are important in seed dissemination. High thousand seed weight is related to the high germination of these seeds (Moshatati & Gharineh, 2012). At a very long phenotypical distance, E21 is the opposite in terms of low DMY and NFE and intermediate fibers (NDF, ADF, ADL, CF).

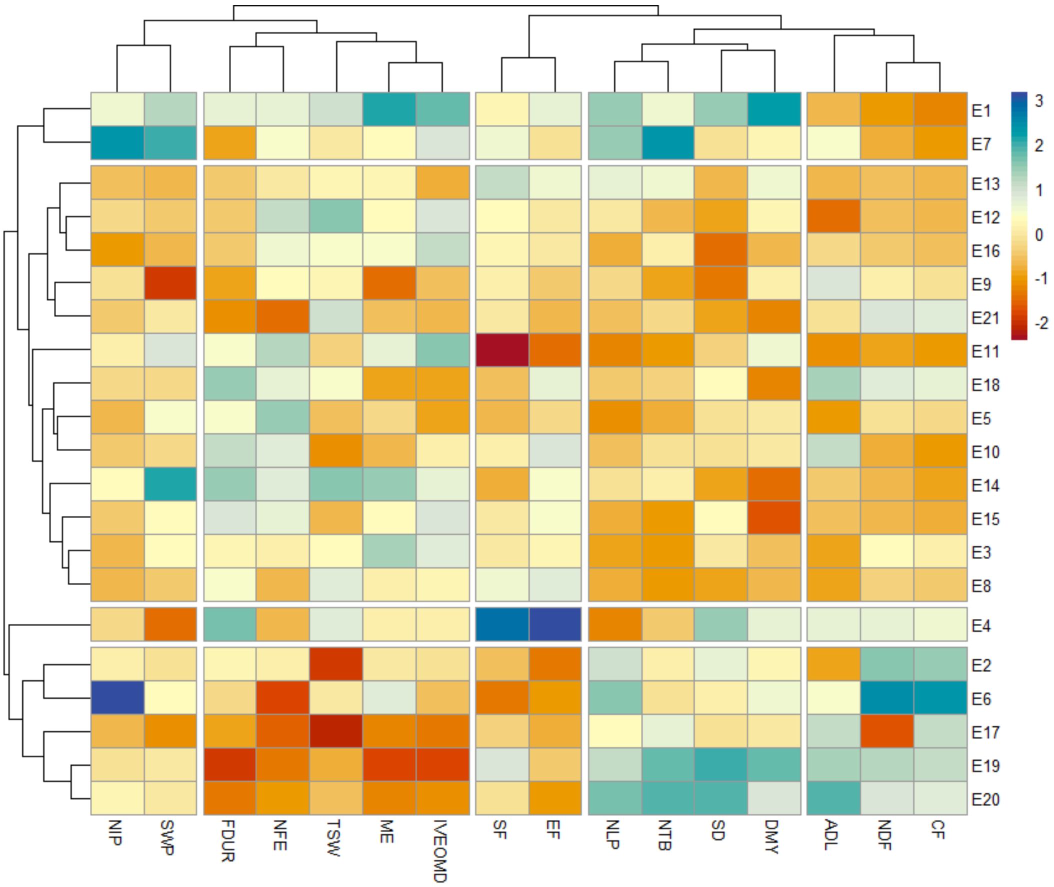

Figure 12 : Cluster heatmap analysis of Sulla flexuosa (L.) Medik. ecotypes' responses to morpho-phenological, agronomic and bromatological characterization.

The heatmap plot describes the relative abundance of each bitter vetch ecotype (rows) within each trait (column). The color code (blue to dark red) displays the values of the parameters: blue color indicates high values while red color indicates low values. The dendrogram (on the left) shows hierarchical clustering of bitter vetch ecotypes based on the Euclidian distance and Ward's clustering method. CF crude fibers (% DM), NDF neutral detergent fibers (% DM), ADL acid detergent lignin (% DM), DMY dry matter yield (T/ha), SD stem diameter (cm), NTB number of total branches, NLP number of leaves per plant, EF end of flowering (days), SF start of flowering (days), IVEOMD enzymatic organic matter digestibility, ME metabolizable energy (kJ/kg DM), TSW thousand seed weight (g), NFE nitrogen free extract (% DM), FDUR flowering duration (days), SWP seed weight per plant (g), NIP number of inflorescences per plant.

E11 could also be interesting related to its precocity to start and end flowering and its long duration of flowering, which is reflected by a high seed weight per plant. E4 is very late (high start and end flowering). In the literature, ecotypes with long vegetative growth tend to have high DMY (Ruisi *et al*., 2011). However, E4 showed intermediate DMY, probably due to its low number of leaves per plant. Porqueddu *et al*., (2001) stated that nutritive value in legumes tends to be higher in late than in early maturing cultivars. Indeed, the early maturing cultivars start to bloom earlier and have a higher proportion of stems; stem proportion being negatively correlated to digestibility. Ecotype 4 could be interesting for breeding because it was the late ecotype, even though it showed chemical composition and nutritive value close to the mean of all ecotypes. The choice of an early or late ecotype will depend on the climatic conditions in which they are seeded. In less humid conditions, an early ecotype will take advantage of the early rains to develop rapidly. In wetter conditions, the development of a late ecotype will allow a cut during a drier period, thus reducing the risk of rain during the drying process in the field in haymaking. Since there is no correlation with forage yield, it will be necessary to choose ecotypes that are early or late and very productive. However, if the aim is seed dissemination to conserve Sulla flexuosa (L.) Medik. in pastures, early ecotypes will be preferred to avoid water stress during seed maturation.

The clustering of these ecotypes had the major objective of creating a Sulla flexuosa (L.) Medik variety. Ceccarelli and Grando (Ceccarelli & Grando, 2020) stated that during selection, despite that the participatory variety selection is technically easier to organize due to the limited number of lines that usually reach the final stage of selection, it is important to use the participatory plant breeding and involve farmers, from the beginning, in most important decisions, during all the stages of a plant breeding program. Otherwise, there is a risk of discarding potentially desirable breeding material to farmers. By involving farmers in the selection process, participatory plant breeding can also help promote local knowledge and empower farmers to take an active role in managing their genetic resources(Ceccarelli & Grando, 2022).

Conclusion. This study demonstrated that it was possible to cultivate wild ecotypes of Sulla flexuosa (L.) Medik. from seeds harvested in Northern Morocco. The agro-, morpho-, pheno-, and nutritional characteristics of these ecotypes grown on the experimental site are close to those reported in the literature for different varieties of Sulla coronaria (L.) Medik. that have been domesticated and selected for a long time. Four groups were distinguished on the basis mainly of the quantitative parameters of plant production, reproduction, and nutritional value. To get the highest protein and dry matter content to better use Sulla flexuosa (L.) Medik. in animal diet, cutting at the start of the flowering stage could be a compromise between dry matter yield, protein content, and digestibility. Future trials are needed to continue the domestication and the selection of sulla flexuosa (L.) Medik., to spread this plant to the breeders and to conserve local genes in a seed bank. Moreover, climate change and recurrent droughts must be considered in the selection scheme.

Material and methods

Plant material, study site, and experimental set-up. *Collection of ecotypes.* The plant material used consisted of 21 Sulla flexuosa (L.) Medik. ecotypes collected on 21 sites in Northern Morocco in June 2018. (Figure 13). They were collected using FAO protocol (FAO, 1993) and according to the relevant institutional, national, and international legislation. Indeed, as the Sulla flexuosa (L.) Medik. is present on the IUCN red list, the Regional Direction of Agriculture of Tangier–Tétouan–Al Hoceima (Morocco) has authorized the National Institute of Agricultural Research (INRA) to collect seeds of local germplasms for a scientific objective of characterization and preservation. At each site, over an area of 50-100 m², mature pods were collected from a minimum of 30 random plants, collecting the maximum number of mature pods per plant. The seeds were kept at room temperature before sowing and then sent to INRA genebank for long-term storage. The seeds are available to seed exchange in research networks at a request addressed to the director.

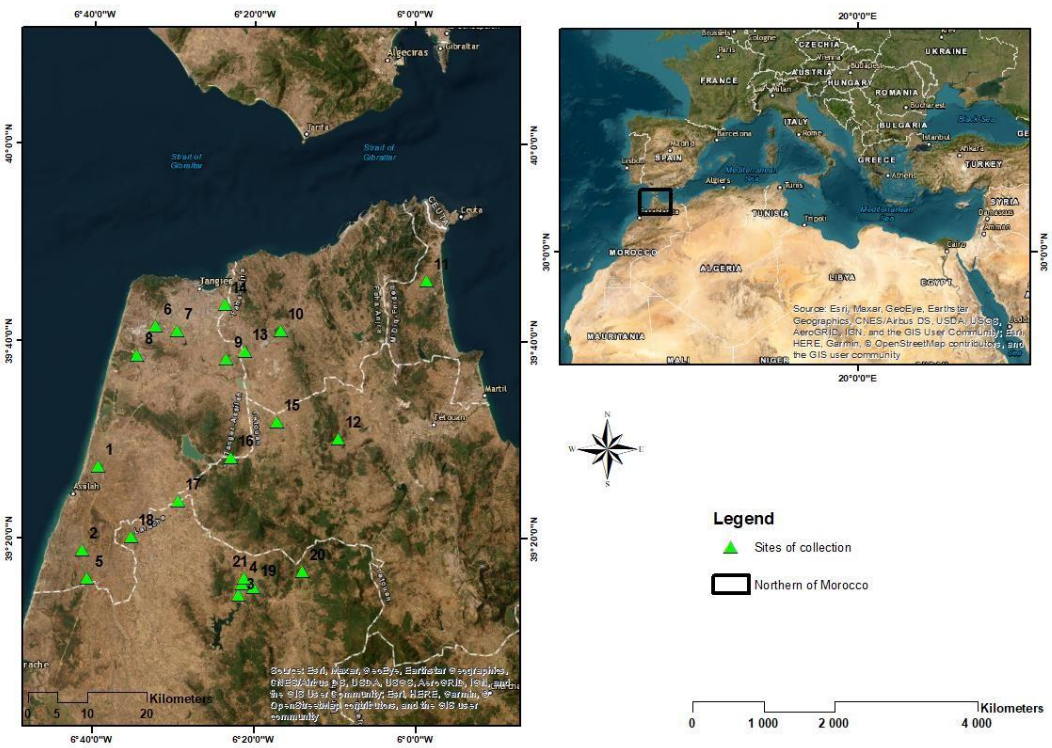

Figure 13: Collection sites of the 21 wild Sulla flexuosa (L.) Medik. ecotypes (ArcGIS Desktop version 10.4.1 - Esri, Redlands, CA, USA)

Ecological characterization. Nineteen bioclimatic data (BIO 1-19) for the period 1950-2000 were estimated for each collection site from major climate databases (www.worldclim.org). The data were extracted from satellite images via ArcGIS Desktop version 9.3 (Esri, Redlands, CA, USA) and were interpolated with a resolution of approximately 1 km (Hijmans *et al*., 2005). Climatic data (minimum, maximum, mean temperatures (°C), rainfall of each month and total rainfall (mm) of the 2019 and 2020 agronomical years) of the experimental site were collected from a climatic station 10 km nearer. The bioclimatic variables of the two years were calculated based on these climatic data. At the collection sites, five samples of soil from the 0-20 cm and five from the 20-40 cm horizons were collected for edaphic analysis. Soil samples were oven dried at 60 °C until constant weight and humidity was calculated as the difference between wet and dry samples. They were ground and passed through a 2 mm sieve to remove larger particles. The pH (water and KCl) was read with a standard calibrated pH meter in 2:1 distilled water and dry soil ratio (Jackson, 1958). Electrical conductivity was measured in the soil extract collected from the saturated soil paste by conductivity meter (Wilcox, 1950). Nitrogen was determined by mineralization and distillation using the Kjeldahl method (Bremner, 1960). Exchangeable potassium (K) was analyzed in 1 N ammonium acetate extract using a flame photometer (Toth & Prince, 1949). Total limestone ($CaCO_3$) was measured by treating the sample with HCl (Nelson, 1983). Carbon was measured through dichromate oxidation and converted to organic matter by multiplying by a factor of 1.72 (Walkley & Black, 1947). Available phosphorus (P) was obtained by the colorimetric method (Olsen *et al*., 1954). Soil texture was determined by using standard Pipette method and wet sieving (Robinson, 1922).

Experimental design. The study was set up during both the agronomic years 2018/2019 (2019) and 2019/2020 (2020) in El Menzla, a related field of Regional Agronomic Research Institute of Tangier (Morocco) (35°31’53’’N; 5°42’36’’W; 128.5 m AMSL). From the collected ecotypes, in greenhouse trays, 3 to 5 seeds were sowed per alveolus on November 1, 2018, and October 29, 2019. Four weeks after sowing, in the field, 60 seedlings were transplanted into a plot in a randomized complete block design with three replications (i.e., 180 seedlings per ecotype a year). In one plot, seedlings were transplanted in six 2-m rows with 30 cm between rows and 22 cm between plants. The distance between plots was 50 cm, and between blocks was 1 m. Sulla flexuosa (L.) Medik. seeds were not inoculated with rhizobium before sowing because prolific nodulation occurs naturally on this site. The trial was conducted under rainfed conditions on fallow plots during 2019 and 2020, and NPK (10-30-10) fertilizer was applied at the rate of 100 kg/ha in the day of transplantation of plants into the plots. Irrigation was applied one time at the same day to avoid nitrogen volatilization. Natural weed was removed manually during the growing season.

Agro-morphological characterization. *Phenological assessment.* The number of days between sowing and emergence (NDE), appearance of the first flower bud (FBA), start of flowering (SF), full flowering (FF) and end of flowering (EF) were recorded during regular visits (every three days) to the experimental site in the first year. The flowering duration (FDUR) was determined by the difference between the start and the end of flowering. For the second year (2020), the confinement due to COVID-19 didn’t allow the phenological characterization. The different phenological stages were expressed in growing degree days (GDD) by considering the base temperature of 10°C30. They were determined by the following formula “equation (1)”:

$$\text{Accumulated GDD} = \Sigma\,[(T_{max} + T_{min})/2] - T_b\ (°C) \quad (1)$$

Where Tmax is the daily maximum temperature (°C), Tmin is the daily minimum temperature (°C), and Tb is the base temperature (10°C).

Morphological assessment. At the start of flowering and the full flowering during the two years, five plants were selected for morphological characterization (number of plagiotropic branches (NPB), plant height (PH), length of the orthotropic axis (LOA), length of the longest plagiotropic branch (LLPB), number of leaves per plant (NLP), number of inflorescences per plant (NIP), number of total branches (NTB), stem diameter (SD).

Agronomical assessment. The fresh and dry matter yields (FMY and DMY) were determined at the budding, start of flowering, and full flowering stages. At each stage, 0.75 m² per plot was harvested and weighed. The plants were cut at the height of 5 cm above the soil to avoid soil contamination. Then 20 g of fresh matter were dried at 102 ± 1.0°C with forced ventilation until constant weight to estimate the DM content of the samples and thus the DMY. In addition, a second sample of 1 kg of fresh matter was used to separate the different constituents of the cut plants: leaves, stems, and flowers. The leaves and stems were then dried at 102 ± 1.0°C with forced ventilation to a constant weight to calculate the leaf-to-stem ratio (LSR) on a DM basis. At the physiological maturity stage, five plants were harvested and threshed to determine seed weight per plant (SWP); one thousand seeds were weighed thrice to determine thousand seed weight (TSW).

Bromatological characterization. *Chemical composition*. Whole plants of Sulla flexuosa (L.) Medik. harvested at budding, early flowering, and full flowering stages that were used to determine FMY and DMY, were also used to determine the bromatological analysis of the forage. Samples were oven-dried at 60°C for 48h, then ground and sieved through a 1 mm sieve, and stored in a desiccator.

The AOAC (1990) methods were used for analyses. Ash content was determined after the incineration of 2 g of dried samples in a muffle furnace at 550°C for 12 h (No. 942.05). Fat content (FC) was obtained by the Soxhlet method using diethyl ether as a solvent (No. 963.15). Crude protein (CP) content was determined by multiplying nitrogen content by 6.25, obtained using the Kjeldahl method (No. 977.02). Fiber content (crude fiber (CF) and fibers (NDF (Neutral Detergent Fiber), ADF (Acid Detergent Fiber) and ADL (Acid Detergent Lignin)) was analyzed using an ANKOM® 200 Fiber Analyser (ANKOM Technology, Macedon, NY, USA) (No. 962.09) for CF and the method of Van Soest *et al*., (1991) for NDF, ADF and ADL. The nitrogen-free extract (NFE) was estimated using the following formula “equation (1)”:

$$\text{NFE (\% DM)} = 100 - (\text{CP} + \text{Fat} + \text{CF} + \text{Ash}) \quad (1).$$

Quantification of total phenols (TP) and total tannins (TT) was performed according to the procedure described by Makkar *et al*., (1993). Briefly, the extraction was done by mixing 10 mL of 70% acetone with 200 mg of finely ground dry sample. The mixture was subjected to ultrasonic treatment for 20 min. and centrifugation for 10 min at 3500 rpm at 4°C. In a tube, 0.05 mL of the extract was mixed with 0.25 mL of Folin Ciocalteu 1N reagent, 0.5 mL of distilled water and 1.25 of 20% sodium carbonate solution. The solution was then vortexed and left in the dark for 40 min; the absorbance was read at 725 nm to determine total phenols. In another centrifuge tube, 3 mL of the extract was mixed with 300 mg of polyvinylpolypyrrolidone and 3 mL of demineralized water. The tube was vortexed, kept at 4°C for 15 min, centrifuged at 4°C at 3500 rpm for 10 minutes, and then read at 725 nm to determine non-tannic phenols (NTP). Total tannins were calculated as the difference between non-tannic phenols and total phenols. Condensed tannins (CT) were analyzed by Porter *et al*., (1985) method. Briefly, in a glass tube, 3 mL of Butanol-HCl reagent and 0.1 mL of ferric reagent were mixed with 0.5 mL of the extract. The tubes were vortexed and put in a water bath at 97°C for one hour. After cooling, the absorbance was read at 550 nm. The difference between TT and CT deduced hydrolysable tannins (HT).

Digestibility. The In Vitro Enzymatic CP Degradability (IVECPD) was determined by the method of Aufrère & Cartailler, (1988). Briefly, in a centrifuge tube, 25 mL of a protease solution was added to 0.5 g of the sample and was incubated at 40°C for one hour. The sample was centrifuged for 5 minutes at 3500 rpm and then filtered. The liquid phase was mineralized for one hour using sulfuric acid at 120°C and for 2 hours at 350°C. The tube containing the mineralized sample was connected to the distiller. Finally, the contents of the Erlenmeyer flask were titrated with 0.1 N HCL to determine the digested nitrogenous matter.

The In Vitro True Digestibility (IVTD) was determined by incubation of feed samples in filter bags in a Daisy II incubator® (ANKOM Technology, Fairport, NY, USA) (Tassone *et al*., 2020). The rumen liquor was obtained from goats at a communal slaughterhouse. Rumen fluid was collected into a pre-warmed thermos and transported to the laboratory, where rumen fluid was purged under CO2 for 30

s. About 500 mg of each feed was placed in ANKOM F57 filter bags with a pore size of 57 mm (ANKOM, Macedon, NY), which were heat-sealed, and subsequently put in jars (24 bags/jar). The rumen liquor was added to artificial saliva in a 1:5 ratio, and then the mixture was added in jars and incubated at 39.5°C for 48 h. The In Vitro Digestibility was estimated by quantifying residual DM compared to incubated initial quantities. The enzymatic pepsin-cellulase method was used to determine the enzymatic dry matter digestibility (IVEDMD) and enzymatic organic matter digestibility (IVEOMD) of Sulla flexuosa (L.) Medik. (Aufrère & Michalet-Doreau, 1983), according to two-step method: 0.5 g of dried sample was incubated at 40°C with 20 ml of a 2% pepsin solution diluted in 0.1 N hydrochloric acid and shaken constantly for 24 h; then the sample was solubilized in 50 ml of a buffer solution containing 1 g/L cellulase and, shaken and incubated at 40°C for 24 h. After incubation, the sample was rinsed with hot distilled water and then placed in an oven at 60°C until constant weight. It was weighed to determine the IVEDMD. The sample was incinerated in the muffle furnace at 550°C for 12 hours to determine IVEOMD.

The metabolizable energy (ME; MJ/kg DM) was calculated using the “equation (2)” of AOAC, (1990):

$$ME = 0.17 \times IVEDMD - 2, (2)$$

where IVEDMD is the enzymatic dry matter digestibility in percentage.

Data analysis. Analysis of variance was carried out to test the years, ecotypes, phenological stages, and their interactions. The variance components were estimated using a general linear model (GLM), using SAS 9.4 version (SAS Inst. Inc., Cary, NC, USA). The phenological stage was considered as a fixed factor in the model. To study the correlations between the different parameters, a correlation matrix was created based on Pearson’s correlation coefficients using the “corrplot” R software (Version 4.2.1). Principal component analysis (PCA) was performed for all parameters using “Factominer and Factoextra” packages of R software. Only variables that were well described on the axes were retained. A heatmap summarising all the morphological, agronomical and bromatological parameters contributing to variation between Sulla flexuosa (L.) Medik. ecotypes was created using “Pheatmap” package of R software, with Euclidean distance as the similarity measure and hierarchical clustering with complete linkage.

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Author Contributions

M.C., J.-F.C., and C.A. received the project funding. S.B., A.N., M.C., J.-F.C., and C.A. conceived and designed the experiment. S.B., A.N., and M.C. collected and provided the germplasm for evaluation and managed the field experiments. S.B. and N.M. analyzed the data. S.B. wrote the draft manuscript. S.B., A.N., N.M., C.A., J.L., J.-L.H., M.C. and J.-F.C. read and reviewed the manuscript for final publication. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

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Section expérimentale

Etude 2 :

Ecological, morpho-agronomical, and bromatological assessment of sorghum ecotypes in Northern Morocco.

Chapitre 4. Étude 2: Ecological, morpho-agronomical, and bromatological assessment of sorghum ecotypes in Northern Morocco

Préambule

Le Sorgho (*Sorghum bicolor* (L.) Moench) est une céréale utilisée pour ses grains comme aliment pour les humains et les animaux ; il est principalement cultivé dans les zones sèches. En raison des changements climatiques et de l’augmentation de la température du globe ces dernières décennies, la prospection de nouvelles ressources alternatives s’avère une nécessité. Pour le sorgho Marocain, quelques études ont été menées sur sa variabilité phénotypique et génotypique. Pourtant, à notre connaissance, aucune étude n’a orienté son analyse vers un objectif d’utilisation en nutrition animale. Ainsi, pour étudier les possibilités d'augmenter sa culture pour son utilisation comme aliment pour le bétail, une caractérisation écologique, morpho-agronomique et bromatologique des écotypes locaux a été menée comme première étape vers la sélection de meilleures variétés. Vingt et un écotypes ont été collectés dans des fermes du Nord du Maroc en 2018. Ces écotypes ont été cultivés en 2019 dans un champ expérimental avec un protocole en blocs aléatoires complets avec trois répétitions. Au stade de la maturité physiologique, les grains et la paille ont été récoltés et analysés. Les résultats ont indiqué des variations significatives entre écotypes pour presque tous les paramètres mais également un rendement en grains intéressant de 3,5 T/ha et une teneur en protéines des grains acceptable (10,5 % MS). Les paramètres génétiques calculés ont souligné la possibilité de sélectionner des cultivars hautement productifs et nutritifs. L'analyse multivariée a regroupé les écotypes en cinq groupes basés sur des paramètres agro-morphologiques, bromatologiques et d’activité antioxydante, où l'écotype E21, appartenant au cinquième groupe, était un candidat de sélection prometteur. Aucun lien de corrélation significatif entre les traits agro-morphologiques et bromatologiques des grains et les distances géographiques n'a été mis en évidence. Le sorgho ne peut donc être amélioré qu'en fonction des caractères agro-morphologiques et bromatologiques recherchés.

Les hypothèses étaient donc :

- ✓ Les caractéristiques du sol des sites de collecte sont similaires ?
- ✓ Les paramètres agro-morphologiques et bromatologiques sont influencés par les écotypes ?

Section expérimentale

Etude 2:

Ecological, morpho-agronomical, and bromatological assessment of sorghum ecotypes in Northern Morocco.

Scientific reports (Soumis)

S. Boukrouh, A. Noutfia, N. Moula, C. Avril, J. Louvieaux, J.L. Hornick, M. Chentouf, J.F. Cabaraux

Abstract

Sorghum Bicolor is a cereal used for grains as feed and food, mainly cultivated in dry areas. To study the possibilities of increasing its cultivation for feed purposes, ecological, morpho-agronomical, and bromatological characterization of the local Moroccan ecotypes was conducted as the first steps toward selecting better cultivars. Indeed, twenty-one ecotypes were collected from farms in Northern Morocco in 2018. The edapho-climatic parameters of the collection sites were evaluated. The ecotypes were cultivated in 2019 in an experimental field with a randomized complete block design with three replicates. At the maturity stage, plants were evaluated for agro-morphological parameters, and grains and straw (leaves and stems) were harvested and analyzed. The results indicated significant variations between ecotypes for almost all parameters and an interesting grain yield of 3.5 T/ha with a 176% yield variation. The nutritive value of grains was interesting compared to straw, especially for mean protein contents (10.5% DM) and organic matter digestibility (81.4%). The calculated genetic parameters emphasized the possibility of selecting highly productive and nutritive cultivars. Multivariate analysis clustered the ecotypes into five groups based on agro-morphological, bromatological, and antioxidant activity parameters; the third group was characterized by high grain-yielding ecotypes, and the fifth one by high nutritive ecotypes. The E21 ecotype, belonging to this last group, was a promising selection candidate as it combines both. No significant correlation link between agro-morphological and bromatological traits of grains and geographical distances was discerned. Sorghum bicolor could thus be improved only according to the researched agro-morphological and bromatological traits.

Introduction

Nowadays, coping with climate change is a major challenge, especially in dry areas where plant breeding faces several obstacles. Arid regions are characterized by limited resources, including water and nutrients (Abraha *et al*., 2015). The challenge of developing more resource-use-efficient cultivars is important for improving crop productivity and sustainability, especially since drought is expected to become more frequent and severe due to climate change. Thus, developing cultivars resilient to changing conditions is a priority for plant breeders to maintain crop productivity and food security.

Sorghum bicolor (L.) Moench, usually called sorghum, is the fifth leading cereal crop in the world after wheat, maize, rice, and barley (FAOSTAT, 2021). It is a multi-purpose crop used as food (grains), feed (grains and fodder), and energy resource (McGinnis & Painter, 2020). It is characterized by high fodder yield in a short time span, requires lower fertilizer inputs than corn, and has the particularity of wide adaptability to various agro-climatic conditions (Prakasham *et al*., 2014).

Sorghum originated and was domesticated in Africa (about 5,000–8,000 years ago), where the highest diversity of cultivars was found (Dossou-Aminon *et al*., 2015). Currently, it is cultivated on 28.42 million hectares of land, with production reaching 28.61 million tons per year (FAOSTAT, 2021), thus, having an average yield of 1 T/ha.

Sorghum grains are known for their high content of minerals, vitamins, carbohydrates, other anti-nutritional secondary compounds such as phenolic compounds and tannins, and their antioxidant activity (Kumari *et al*., 2021). Other studies (Djè *et al*., 1999) reported numerous genotypes with specific sizes (high, medium, or low), cycles (early or late), and aptitude (forage, grain, or dual-purpose), having a strong influence on the nutritional value of the fodder and grains. Djé *et al*., (1999) assessed the level of genetic diversity of Northern Moroccan sorghum ecotypes based on direct field sampling using allozyme and microsatellite markers and suggested that individual sorghum fields constitute valuable conservation units.

In Morocco, sorghum is used as a staple animal feed for cattle, sheep, and poultry for its grains, especially as a supplement in the summer season. It is also used as an energy source in the finishing phase of the fattening of calves and sheep (Boukrouh *et al*., 2021). It is conducted in rainfed conditions in marginalized areas as a secondary crop (Boukrouh *et al*., 2021). In 2021, the Moroccan produced quantities reached 5,000 tons in an area of 4,600 ha, with a mean yield of 1.1 T/ha (FAOSTAT, 2021), ranging from 200kg to 2T/ha (Boukrouh *et al*., 2021). Low yields are due to bad weather conditions, traditional agricultural techniques based on old methods, and parceled-out lands (Kadiri & Ater, 1997). The phenotypic selection exerted by farmers since the introduction of sorghum in Morocco has engendered locally adapted ecotypes (Kadiri & Ater, 1997). The ease of farming techniques encouraged its cultivation (Boukrouh *et al*., 2021).

Due to climate change with more prolonged drought periods, the decrease in water supply, and the increasing world population, this drought-resistant crop, like other available local feed alternatives (Boukrouh *et al*., 2023), could quickly become crucial and see its global use increase. Previous studies emphasized the agro-morphological and genetical diversity of some Moroccan ecotypes (Bouargalne *et al*., 2022; Djè *et al*., 1999; Medraoui *et al*., 2007). However, no studies assessed the nutritive value as feed on these ecotypes. This study collected twenty-one sorghum local germplasm from the traditional production area in the north of Morocco. It aimed thus to link the ecological and agro-morphological variability to the nutritive value (grain and straw) of twenty-one Moroccan sorghum ecotypes. This characterization could allow the preservation of local germplasms, help to choose the more productive and interesting ecotypes for feed, and enrich the biodiversity of the Moroccan sorghum gene bank.

Results & discussion

Ecological characterization. The soil results concerning the collection and experimental sites are shown in Table 5. The collection sites were located on different altitudes varying from 26 to 943 m. The results showed that sorghum ecotypes could grow on various ranges of soil pH (4.5-8.7 for pH water and 4.4-8.5 for pH KCl), electrical conductivity (1.0-76.3 mS/m), organic matter (0.7-5.2%) and total limestone (0.8-7.6%). One author reported that Sorghum plants cannot grow well in acidic soils (Abdulmajeed & Ezekiel Akinkunmi, 2016). In the present study, the observation of sorghum cultivation on acidic soils in some farms could nuance these results. Sorghum was also found on a variable range of soil parameters related to nitrogen (0.1-0.4%), carbon to nitrogen ratio (4.6-16.9%), exchangeable potassium (4.1-391.8 ppm), and available phosphorus (32.0-128.5 mm). These variations are obviously due to the differences in farm fertilization strategies. Compared to the farms, the nitrogen was similar, exchangeable K higher (705.3 vs. 90.7 ppm), and available phosphorus lower (39.7 vs 73.5 ppm) in the experimental station soil. Phosphorus being the second most growth-limiting macronutrient after nitrogen, fertilizer was applied to correct the difference on the sowing day. The textural composition also varied for clay (0.0-82.5%), coarse sand (0.2-19.2 %), fine sand (0.2-29.0%), coarse silt (17.2-80.2%), and fine silt (0.0-24.5%). Similar variability was observed between different sites where Ethiopian sorghum landraces were collected (Praptiningsih *et al*., 2020). The 2019 annual precipitations were lower than the 1950-2000 mean values of the collection sites (563.4 vs. 784 mm), which could expose ecotypes to drought stress conditions at the experimental site.

Table 5: Climatic and physico-chemical characteristics of the soils of the 21 collection farms and the experimental cultivation site in Northern Morocco.

	Collection farms							Experimental site
	Mean	Min	Max	Standard deviation	1st quartile	Median	3rd quartile	2019
Altitude (m)	333.7	26.0	943.0	267.6	69.0	304.0	540.0	129.0
Edaphic parameters								
Humidity (%)	5.9	3.6	9.5	1.6	5.0	5.9	6.6	5.2
pH water	7.9	4.5	8.7	1.1	7.9	8.4	8.5	8.4
pH KCl	7.5	4.4	8.5	1.1	7.4	7.6	8.3	7.4
Electrical conductivity (mS/m)	22.9	1.0	76.3	28.2	2.2	7.3	50.2	31.0
Organic matter (%)	3.0	0.7	5.2	1.3	2.0	2.8	4.0	2.2
Total limestone ($CaCO_3$, %)	2.5	0.8	7.6	1.9	1.1	2.0	2.4	7.4
Exchangeable potassium (K, ppm)	90.7	4.1	391.8	103.4	13.0	24.8	165.2	705.3
Available phosphorus (P, ppm)	73.5	32.0	128.5	37.7	35.0	77.6	107.0	39.7
Carbon to nitrogen ratio (C/N)	10.6	4.6	16.9	3.7	7.3	10.3	13.7	6.4
Nitrogen (N, %)	0.2	0.1	0.4	0.1	0.2	0.2	0.2	0.2
Clay (%)	30.9	0.0	82.5	28.2	0.0	27.9	56.2	50.0
Coarse sand (%)	4.4	0.2	19.2	6.2	0.4	0.7	4.8	1.6
Fine sand (%)	5.4	0.2	29.0	9.4	0.5	1.0	3.6	1.1
Coarse silt (%)	47.5	17.2	80.2	19.2	36.5	39.5	62.1	35.0
Fine silt (%)	11.6	0.0	24.5	7.3	6.7	10.0	20.0	10.0
Bioclimatic parameters								
Average annual temperature (BIO1, °C)	17.7	16.4	18.9	0.8	17.1	18.0	18.3	18.1
Average diurnal variation (BIO2, °C)	10.7	8.6	11.9	1.2	9.7	11.4	11.6	22.9
Isothermality (BIO3 = BIO2/BIO7×100, %)	40.3	39.2	41.0	0.5	40.0	40.3	40.7	57.3
Temperature seasonality (BIO4)	545.8	443.5	618.1	63.8	486.7	571.9	600.9	542.1
Maximum temperature of the warmest month (BIO5, °C)	32.2	29.3	35.1	1.8	30.6	32.7	33.8	40.7
Minimum temperature of the coldest month (BIO6, C°)	5.7	3.7	8.2	1.4	4.3	5.7	6.8	0.7
Temperature Annual Range (BIO7 = BIO5-BIO6, °C)	26.5	21.1	29.6	3.0	24.0	28.2	29.0	40.0

Average Temperature of the wettest Quarter (BIO8, °C)	11.4	9.9	13.2	1.2	10.0	11.6	12.5	16.2
Average temperature of the driest quarter (BIO9, °C)	24.5	23.2	26.6	0.9	24.0	24.2	25.0	21.8
Average temperature of the warmest quarter (BIO10, °C)	24.7	23.4	26.6	0.7	24.3	24.6	25.0	24.4
Average temperature of the coldest quarter (BIO11, °C)	11.3	9.4	13.2	1.3	10.0	11.6	12.4	11.8
Annual precipitation (BIO12, mm)	784.0	627.0	940.0	94.0	742.0	774.0	864.0	563.4
Precipitation of the wettest month (BIO13, mm)	140.4	97.0	169.0	20.5	134.0	142.0	153.0	152.0
Precipitation of the driest month (BIO14, mm)	0.4	0.0	1.0	0.5	0.0	0.0	1.0	0.0
Precipitation seasonality (BIO15, mm)	80.4	68.0	88.3	6.4	77.4	82.6	85.4	117.8
Precipitation of the wettest quarter (BIO16, mm)	389.8	267.0	479.0	60.6	377.0	398.0	421.0	129.8
Precipitation of the driest quarter (BIO17, mm)	12.1	7.0	18.0	3.4	9.0	13.0	15.0	0.1
Precipitation of the warmest quarter (BIO18, mm)	16.6	13.0	20.0	1.9	16.0	17.0	17.0	1.3
Precipitation of the coldest quarter (BIO19, mm)	389.4	261.0	479.0	61.1	377.0	398.0	421.0	44.9

Qualitative parameters. The frequencies of the different qualitative traits of the tested plants are reported in table 6. The knowledge of phenotypic diversity among sorghum is an essential tool for their efficient utilization in plant breeding models and effective conservation. Leaf rolling, a mechanism to reduce water evaporation when water stress is present (Matschi *et al*., 2020), was absent in 87.3% of the plants. It could be explained by optimal climatic conditions or drought resistance due to osmotic adjustment (Badigannavar *et al*., 2018). Peduncle exertion is also an indication of water stress occurrence (Enninful *et al*., 2019). In the present study, only one-third of the plants had recurved peduncles. The 2019 precipitations during the growing season were 28% lower than the long-term average, corroborating the plant's drought resistance hypothesis. Stay green is another characteristic of drought resistance, measuring the ability of the plant to retain greenness during grain ripening under water-limited conditions (Badigannavar *et al*., 2018). In the present study, less than 2% of the plants were completely senescent, while almost 70% were very slightly senescent. The north of Morocco is a region where high wind speeds routinely occur (Benkhattab *et al*., 2020). The lodging resistance is an important parameter that should be targeted, especially in lowlands where machines harvest. Fortunately, more than 80% of plants manifested low lodging susceptibility. For leaves color, almost all the plants (95.2%) had dark green leaves, which testify to the probable higher photosynthesis activity. It could also be reinforced by the erect orientation of the leaves (74.0%).

In the literature, midrib color can be white, light, and dark green, brown, or yellow (Naoura *et al*., 2020). More than 80% of the leaves had white midrib. Green midrib color was reported to indicate juicy stems, while white midrib color indicates pithy stems, i.e., less palatable, and less digestible (Li et al., 2015). Almost two-thirds of the plants had semi-compact and compact elliptic branches for panicle compactness. It could be due to the selection by the farmers for high-yielding cultivars. Grain colors were also variable, with almost half of the plants having a white color. Grain luster was absent in more than 90% of the plants, and 80.0% of the plants had middle-size grains. Half of the plants had circular, and the other half had elliptic grains shape. For grain covering, only less than 8% of grains were fully covered; some had glumes longer than grains. The fact that a major part of grains were not fully covered seems to be a selected feature that facilitates drying in order to minimize grain mold (GebrieTsige, 2019). It could also facilitate shattering, as observed with about 75% of the panicles. Glumes color also varied, and almost 57% were pale. However, darker glumes are reportedly more resistant to grain mold incidence (Aruna *et al*., 2021). Half of the glumes were hairy, while more than 90% had an absence of aristation. Almost 70% of the grains had mostly starchy endosperm.

Table 6: Frequencies of the different qualitative parameters considered to describe the 21 cultivated Moroccan Sorghum ecotypes.

Parameters and codes	Variables and scores	Frequency (%)
Leaf rolling	Non rolled leaves	87.3
	25% of rolled leaves	7.9
	50% of rolled leaves	4.8
Stay green	Very slightly senescent	68.3
	Slightly senescent	15.6
	Intermediate (about half of the leaves are dead)	10.5
	Mostly senescent	4.1
	Completely senescent leaves and dead stalk	1.6
Leaf color	Dark green	95.2
	Light green	4.8
Leaf orientation	Erect	74.0
	Drooping	26.0
Lodging susceptibility	Low	82.2
	Intermediate	14.0
	High	3.8
Midrib color	White	85.0
	Light green	15.0
Panicle compactness	Very lax panicle	4.4
	Very loose erect primary branches	2.2
	Very loose drooping primary branches	0.6
	Loose erect primary branches	3.5
	Semi-loose erect primary branches	27.6
	Semi-loose drooping primary branches	1.0
	Semi-compact elliptic	23.2
	Compact elliptic	33.3
	Compact oval	4.1
Exertion peduncle	Slightly exserted	30.8
	Exserted	4.8
	Well exserted	29.8
	Peduncle semi-recurved	4.4
	Peduncle recurved	30.2
Grain color	White	45.6
	Yellow	25.8
	Red	20.7
	Black	5.3
	brown	2.6
Grain luster	Present	7.3
	Absent	92.7
Grain shape	Narrow elliptic	10.1
	Elliptic	45.7
	Circular	44.3
Grain size	Small (<5mm)	20.0
	Middle (5-10 mm)	80.0
	Big (>5 mm)	0.0
Aristation	Present	12.7
	Absent	87.3
Endosperm texture	Mostly corneous	4.8
	Intermediate	4.1
	Mostly starchy	66.7
	Completely starchy	24.4
Grain covering	25% of grain covered	13.0
	50% of grain covered	26.7
	75% of grain covered	52.7
	Full grain covered	7.7
Glume color	White	22.2

	Beige Purple Black red	35.0 14.7 18.5 9.6
Glume hairiness	Present Absent	47.3 52.7
Shattering	Low Intermediate High	10.1 14.5 75.4

Quantitative parameters. *Agro-morphological characterization.* For agro-morphological parameters, the variation between the ecotypes was statistically significant ($p < 0.05$) for all the traits, indicating considerable phenotypic variability between the ecotypes (Table 7). The selection of performant ecotypes is usually based on phenotypic characterization, and the success would naturally depend upon the relationship between the phenotype and the genotype. To explain the variability, Chithra *et al*., (2022) used the estimated genotypic and phenotypic coefficients of variation (GCV and PCV) and the degree of heritability. Heritability (H^2) estimates remain extremely useful in studying the inheritance of quantitative traits following selection and deciding suitable breeding procedures for improving a crop plant. The selection could be more effective for a specific trait improvement by using both H^2 and genetic advance than the unique use of H^2. So, GCV and PCV are categorized as low (0-10%), moderate (10-20%), and high (>20%) (Chithra *et al*., 2022). Most of the parameters in the present study, including plant height, percentage of leaves, peduncle length, panicle length, width and weight, grain filling period, grains per plant and yield, harvest index, and thousand seed weight, had high GCV and PCV which indicates that a selection could be applied based on those traits to isolate more promising cultivars. The number of leaves and internodes, leaf length, stem diameter, and days to flowering exhibited intermediate GCV and PCV, which suggested that a vigorous selection could improve these traits. The days to maturity had low GCV and PCV, indicating less variability for this character and low options for breeders to diverse varieties for this trait. The heritability (H^2) is categorized as low (0-30%), moderate (30-60%), and high (60% and above) (Chithra *et al*., 2022). All the parameters were highly heritable except for leaf width and stem diameter, which had moderate heritability. Therefore, the response to direct selection could successfully improve all these traits. The observed variation in a population is due to both genetics and environmental factors, whereas genetic variability is the only heritable part from generation to generation. Thus, heritability alone does not provide an idea about the expected gain in the next generation, but it must be considered in conjunction with the genetic advance.

Table 7: Descriptive statistics and genetic parameters of 20 quantitative agro-morphological traits in 21 sorghum studied ecotypes.

Trait	Max	Min	Mean	SEM	GCV	PCV	H²	GA	GAM
Plant height (cm)	168.9	43.0	108.5***	1.8	22.6	23.7	91.0	48.1	44.4
Internodes number	11.0	5.8	7.6***	0.1	12.6	14.2	78.1	1.7	22.9
Leaves number	10.4	5.2	6.7***	0.1	11.2	13.0	73.7	1.3	19.8
Leaf length (cm)	43.8	17.5	31.7***	0.4	13.7	15.1	82.0	8.1	25.6
Leaf width (cm)	6.8	2.5	3.4***	0.1	6.2	12.1	26.4	0.2	6.6
Stem diameter (cm)	1.6	0.6	0.8*	0.0	14.1	19.1	54.1	0.2	21.3
Percentage of leaves (%)	18.2	3.1	7.6***	0.2	37.7	40.8	85.5	8.3	71.8
Percentage of stem (%)	24.0	2.4	41.4***	0.4	17.6	20.5	73.8	12.9	31.1
Percentage of panicle (%)	68.6	16.5	47.1***	0.7	17.8	20.5	75.6	15.0	31.9
Peduncle length (cm)	81.7	15.6	36.0***	1.0	38.2	39.5	93.3	27.3	76.0
Panicle length (cm)	30.9	8.7	17.6***	0.3	26.3	27.0	94.6	9.3	52.6
Panicle width (cm)	9.7	3.0	6.6***	0.1	23.7	25.6	85.7	3.0	45.3
Panicle weight (g)	112.1	3.6	39.5***	1.5	40.8	46.6	76.6	29.0	73.5
Days to flowering (days)	117.0	65.0	81.6***	1.6	15.3	15.4	98.0	25.4	31.1
Days to maturity (days)	142.0	94.0	113.0***	1.3	9.3	9.5	95.9	21.2	18.8
Grain filling period (days)	55.0	20.0	31.4***	1.1	27.8	28.1	98.1	17.8	56.7
Grains per plant	3,485.6	182.6	1,472.9***	93.4	42.9	44.8	91.9	1,248.8	84.8
Grain yield (T/ha)	7.7	0.5	3.5***	0.2	48.7	53.2	84.0	3260.2	92.0
Harvest index (%)	75.0	13.6	51.8***	1.8	27.6	28.6	93.6	28.6	55.1
Thousand seed weight (g)	34.4	12.7	22.5***	0.8	26.2	26.8	95.9	11.9	52.9

PCV phenotypic coefficient of variation (%), *GCV* genotypic coefficient of variation (%), *H²* broad-sense heritability (%), *GA* genetic advance, *GAM* genetic advance as percentage of the mean (%), *Max* maximum, *Min* minimum, *SEM* standard error of the mean. *, ** and *** represent significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

According to Chitra et al. (2022), genetic advance as a percent of the mean (GAM) is classified as low (<10%), moderate (10-20%), and high (>20%). Among the characters under study, except for the number of leaves and days to maturity that showed moderate GAM and leaf width that showed low GAM, all the other parameters exhibited high GAM coupled with high heritability. It could be used as a powerful tool in the selection process as such characters are controlled by the additive genes and are less influenced by the environment. Hence, the direct selection of such traits could also be effective in improving the yield. Close results were reported with Ethiopian ecotypes. On the other hand, low to moderate heritability and genetic advance values could hinder the selection due to high environmental effects over the genetic effects. So, only an effective selection can be obtained with the traits having higher GCV, PCV, H², and GAM, meaning that the additive genetic effects are sufficiently robust to the environmental effect. The improvement of the traits with low heritability and genetic advance can also be boosted over heterosis breeding.

North Moroccan ecotypes were shorter and less leafy than Pakistani ones (108.5 vs. 145.0 cm and 7 vs. 12, for plant height and leaves number, respectively) (Abdus Sattar *et al.*, 2012). Also, despite the lower leaf length and width that are responsible for photosynthesis, compared to ecotypes from Benin (31.7 vs. 76.4 cm and 3.4 vs. 7.5, respectively), the mean grain yield of the ecotypes (3.5 T/ha) was in the range of the value reported for traditional sorghum cultivars under restricted water conditions (Sugg *et al.*, 2017). Additionally, the harvest index was higher compared to several Ethiopian landraces

(Derese et al., 2018; Mengistu *et al*., 2020), which implies that Moroccan ecotypes were selected by farmers for grain production at the expense of fodder production. Grains per plant (1473) and thousand seed weight (22.5 g) were in the range of values for the Ethiopian sorghum (Wondimu *et al*., 2020). The days to flowering (82) were in the range of values reported in Ethiopia (Derese *et al*., 2018). However, the ecotypes in the present study were earlier to reach maturity, which lowered the grain filling period compared to their study (31 vs. 55 days) (Derese *et al*., 2018). The stem diameter in the present study was lower than other sorghum ecotypes (Chavan *et al*., 2018; Naoura *et al*., 2015). It could be interesting from a bromatological perspective as it was reported to be negatively correlated to digestibility (Khalilian *et al*., 2022). Low stem diameter was reported to be correlated to low resistance for lodging (Vki *et al*., 2021). However, despite the low stem diameter, the ecotypes of the present study were lodging resistant (82%), probably due to their short height, explained by a selection of grains rather than fodder.

Bromatological characterization. The choice of the best ecotypes to use in a particular region or a selection program depends not only on their productivity. The nutritive value plays a crucial role in supporting plant breeding programs. The long-term objective of the present study is to develop grain cultivars with high nutrient value since sorghum grains are principally used as feed in Northern Morocco. Moreover, using the straw after harvest could add extra interest. At the maturity stage, except for the grain DM content, the difference between the ecotypes was highly significant ($p < 0.001$) for all the bromatological parameters analyzed for the three parts of the plants (grains, leaves, and stems) (Table 8).

The bromatological parameters showed weaker variation than agro-morphological parameters. For sorghum grains, high GCV and PCV were found for ADF, ADL, CF, phenols, tannins (CT, TT), antioxidant activities (DPPH, FRAP), and CP digestibility. However, except for DM, NFE, and true digestibility, high heritability coupled with high GAM was found for all the parameters. It suggests that the selection based on these parameters will improve the nutritive value of the grains of the selected cultivars. For the leaves, only CP and Ash had high GCV and PCV. However, most parameters, including ADF, ADL, CF, EE, Ash, ME, and OM digestibility had high heritability coupled with high GAM. It testifies that improving the nutritive value of the sorghum leaves at maturity is also possible through selection. For the stems, only EE, CP, and ME showed high GCV and PCV, and except for DM, NDF, ADF, NFE, and true digestibility, all the other parameters had high heritability coupled with high GAM, suggesting that the stem nutritive value could also be improved through selection.

Table 8: Descriptive statistics and genetic parameters of bromatological traits in grains, leaves, and stems of 21 sorghum ecotypes studied in 2019 in Northern Morocco.

Trait	Max	Min	Mean	SEM	GCV	PCV	H^2	GA	GAM
Grains									
Dry matter (DM, %)	89.6	81.3	85.9	0.7	0.6	1.5	13.6	0.4	0.4
Neutral detergent fiber (% DM)	37.1	16.4	26.0***	1.1	18.2	19.7	85.3	9.0	34.6
Acid detergent fiber (% DM)	15.4	5.2	6.7***	0.5	25.3	26.8	89.0	4.8	49.2
Acid detergent lignin (% DM)	10.1	2.9	5.5***	0.3	34.1	35.4	93.1	3.7	67.9
Crude fiber (% DM)	6.1	1.1	2.9***	0.2	44.0	45.4	93.9	2.6	87.8
Ether extract (% DM)	6.8	2.7	4.5***	0.3	18.6	21.7	73.3	1.5	32.8
Ash (% DM)	2.4	1.0	1.6***	0.1	19.7	20.3	93.8	0.6	39.2
Crude protein (% DM)	19.8	7.1	10.5***	0.7	14.0	18.2	58.8	2.3	22.1
Nitrogen-free extract (% DM)	84.5	58.0	80.3***	1.7	2.6	4.4	34.9	2.5	3.2
In vitro enzymatic organic matter digestibility (% DM)	96.9	60.3	81.4***	1.5	10.5	11.0	91.6	16.8	20.7
In vitro true digestibility (% DM)	98.7	79.1	92.2***	1.1	4.4	4.9	81.4	7.5	8.2
In vitro enzymatic crude protein digestibility (% DM)	53.3	22.0	35.3***	1.2	27.0	27.1	99.4	19.6	55.5
Metabolisable energy (KJ/kg DM)	13.8	8.1	11.4***	0.3	11.9	12.8	86.1	2.6	22.7
Total phenols (mg TAE/g DM)	180.8	49.4	110.2***	0.5	35.4	36.6	93.8	77.9	70.7
Condensed tannins (mg TAE/g DM)	9.1	2.5	5.0***	0.3	47.1	47.3	99.1	4.8	96.6
Total tannins (mg TAE/g DM)	12.1	2.8	6.0***	0.3	43.8	44.3	97.5	5.3	89.0
DPPH (IC50; µg/mL)	50.6	17.6	72.7***	0.0	39.8	40.0	99.2	59.4	81.7
FRAP (mg F_eSO_4/g DM)	27.6	12.0	17.1***	0.6	29.0	29.1	99.0	10.1	59.3
Leaves									
Dry matter (DM, %)	93.2	81.4	87.7***	1.4	2.3	3.5	42.7	2.7	3.1
Neutral detergent fiber (% DM)	75.8	55.5	65.5***	1.6	4.8	6.3	57.5	4.9	7.5
Acid detergent fiber (% DM)	49.0	21.0	36.9***	0.6	15.4	15.6	97.3	11.6	31.3
Acid detergent lignin (% DM)	33.0	13.0	25.5***	0.5	18.1	18.4	96.8	9.3	36.7
Crude fiber (% DM)	40.6	24.6	31.4***	0.5	13.0	13.3	95.6	8.2	26.1
Ether extract (% DM)	3.9	2.0	2.7***	0.1	18.2	18.7	95.0	1.0	36.6
Ash (% DM)	17.1	5.7	8.9***	0.7	21.8	25.5	72.8	3.4	38.2
Crude protein (% DM)	12.3	4.0	7.1***	0.3	28.6	29.5	93.8	4.0	57.0
Nitrogen-free extract (% DM)	61.1	37.0	49.9***	0.9	9.8	10.3	90.6	9.6	19.1
In vitro enzymatic organic matter digestibility (% DM)	50.3	28.5	40.9***	1.0	11.4	12.1	89.0	9.1	22.2
In vitro true digestibility (% DM)	82.9	61.8	72.8***	1.5	5.0	6.1	67.0	6.1	8.4
Metabolisable energy (KJ/kg DM)	6.5	1.7	4.1***	0.3	17.1	20.8	67.8	1.2	29.1
Stems									
Dry matter (DM, %)	95.8	83.0	88.8***	1.0	3.0	3.6	70.4	4.6	5.2
Neutral detergent fiber (% DM)	89.6	57.4	71.8***	3.0	6.9	10.0	46.8	7.0	9.7
Acid detergent fiber (% DM)	57.0	34.4	45.3***	2.1	7.2	10.7	45.3	4.5	10.0
Acid detergent lignin (% DM)	38.2	20.3	27.8***	0.7	13.9	14.6	91.6	7.6	27.5
Crude fiber (% DM)	49.9	31.4	40.4***	1.1	11.0	11.9	85.0	8.4	20.8
Ether extract (% DM)	1.8	0.6	1.1***	0.0	22.9	23.8	93.0	52.1	45.5
Ash (% DM)	8.3	2.9	5.1***	0.5	17.8	25.0	50.5	1.3	26.0
Crude protein (% DM)	3.8	1.0	2.4***	0.1	26.1	27.8	87.8	1.2	50.3
Nitrogen-free extract (% DM)	60.0	41.2	51.0***	1.2	8.8	9.6	83.1	8.4	16.5
In vitro enzymatic organic matter digestibility (% DM)	31.0	15.1	24.7***	0.9	14.0	15.4	82.5	6.5	26.1
In vitro true digestibility (% DM)	82.9	49.6	60.8***	2.4	10.4	12.5	69.0	10.8	17.8
Metabolisable energy (KJ/kg DM)	4.4	1.5	2.8***	0.2	23.8	27.6	74.4	1.2	42.2

PCV phenotypic coefficients of variation (%), GCV genotypic coefficients of variation (%), H^2 broad-sense heritability (%), GA genetic advance, GAM genetic advance as percentage of the mean (%), Max maximum, Min minimum, SEM standard error of the mean. *, ** and *** represent significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

The grains' CP, EE, and Ash (10.5, 4.5, and 1.6% DM, respectively) were in the range of values in the Mediterranean area56. However, NDF values were higher than values reported in the USA (26.0 vs. 16.9 % DM) (Douglas *et al*., 1990). Probably because the traditional Moroccan ecotypes contain a thick pericarp characterized by a high fiber quantity (Chantereau *et al*., 2013). Despite this high NDF content, OM and true digestibilities (81.4 and 92.2%) were high but in the range of values reported for North-American ecotypes (Jaworski *et al*., 2015). The leaf NDF and ADF values (65.5 and 36.9% DM, respectively) were in the range of values reported for USA hybrids (Vietor *et al*., 2010a). According to Kamal *et al*., (2019), low fiber content is related to the leaf stay-green character, which was observed in almost 70% of the ecotypes. However, the stem NDF and ADF values (71.8 and 45.3% DM, respectively) were higher than the ones reported by the same authors (Vietor *et al*., 2010b). These two parameters, in addition to NFE, CF, EE, and CP (51.0, 40.4, 1.1, and 2.4 %DM, respectively), were in the range of values reported with Ethiopian ecotypes (Haile *et al*., 2016). However, ADL content was higher (27.8 vs. 6% DM) and explained the lower stem ME for the ecotypes in the present study (2.8 vs. 7.1 MJ/kg DM). This higher stem ADL content could also strengthen the lodging resistance.

The sorghum grains and straw (leaves and stems) have different nutritive values and can be used for different purposes. The grains can be a good source of energy (11.4 MJ/kg DM for grains vs. 4.1 and 2.8 MJ/kg DM for leaves and stems, respectively). Alternatively, the straw can be a good source of fibers (higher NDF (26% DM for grains vs. 65.5 and 71.8 % DM for leaves and stems, respectively), ADF (6.7% DM for grains vs. 36.9 and 45.3% DM for leaves and stems, respectively) and ADL (5.5% DM for grains vs. 25.5 and 27.8% DM for leaves and stems, respectively), but it was negatively reflected into a lower digestibility (81.4% DM for grains vs. 40.9 and 24.7% DM for leaves and stems respectively). The grains and leaves had high protein content (10.5 and 7.1% DM, respectively), while the stems had lower protein content (2.4% DM). Manifestly, the leaves were more nutritious compared to the stems. Similar results were reported for corn and pearl millet (Cetinkaya *et al*., 2020; Umutoni *etal*., 2021).

Total phenols of the grains (110.2 mg TAE/g DM) were lower than values reported for other Moroccan ecotypes (Bouargalne *et al*., 2022), probably due to the differences in the collected ecotypes. Their ecotypes grains were darker (brown and light brown) than present grains, where almost half of the grains were white, phenol contents being positively correlated to the darkness (Adarkwah-Yiadom & Duodu, 2017). Condensed tannin content (5 mg TAE/g DM) was lower than values reported for type II and type III sorghum grains rich in phenols and tannins, which confirms that most of the ecotypes present in this study probably belong to type I sorghum grains (Adarkwah-Yiadom & Duodu, 2017). Antioxidant activity (DPPH and FRAP) values were in the range of values reported by Kumari *et al*., (2021) for different sorghum ecotypes.

Correlation analysis. The knowledge of the association between yield, yield components, and bromatological parameters of the grains can help in the simultaneous selection of traits of interest for Moroccan sorghum crop improvement. The correlation matrix is reported in Figure 14. Only significant ($p < 0.05$) correlations are discussed. As expected, positive correlations were found between grains per plant and grain yield and between thousand seed and panicle weights. Some negative correlations were reported between grain yield and days to flowering and to maturity, which could be explained by a drought resistance strategy of the ecotypes. The negative correlations of grain yield with NDF, ADL, CT, and TT were interesting and showed that farmers had selected grain high-yielding cultivars but also low fiber and tannin contents in their grains. Contrary to several studies that reported a negative association between grain yield and plant height (Mengistu *et al*., 2020), plant height in the present study positively correlated with yield components, including grains per plant, grain yield, thousand seed weight, and panicle weight, indicating that the selection for higher plants could improve grain yield of the selected ecotypes. The strong positive correlation between thousand seed weight and grain filling period confirmed that a long maturation period could favor a good grain filling. Moreover, significant positive correlations were reported for leaf length and width with OM digestibility of the grains, possibly due to enhanced photosynthesis. The stem diameter also had positive correlations with grain OM digestibility and ME, showing that the breeders selected for high stem diameter (probably to protect the plants from lodging (Vki *et al*., 2021)) and also for grain nutritive value. Phenols, tannins, and antioxidant activities were positively correlated with ADF. Several authors reported the antioxidant activities of the phenols and tannins (Mohamed *et al*., 2016). The higher antioxidant activity was observed in the “darkest” grains in the present study, these grains having the thickest pericarp and thus the highest ADF content. ADF had positive correlations with NDF and ADL, as also reported in other studies (Sugg *et al*., 2017). In grains, true digestibility was negatively correlated to TT, and CP digestibility was negatively correlated to Phenols. It could be explained by some grains' high phenol content (up to 18%) and its negative impact on the rumen microbiota (Kondo *et al*., 2014).

Figure 14: The correlation matrix of agro-morphological and bromatological traits evaluated in 21 sorghum ecotypes studied in 2019 in Northern Morocco.

Color intensity and the size of the circle are proportional to the correlation coefficients. In the right side of the correlogram, the legend color shows the correlation coefficients and the corresponding colors. Positive correlations are displayed in green and negative correlations in purple color. TSW thousand seed weight (g), GFP grains filling period (days), PanL panicle length (cm), PH plant height (cm), PedL peduncle length (cm), pStem % of stems (%), HI harvest index (%), CP crude protein (% DM), GPL grains per plant, PanW panicle weight (g), GRY grains yield (T/ha), CF crude fiber (% DM), DayF days to flowering (days), DayM days to maturity (days), pLeav perventage of leaves (%), pPan percentage of panicle (%), IVTD in vitro true digestibility (%), SD stem diameter (cm), IVEOMD in vitro enzymatic organic matter digestibility (%), ME metabolizable energy (KJ/kg DM), LWi leaf width (cm), LL leaf length (cm), IntN internodes number, LN leaves number, DPPH 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity (IC50, µg/ml), FRAP Ferric reducing ability of plasma (mg FeSO4/g DM), Phenols total phenols (mg TAE/g DM), CT condensed tannins (mg TAE/g DM), TT total tannins (mg TAE/g DM).

Principal component analysis. The principal component analysis was conducted to cluster ecotypes based on parameters that participate mostly in the variation. The first three components explained 56.46% of the variability. Figure 15 represents the distribution of variables and individuals in the first two dimensions. The first component (PC1) participated with 28.0% in the variability. It was positively correlated to grains per plant and grain yield, panicle weight, and plant height and was negatively correlated to ADL, ADF, tannins, FRAP activity, Phenols, and days to flowering and maturity.

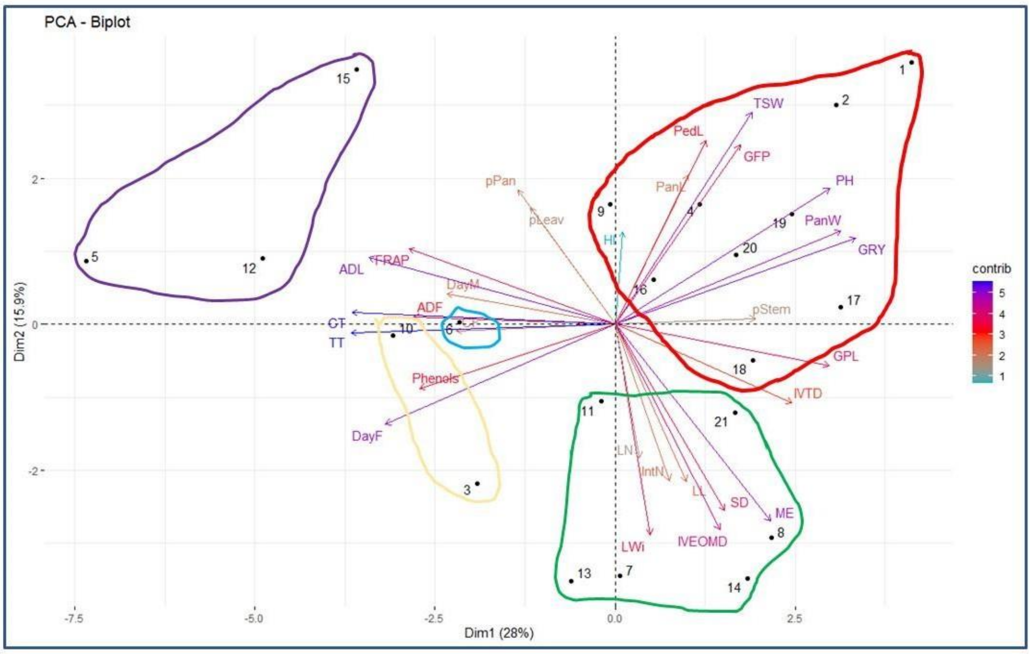

Figure 15 : Graph of the variables and individuals of the principal component analysis.

TSW thousand seed weight (g), GFP grain filling period (days), PanL panicle length (cm), PH plant height (cm), PedL peduncle length (cm), pStem, % of stems (%), HI harvest index (%), CP crude protein (% DM), GPL grains per plant, PanW panicle weight (g), GRY grain yield (T/ha), CF crude fiber (% DM), DayF days to flowering (days), DayM days to maturity (days), pLeav % of leaves (%), pPan % of panicle (%), IVTD in vitro true digestibility (%), SD stem diameter (cm), IVEOMD in vitro enzymatic organic matter digestibility (%), ME metabolizable energy (KJ/kg DM), LWi leaf width (cm), LL leaf length (cm), IntN internodes number, LN leaves number, DPPH 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity (IC50, µg/mL), FRAP Ferric reducing ability of plasma (mg FeSO4/g DM), Phenols total phenols (mg TAE/g DM), CT condensed tannins (mg TAE/g DM), TT total tannins (mg TAE/g DM). Five clusters were determined by the cluster heatmap analysis and represented via the five coloured circles (red, green, purple, yellow and blue) on this figure.

The second component (PC2) explained 15.9% of the variability. It was positively correlated to thousand seed weight, peduncle length, and grain filling period and was negatively correlated to leaf length and width, internodes number, stem diameter, ME, and OM digestibility. According to these correlations, the PC1 was a positive agronomical (yield) and negative bromatological (fibers and antioxidants) component, while the PC2 was a negative agronomical (plant morphology) and negative bromatological (ME and OM digestibility) component. The opposite distribution of yield components with antioxidant content and activity could indicate that although the concentration of phenolic compounds in the grains could increase the antioxidant capacity, it could also negatively affect the absorption of essential nutrients (Fe, Mn, and P) by the plant, which hinders physiological processes, and thus reduce the yield (Adarkwah-Yiadom & Duodu, 2017).

Heatmap analysis. A heatmap was conducted to cluster the ecotypes based on the agro-morpho-phenological and bromatological parameters (Figure 16). The heatmap analysis structured the dendrogram on the left side of the figure according to sorghum ecotypes, and the second dendrogram at the top side of the figure showed the parameters that affected this distribution. The heatmap described five clusters, also represented in Figure 16 by five colored circles. The explanatory variables were divided into six groups: the first group of high nutritive value and vegetative parameters (ME, OM digestibility, stem diameter, leaves number, length and width, and internodes number), the second group of true digestibility and percentage of stems, the third group of grain yield characteristics (grains per plant, grain yield, panicle length and weight, plant height, and peduncle length), the fourth group of grain weight (percentages of panicle and leaves, grain filling period, and thousand seed weight), the fifth group of phenology (days to flowering and to maturity, and CF) and the sixth group of fibers, and antioxidant content and activity (ADL, ADF, TT, CT, phenols, and FRAP). The first group's three ecotypes (E5, E12, and E15) were characterized by grains having low nutritive value and yield, and high content of fibers and phenols. The second group was similar to the first one except for the phenology (later ecotypes) and low fiber content. Opposite traits of the first group characterized the third group. High antioxidant factors and intermediate values for the other components characterized the only ecotype (E6) present in the fourth group. The fifth group differed by its higher nutritive value, more interesting vegetative parameters, and lower values for the other parameters.

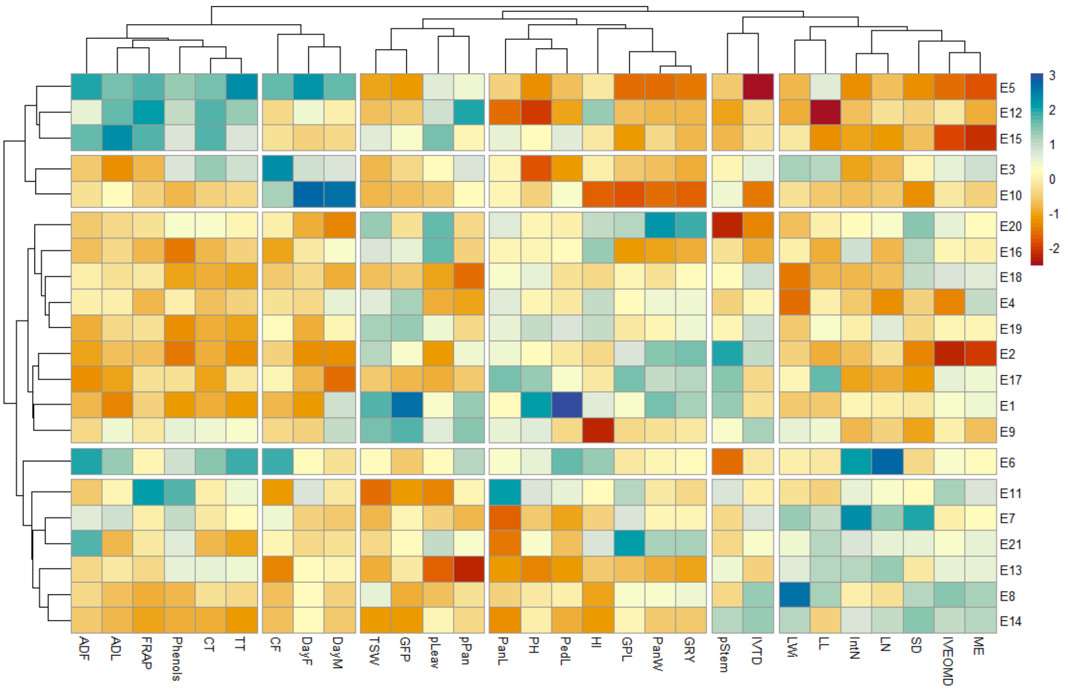

Figure 16: Cluster heatmap analysis of sorghum ecotypes' responses to morpho-phenological, agronomic and bromatological characterization.

The heatmap plot describes the relative abundance of each bitter vetch ecotype (rows) within each trait (column). The color code (blue to dark red) displays the values of the parameters: blue color indicates high values while red color indicates low values. The dendrogram (on the left) shows hierarchical clustering of bitter vetch ecotypes based on the Euclidian distance and Ward's clustering method. *TSW* thousand seed weight (g), *GFP* grains filling period (days), *PanL* panicle length (cm), *PH* plant height (cm), *PedL* peduncle length (cm), *pStem* % of stems (%), *HI* harvest index (%), *CP* crude protein (% DM), *GPL* grains per plant, *PanW* panicle weight (g), *GRY* grain yield (kg/ha), *CF* crude fiber (% DM), *DayF* days to flowering (days), *DayM* days to maturity (days), *pLeav* % of leaves (%), *pPan* % of panicle (%), *IVTD* in vitro true digestibility (%), *SD* stem diameter (cm), *IVEOMD* in vitro enzymatic organic matter digestibility (%), *ME* metabolizable energy (KJ/kg DM), *LWi* leaf width (cm), *LL* leaf length (cm), IntN internodes number, *LN* leaves number, *DPPH* 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity (IC50, µg/mL), *FRAP* Ferric reducing ability of plasma (mg $FeSO_4$/g DM), *Phenol*s total phenols (mg TAE/g DM), *CT* condensed tannins (mg TAE/g DM), *TT* total tannins (mg TAE/g DM).

According to this map, the choice of interesting ecotypes for feed should be based on the balance between plant and yield components, combined with high nutritive value. Ecotype 21 could be interesting as it combines high grain yield and high ME. Several studies highlight the importance of these dual-purpose genotypes (Ndiaye *et al*., 2019).

Mantel test. The Mantel test was conducted to link the environmental and agro-morphological data to bromatological values. The ecotypes were clustered into different groups irrespective of the region where they were collected. The Mantel test showed that no significant correlations were found between morpho-pheno-agronomic and bromatological parameters and geographical data (latitude and longitude) of the ecotype collection sites (r= -0.08, p=0.82), nor with environmental data (r=-0.08, p=0.81). However, as expected, geographic distance and environmental data were correlated (0.72, p <0.001) for each collection site. The absence of significant correlation for the previous parameters could be due to the collection of ecotypes in a wider area covering all the Northern region of Morocco or to seed exchange between farmers from the different regions. Consequently, choosing interesting ecotypes should be based on ecotypes level rather than geographical area.

Conclusion. The present study showed that Moroccan ecotypes of *Sorghum bicolor* were highly variable for agro-morphological and bromatological parameters, with some resistance to drought. The grains presented interesting protein contents and metabolizable energy. The multivariate analysis distinguished five clusters based on agro-morphological, bromatological, and antioxidant activity. Selecting the better ecotypes could be based on ecotypes level rather than geographical area. This work being the first step, future multi-location trials across multiple cropping cycles are needed to confirm and strengthen the present results in order to improve sorghum selection and spread the best ecotypes. Moreover, conserving these local genes in a seed bank is useful since climate change and increasing recurrent droughts require maintaining a wide sorghum biodiversity bank.

Materials and methods

All experiment and analysis methods were performed following relevant regulations and guidelines.

Plant material, study site, and experimental set-up. In 2018, seeds of 21 Sorghum ecotypes were collected using a simple random sampling from 21 farms in Northern Morocco (Figure 17). Prospection missions were conducted just after the end of the sorghum harvest season. In each farm, 3 kg of grains were collected from the grain stock.

The seeds were sown in 2019 in the El Menzla experimental field (35°31’53’’N; 5°42’36’’W; 128,5 m), a related research station to INRA of Tangier, Morocco.

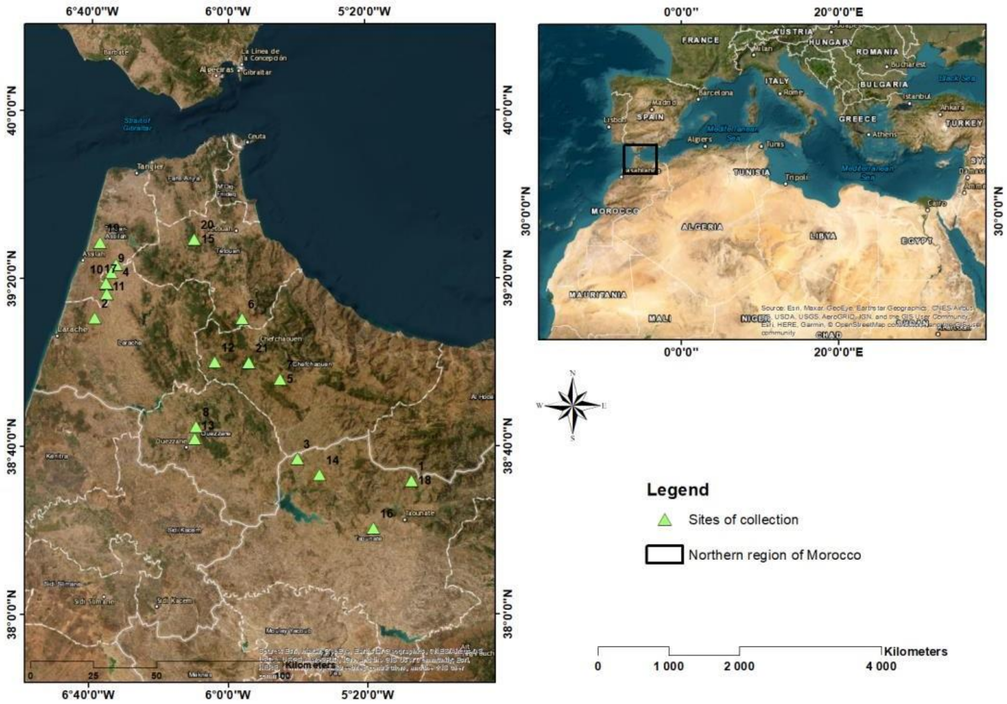

Figure 17: Collection sites of the 21 Sorghum bicolor ecotypes (This map was generated using ArcGIS Desktop version 10.4.1 - Esri, Redlands, CA, USA, https://en.freedownloadmanager.org/Windows-PC/Portal-for-ArcGIS.html)

Ecological characterization. At the collection and experimental sites, five soil samples were collected from 0-20 cm and 20-40 cm horizons for physico-chemical analysis (Table 1). Soil samples were oven dried at 60°C until constant weight to determine soil humidity (%), then grounded and passed through a 2 mm sieve. The pH (water and KCl) was read with a standard calibrated pH meter in 2:1 distilled water solution and dry soil ratio (Jackson, 1958). The nitrogen was determined by mineralization and distillation using the Kjeldahl method (Bremner, 1960). The electrical conductivity was measured in the collected soil extract from the saturated soil paste by conductivity meter (Wilcox, 1950). Exchangeable potassium was analyzed in 1 N ammonium acetate extract using a flame photometer (Toth & Prince, 1949).

Total limestone ($CaCO_3$) was measured using HCl (Nelson, 1983). Carbon was measured through dichromate oxidation and converted to organic matter (OM) by multiplying by a factor of 1.72 (Walkley & Black, 1947), and available phosphorus was determined by the colorimetric method (Olsen et al., 1954). Soil texture was determined by using the standard Pipette method and wet sieving (Robinson, 1922).

Nineteen bioclimatic data (BIO 1-19) for 1950-2000 were estimated from major climate databases for each farm collection site (www.worldclim.org). The data were extracted from satellite images via ArcGIS Desktop version 9.3 (Esri, Redlands, CA, USA) and were interpolated with a resolution of approximately 1 km (Hijmans *et al*., 2005) (Table 2). The climatic data (minimum, maximum, and mean temperatures (°C), rainfall (mm) of each month, and total rainfall (mm)) of the 2019 agronomical year at the experimental site were collected from a climatic station 10 km nearer. The 2019 bioclimatic variables (BIO 1-19) were determined based on these climatic data.

Experimental design. The experiment was conducted under a randomized block design with three replicates. Ecotypes were sown on 10th April 2019, with a density of 10 plants/m² with a spacing of 50 cm between lines and 20 cm between plants in a 3×2 m² plot. One meter was the distance between plots and between blocks. The trial was conducted under rainfed conditions on a fallow plot. As soil analysis indicated low phosphorus concentration compared to potassium and nitrogen, NPK (10-30-10) fertilizer was applied at the rate of 100 kg/ha on the day of sowing. No watering was needed at sowing because rainfall occurred immediately after.

Agro-morphological characterization. *Phenological assessment.* Every three days, the phenological parameters were observed during the growing season. Days to flowering (DayF) and days to maturity (DayM) were recorded as the days from planting until 50% of the plants reached the flowering stage and the physiological maturity, respectively (Abraha *et al*., 2015). The grain filling period (GFP) was measured as the difference between these two parameters.

Morphological assessment. Fourteen quantitative and seventeen qualitative traits were recorded at the ecotype maturity stage. These traits were described based on the recommendations of the International Board for Plant Genetic Resources and the International Crops Research Institute for the Semi-Arid Tropics (IBPGR & ICRISAT, 1993), from 5 randomly individual plants per ecotype in each plot. The plant height (PH) was recorded as the height of the plant from the ground to the tip of the panicle. The peduncle length (PedL) was measured as the average exertion of the panicle from the flag leaf's blade to the base of the lowest panicle branch. The leaves number (LN) and internodes number

(IntN) were counted on each plant. The length and width of the leaf (LL and LWi, respectively) and the stem diameter (SD) were measured at the third internode. The panicle length (PanL) was recorded as the average length of the panicle from the lower panicle branch to the tip of the panicle, and the panicle width (PanWi) was measured as the average width of the panicle at its widest section. The panicle weight (PanW) was recorded as the weight of an unthreshed panicle. The panicles were threshed to evaluate the number of grains per plant (GPL). Ultimately, the plants were separated into their different parts, dried in an oven at 102°C until constant weight, and were transformed into percentages of panicle (pPan), leaves (pLeav), and stems (pStem). The 18 qualitative variables were assessed for sorghum. Leaf rolling (non-rolled to fully rolled), color (dark and light green), and orientation (erect or drooping) were visually rated on the leaves of five identified plants at the flowering stage. Before harvesting, stay green (very slightly senescent, slightly senescent, intermediate (about half of the leaves are dead), mostly senescent, completely senescent leaves and dead stalk), and lodging susceptibility (low, intermediate, and high) were observed on five whole plants. Then after harvesting the panicles, the other parameters were evaluated using IBPGR & ICRISTAT (1993) descriptors. Their compactness was classified into (very lax panicle, very loose erect primary branches, very loose drooping primary branches, loose erect primary branches, semi-loose erect primary branches, semi-loose drooping primary branches, semi-compact elliptic, compact elliptic, compact oval), and the exertion of the peduncle was classified to (slightly exserted, exserted, well exserted, peduncle semi-recurved, peduncle recurved). These two parameters were classified by assigning them to different shapes shown by the descriptors. The frequency of grain color (white, yellow, red, black, and brown), grain luster (present or absent), grain shape (narrow elliptic, elliptic, circular), grain size (small (<5mm), middle (5-10 mm), and big (>5 mm)), aristation (present or absent), endosperm texture (mostly corneous, intermediate, mostly starchy, completely starchy), grain covering (25% of grain covered, 50% of grain covered, 75% of grain covered, full grain covered) and glume color (white, beige, purple, black, red), glume hairiness (present or absent) and shattering (low, intermediate or high) were evaluated on ten randomly selected grains per plant by visually comparing them to the morphological descriptors present in the IBPGR and ICRISAT options (IBPGR & ICRISAT, 1993).

Agronomical assessment. All the plants in a plot (n = 60 plants per plot) served to determine the grain yield (GRY) as the dry weight of the grains. Thousand seed weight (TSW) was recorded as the weight of one thousand seeds sampled thrice from bulked seeds in each plot. The straw was measured as the weight of the above ground plant parts. The harvest index (HI) was calculated as the ratio of GRY to straw.

Bromatological analysis. *Chemical composition*. At the maturity stage, the plots were harvested (n = 55 plants per plot) and separated into grains, leaves, and stems. The separated material was dried at 50°C for 48h, ground, and passed through a 1 mm sieve for bromatological analysis. The samples were evaluated according to AOAC (1997) to determine the contents of dry matter (DM) by drying 5 g of the sample at 102°C until constant weight (method 934.01), ash by incinerating 5g of the sample at 550 °C for 12h (method 942.05), ether extract (EE) by extraction with diethyl ether in Soxhlet apparatus (method 963.15). The crude protein (CP) content was determined by multiplying the nitrogen content by 6.25, obtained after mineralization with H_2SO_4 and distillation with NaOH using the Kjeldahl method (method 977.02). Fiber content (crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL)) were analyzed using an ANKOM® 200 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA), following the method of AOAC (1990) and Van Soest *et al*. (1991). The nitrogen-free extract (NFE) was estimated using the following "equation 1":

$$\text{NFE (\% DM)} = 100 - (\text{EE} + \text{CP} + \text{CF} + \text{Ash}) \quad (1).$$

Total phenols (TP) and total tannins (TT) were quantified in a methanol extract solution using the Folin–Ciocalteu method (Palacios *et al*., 2021). Total phenolic substances were determined by combining 40 µL of the sorghum methanol extract, 60 µL of distilled water, 50 µL of Folin–Ciocalteu reagent, and 225 µL of sodium carbonate (20%). The mixture was vortexed and kept in the dark for 40 min at room temperature. The tube was centrifuged, and an aliquot of 250 µL of the supernatant was transferred to a microplate well. The absorbance was read at 725 nm. Non-tannic phenols were determined in another centrifuge tube: 750 µL of the extract was mixed with 100 mg of polyvinylpolypyrrolidone (PVPP) and 1 mL of demineralized water. The tube was vortexed, kept at 4°C for 15 min, centrifuged at 4°C at 3,500 rpm for 10 minutes, and then read at 725 nm. Tannic acid at different concentrations (5-16 µg/mL) was used to construct the calibration curve. Total tannins were calculated as the difference between total and non-tannic phenols after precipitation with PVPP. The condensed tannins (CT) were analyzed by Porter *et al*. (1985) method. Briefly, 3 mL of Butanol-HCl reagent were mixed in a glass tube with 0.1 mL of ferric reagent and 0.5 mL of the extract. The tubes were vortexed and put in a water bath at 97°C for one hour. After cooling, the absorbance was read at 550 nm.

Digestibility. The *in vitro* enzymatic dry and organic matter digestibilities (IVEDMD and IVEOMD) were determined by the enzymatic method in a two-step method (Aufrère & Michalet-Doreau, 1983). The first step concerned the incubation of 0.5 g of dry sample with 20 mL of a 2% pepsin solution diluted in 0.1 N hydrochloric acid. After 24h of incubation at 40°C, the sample was solubilized in 50 mL of a buffer solution containing 1 g/L cellulase and again incubated at 40 °C for 24 h. After incubation, the sample was rinsed with hot distilled water and then placed in an oven at 60°C until constant weight. It was weighed to determine the IVEDMD. The sample was incinerated in the muffle furnace at 550°C for 12 hours to determine IVEOMD. The *in vitro* true digestibility (IVTD) was determined by incubation of feed samples in filter bags in a Daisy II incubator® (ANKOM Technology, Fairport, NY, USA) (Kowalski *et al*., 2014). All the study procedures were approved by the Regional

Center of Agricultural Research of Tangier (permit number: 01/CRRAT/2017). Rumen fluid was collected from five animals at a communal slaughterhouse. The animals were fed a conventional diet based on oat hay, barley, and fava bean grains, as distributed by regional farmers. The animals were slaughtered almost 12 hours after feeding, and rumen fluid was immediately collected and sieved using a double cheese filter. Then, it was kept in a thermos at 39 °C to maintain the viability of rumen microflora. After arriving at the laboratory, it was added to artificial saliva in a 1:5 ratio containing samples heat-sealed in ANKOM F57 filter bags and incubated at 39.5°C for 48h. The IVTD was estimated by quantifying residual DM compared to incubated initial quantities. The metabolizable energy (ME) was calculated according to AOAC (1990) using "equation 2":

$$\text{ME (MJ/kg DM)} = 0.17 \times \text{IVEDMD} - 2 \quad (2),$$

where IVEDMD is the in vitro enzymatic dry matter digestibility in percentage. The in vitro enzymatic crude protein digestibility (IVECPD) was determined according to the procedure described by Aufrère & Cartailler (1988). Briefly, 1.0 g of ground sample was added to 50 ml of enzyme solution (0.1 g protease per 1L of borate–phosphate buffer; pH 6.8). Then, the tubes were sealed and incubated at 40 °C for 24 h under permanent stirring. Subsequently, samples were filtered, and residual N content was analyzed. The IVECPD was calculated according to the following "equation 3":

$$\text{IVECPD (\%)} = \frac{(\text{Nsample} - \text{Nresidue})}{\text{Nsample}} \times 100 \quad (3),$$

where N_{sample} represents the nitrogen content of the sample and $N_{residue}$ represents the nitrogen remaining after digestion.

Antioxidant activity. The ground and sieved sorghum grain (2 g) was mixed with 20 mL of 80% methanol in a shaker at room temperature for 12h. The resulting supernatants were filtered using Whatman filter paper and centrifuged at 6000 rpm for 10 min. Finally, the filtrate was evaporated at 30 °C. The dried extract was weighed and redissolved in methanol to a concentration of 1 mg/ml, then stored at 20°C until analysis. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined according to the procedure reported by Tepe *et al.*, (2005). For this purpose, 100 µL of various concentrations of the extract were added to 300 µL of a 100 mM methanol solution of DPPH. The absorbance was read after a 30 min incubation period against a blank at 517 nm at room temperature. Inhibition of free radical DPPH in percent (%) was calculated in the following way "equation 4":

$$\text{DPPH scavenging activity (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100 \quad (4),$$

where $A_{control}$ is the absorbance of DPPH in the absence of a sample and A_{sample} is the absorbance of DPPH in the presence of a sample. Extract concentration providing 50% inhibition (IC50) was calculated from the graph of inhibition percentage against extract concentration. The Ferric-reducing ability of plasma (FRAP) of grain extract was determined according to the method of Benzie & Strain, (1996). Briefly, FRAP solution was prepared by mixing acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM in 40 mM HCl), and $FeCl_3.6H_2O$ solution (20 mM) in a 10:1:1 ratio and was incubated at 30°C for 30 min. Then, 40 µl of the extract solution was mixed with 360 µl of the freshly prepared FRAP solution. The mixture was shaken and incubated for 4 min at 37°C in a water bath, and the absorbance was read at 593 nm.

Data analysis. Analysis of variance was carried out to test the ecotype effect. The variance components were estimated using a general linear model (GLM), using SAS 9.4 version (SAS Inst. Inc., Cary, NC, USA). Phenotypic coefficients of variation (PCV), genotypic coefficients of variation (GCV), broad-sense heritability (H^2), genetic advance, genetic advance as a percentage of the mean (GAM), and genotypic and phenotypic correlation matrix were estimated following the formula given by Shariatipour *et al*., (2022). All genetic parameters were estimated using the following equations “5, 6, 7, 8, 9, 10, 11”:

$$\sigma^2 g = \frac{(MSg - MSe)}{r} \quad (5),$$

$$\sigma^2 p = \frac{MSg}{r} \quad (6),$$

$$\text{GCV}\ (\%) = \frac{\sqrt{\sigma^2 g}}{\bar{x}} \times 100 \quad (7),$$

$$\text{PCV}\ (\%) = \frac{\sqrt{\sigma^2 p}}{\bar{x}} \times 100 \quad (8),$$

$$H^2 (\%) = \frac{\sigma^2 g}{\sigma^2 p} \times 100 \quad (9),$$

$$\text{GA} = H^2 \times k \times \sqrt{\sigma^2 p} \quad (10),$$

$$\text{GAM}\ (\%) = \frac{\text{GA}}{\bar{x}} \times 100 \quad (11),$$

where $\sigma^2 g$ = genotypic variance, MSg = mean square of genotypes, MSe = mean square of error, r = number of replications, $\sigma^2 p$ = phenotypic variance, $\bar{x}$ = general mean of the trait, and k = Selection differential, which is equal to 2.06 at 5% intensity of selection. To summarize and visualize the relationship between agro-morphological and bromatological parameters, a correlation matrix was generated using the “corrplot” package of R software, version 4.2.1. Principal component analysis (PCA) was performed using the “FactoMineR” and “Factoextra” packages. A heatmap was created using the “Pheatmap” package, with Euclidean distance as the similarity measure and hierarchical clustering with complete linkage. The Mantel test was used to assess the correlation between the phenotypic distance of the ecotypes with the geographic and environmental one. The Tidyverse”, “Vegan”, and “Geosphere” packages were used.

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Author Contributions

M.C., J.-F.C., and C.A. received the project funding. S.B., A.N., M.C., J.-F.C., and C.A. conceived and designed the experiment. S.B., A.N., and M.C. collected and provided the germplasm for evaluation and managed the field experiments. S.B. and N.M. analyzed the data. S.B. wrote the draft manuscript. S.B., A.N., N.M., C.A., J.L., J.-L.H., M.C. and J.-F.C. read and reviewed the manuscript for final publication. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

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Addendum

Table 9. Descriptive statistics and genetic parameters of phenological traits expressed in days and GDD of 21 sorghum ecotypes studied in 2019 in Northern Morocco.

Trait	Mean	Max	Min	SEM	GCV	PCV	H²	GA	GAM
Days to flowering (days)	81.6***	117.0	65.0	1.6	15.3	15.4	98.0	25.4	31.1
Days to flowering (GDD, °C)	883.9***	1424.5	665.4	24.0	21.7	21.8	99.4	394.7	44.6
Days to maturity (days)	113.0***	142.0	94.0	1.3	9.3	9.5	95.9	21.2	18.8
Days to maturity (GDD, °C)	1385.1***	1852.1	1091.6	23.0	13.1	13.2	98.6	371.1	26.8
Grain filling period (days)	31.4***	55.0	20.0	1.1	27.8	28.1	98.1	17.8	56.7
Grain filling period (GDD, °C)	501.1***	858.4	343.4	17.5	27.8	27.9	99.2	285.7	57.0

PCV phenotypic coefficient of variation (%), GCV genotypic coefficient of variation (%), H^2 broad-sense heritability (%), GA genetic advance, GAM genetic advance as percentage of the mean (%), Max maximum, Min minimum, SEM standard error of the mean. *, ** and *** represent significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Section expérimentale

Etude 3 :

Ecological, agro-morphological and bromatological characterization of bitter vetch ecotypes in Northern Morocco

Chapitre 5. Étude 3 : Ecological, agro-morphological and bromatological characterization of bitter vetch ecotypes in Northern Morocco

Préambule

L'orobe est une légumineuse utilisée dans la région Méditerranéenne comme source de protéines, principalement pour ses grains, dans l'alimentation animale. Au Nord du Maroc, elle est utilisée pour valoriser les terres marginalisées. Dans la région Méditerranéenne, elle a été rapportée comme résistante à la sécheresse et nécessitant moins d'intrants. Elle peut être un bon précédent culturale pour sa capacité à fertiliser les sols en azote naturellement. Elle a également montré des concentrations en protéines intéressantes, mais des teneurs en facteurs antinutritionnels élevées qui seront un obstacle à son utilisation. D'ailleurs, son nom commun vesce amère vient de cette observation. Au Maroc, aucune étude n'a essayé d'évaluer le potentiel des écotypes à des fins de nutrition animale. Pour ses possibles bienfaits environnementaux et économiques, et le besoin de chercher de nouvelles ressources alternatives dans la nutrition animale, la prospection de gènes Marocains s'avérait une nécessité. Pour étudier son potentiel afin de l'introduire dans le calendrier alimentaire des caprins dans la région, des évaluations de la diversité agro-morphologique et bromatologique ont été réalisées. Dix-sept écotypes ont été collectés dans dix-sept fermes situées dans le Nord du Maroc. La culture a été réalisée au cours des saisons de croissance 2019 et 2020 en utilisant un protocole en blocs aléatoires complets avec trois répétitions. Les données agro-morphologiques ont été recueillies aux stades début de la floraison, la pleine floraison et la formation des gousses. Au stade de la maturité physiologique, les données sur le rendement en grains et ses composantes ont été enregistrées. La caractérisation bromatologique a été réalisée pour les grains et la paille. Les résultats ont indiqué des variations significatives entre les écotypes pour presque tous les paramètres et des résultats intéressants sur le rendement (1 T/ha) mais une faible teneur en protéines (22,9% MS) par rapport aux écotypes de la région Méditerranéenne. Les analyses multivariées (analyse en composantes principales et heatmap) ont permis de privilégier le second groupe d'écotypes présentant des rendements en grains et des paramètres végétatifs intéressants, notamment l'écotype E11 à meilleure valeur nutritive. La variabilité entre les écotypes a été plus élevée pour les paramètres des composants du rendement en grains par rapport aux paramètres morphologiques et bromatologiques, qui sont probablement influencés par les variations environnementales interannuelles. Ces variations environnementales vont possiblement rendre la sélection des écotypes plus difficile. En conclusion, la collection des écotypes Marocains présente certains écotypes intéressants, oriente la recherche future et ouvre de nouvelles perspectives d'évaluation. Elle souligne également la possibilité d'utiliser les écotypes en nutrition animale.

Les hypothèses étaient donc :

- ✓ Les caractéristiques du sol des sites de collecte sont similaires ?
- ✓ Les paramètres agro-morphologiques et bromatologiques sont influencés par les écotypes ?

Section expérimentale

Etude 3 :

Ecological, agro-morphological and bromatological characterization of bitter vetch ecotypes in Northern Morocco

À soumettre

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Abstract

Bitter vetch is a promising legume mainly used in the Mediterranean area for its grains as a source of proteins in animal feed. However, it is an underused crop conducted in marginal soils in Northern Morocco. To study its reintroduction potentials, agro-morphological and bromatological diversity evaluations were conducted. Seeds of seventeen ecotypes were collected in seventeen farms located in Northern Morocco in 2018. The cultivation was realized during the 2019 and 2020 growing seasons using a randomized complete block design with three replicates. Agro-morphological data were collected at the flowering, full flowering, and pod setting phenological stages. Yield component assessment and grain and straw bromatological characterization were performed at maturity. The results indicated significant variations between ecotypes for almost all parameters and interesting results about yield (1 T/ha) but low protein content (22.9 % DM) compared to other ecotypes of the Mediterranean region. The estimated genetic parameters could emphasize the possibility of selecting highly productive and nutritive cultivars. However, interannual variations were also detected, making the selection of the ecotypes harder. No significant correlations were observed between agro-morphological and bromatological traits of grains and geographical distances. Multivariate analyses (PCA and Heatmap) clustered ecotypes into five groups, where the second cluster was the more interesting candidate for developing high yielding and nutritive varieties in Mediterranean areas.

Introduction

The aim of world food security involves the selection of highly productive plant varieties and the use of large amounts of chemicals and water (Tilman *et al*., 2011). This approach led to gene and biodiversity losses and environmental pollution (Marouane et al., 2015). Moreover, due to climate change, plants are suffering from rising temperatures and drought periods (Schilling *et al*., 2020). In the Mediterranean area, small ruminants are mainly conducted on silvopastoral systems, which have undergone several changes due to different drivers (wildfire, cultivation, urban expansion,…), leading to decreased rangeland forage availability and sustainability (Chebli *et al*., 2021). The use of ancient plants, neglected because of lower yields but more adapted to the local environment, must be investigated to feed animals. It could be a sustainable alternative because their reintroduction could increase plant biodiversity in farming systems (Boukrouh *et al*., 2021, 2023).

Vicia ervilia, or bitter vetch, is one of the earliest domesticated plants. It belongs to the genus Vicia L., which contains about 210 species (Kupicha, 1976; Hanelt & Mettin, 1989). Archaeobotanical studies indicated the Mediterranean and Middle East areas as possible centers of origin of the genus Vicia L., at least since the Neolithic period (Peña-Chocarro & Zapata, 2010). It has been marginalized since the 16th and 17th centuries, parallel to the introduction of new crops from the New World in the Mediterranean area (FAO, 1994). In Morocco, bitter vetch is an underutilized crop mainly cultivated in marginal soils for grain and straw use in animal production (Larbi *et al*., 2011; El Fatehi & Ater, 2017). However, this legume has a high capacity for fixing nitrogen (Romanyà and Casals, 2020) and is highly drought tolerant (some ecotypes were more constant in biomass and grain yield production across all irrigation levels) (Ghanipour Govarki *et al*., 2019). Considering the effect of climate change on agriculture, the adaptability of bitter vetch could help farmers achieve feed security.

Many studies have been focused on the nutritional value of grains and straw, especially on proteins, free amino acids, fatty acids, and anti-nutritional factors of whom polyphenols (Berger *et al*., 2003; Kaplan *et al*., Larbi *et al*., 2011, 2014; Irakli *et al*., 2018). Bitter vetch grains were used directly for feeding sheep (Abdullah *et al*., 2010) or after different processings for poultry (Sadeghi *et al*., 2009).

The analysis of genetic diversity is important for deciphering the nature and the magnitude of the variability between traits for an efficient selection of ecotypes. This evaluation for bitter vetch ecotypes was done using molecular techniques or agro-morphological traits (Larbi *et al*., 2011; Livanios *et al*., 2018a) or combining both (El Fatehi *et al*., 2014; Russi *et al*., 2019). In Morocco, only two studies (El Fatehi *et al*., 2014; 2016) about genetic characterization were realized under greenhouse conditions but without nutritional value assessment. Nevertheless, the bromatological analysis could determine better ecotypes to use in animal production.

This study aimed thus to determine the ecological, agro-morphological, and nutritive value (grain and straw) of seventeen north Moroccan bitter vetch ecotypes and the possible link to the original environment. This characterization could help to revalorize performant ecotypes as feed and improve biodiversity by enriching the Moroccan bitter vetch seed bank.

Results

Ecological characterization. The soil results concerning the collection and experimental sites are shown in Table 10. The collected ecotypes of bitter vetch were found on farms with altitudes varying from 219 to 1154 m. Concerning edapho-climatic parameters, bitter vetch ecotypes were sampled from neutral (6.5) soils to highly alkaline (8.7) ones on farm sites. The mean electrical conductivity varied from 5.4 to 98.4 mS/m, OM from 0.9 to 5.4%, and total limestone from 0.5 to 6.9%. For soil minerals, exchangeable potassium varied from 113.0 to 480.7 ppm, available phosphorus from 0.4 to 1.2 ppm, and nitrogen from 0.1 to 0.2%. All the parameters in the collection farms were comparable to experimental sites in the two years, except for total limestone, exchangeable potassium, and available phosphorus, which were lower.

For mean textural composition, the soil of the collection farms presented a predominance of coarse silt (62%) and a lower presence of fine and coarse sand (0.7 and 1.6%, respectively). The average annual temperatures (BIO1) of the 2019 and 2020 years were close (18.1 and 18.5°C, respectively). The long-term isothermality (BIO3) was 33.3% lower than the mean isothermality of the two experimental seasons. The long-term maximum temperature of the warmest month (BIO5) was lower than for the two studied seasons (33.0 vs. 38.2°C), while the minimum temperature of the coldest month (BIO6) was higher (4.6 vs. 1.2 °C). The two tested seasons' temperature annual range (BIO7) increased by 41% compared to the long-term range. On the other hand, 2020 was 31% wetter than 2019 (563 and 737 mm, respectively), and they were within the defined limits of the long-term precipitation range of the collection sites (BIO12), varying between 422 and 947 mm for 1950-2000 period.

Agro-Morphological characterization. *Qualitative variables*. The frequencies of the different qualitative traits of the tested plants are reported in Table 10. About 82% of the plants in the present study showed high germination percentages. Half the plants showed a semi-erect growth habit, while only 13% were prostrate. Almost 80% of plants did not have a pigmented stem. The pattern of testa was absent for almost all plants (more than 80%), while lower proportions of grains were marbled, spotted, or with no testa. The plants showed a high variability of grain testa colors, with one-third having light brown color and less than 1% showing greenish orange color. Some grains had the brown or black color of the pattern, close to 10% each. More than 80% of the grains had a pyramidal shape.

Quantitative variables. The results revealed that all the agro-morphological parameters of bitter vetch were significantly ($P < 0.01$) influenced by the ecotype (Table 11). The year had a significant effect ($P < 0.05$) on almost all parameters except the number of primary branches, stem diameter, leaf width, and number of pods per plant. Indeed, during the second year, plant height and leaf length increased by 12% and 8%, respectively, while leaf number and root length decreased by 9 and 37%, respectively. For grain yield parameters, grain per plant and grain yield increased by 15% and 22%, respectively, in 2020. Except for the leaf width, the phenological stage influenced all parameters. So, from the start of flowering to the pod setting, the number of total branches decreased by 3%. Plant height, internode number, and leaflet number increased by almost 10%, while the stem diameter and leaf number increase exceeded 30%. The effect interactions were significant ($P < 0.05$) for many parameters.

Genetic parameters are also reported in Table 11. The phenotypic variance (PCV) was slightly higher than the genotypic variance (GCV) in all the traits. Harvest index showed the highest PVC (30.2%) and GCV (30.5%), while the lowest values were reported for full flowering (3.4 and 3.3%, respectively). Several parameters, including flowering duration, root length, and grain yield-related parameters (grains per pod and per plant, pod number, grain yield, and harvest index), were characterized by a GCV and PCV higher than 20%. Almost all studied parameters presented a heritability estimation higher than 90.0%. The trait harvest index (61.5%) had the highest genetic advance as a percentage of the mean (GAM), while the lowest value was met for the full flowering trait (6.6%).

Bromatological characterization. For grains, the difference between ecotypes was significant ($P < 0.05$) for all bromatological parameters except for DM (Table 13). The effect of year was significant ($P < 0.05$) for all parameters except for DM, ADL, NFE, and DPPH. During the second year, grain fibers decreased (NDF by 9.9%, ADF by 9.3%, and CF by 17.0%). The second year was also characterized by a decrease in ash content (40.7%), ME (1.5%), and DPPH antioxidant activity (25%). Interestingly, CP and EE increased during the second year by 13.0 and 7.1%, respectively. Grain in vitro digestibilities slightly increased during the second year (CP by 2.6%, OM by 1.6%, and true by 3.3%). For straw, the difference between the ecotypes was also significant ($P < 0.05$) for all parameters except for DM and ADL. Unlike grains, straw fibers increased during the second year (NDF by 8.3%, ADF by 14.2%, and CF by 6.8%). The nitrogen-free extract increased by 2.9%, while CP and EE decreased by 28.8 and 7.7%, respectively.

According to genetic parameters, bromatological variability was lower compared to agro-morphological one. Only GCV and PCV of DPPH and FRAP antioxidant activities were higher than 20% for grains. The parameters such as NDF, ADF, CF, ash, total and condensed tannins showed GCV and PCV between 10 and 20%, while most parameters showed values lower than 10%. The heritability estimation was higher than 75% for all the parameters except for DM. The DM and IVTD traits showed the highest GAM (92.8%), while the lowest value was observed for the EE trait (1.4%). Compared to the grains, in a general way, the straw showed weaker coefficients of variation. On the other hand, like for grains, the heritability of the straw bromatological parameters was higher than 70%, except for DM and ADL, and the highest and lowest GAM were also DM (89.4%) and EE (1.2%), respectively.

Correlation analysis. The significant correlation coefficients for the vegetative parameters, yield components, and bromatological values of bitter vetch grains are shown in Figure 18. Plant height was significantly correlated to leaf length (r = 0.74) and number (r = 0.53), pod length (r = 0.53), thousand seed weight (r = 0.55), EE (r = 0.48), and ADL (r = -0.58). Leaflet number was correlated to CP (r = 0.51) and leaf number (r = 0.71). The results showed a significant positive correlation between grains per pod with pod number (r = 0.74) and grains per plant (r = 0.69). Pod length was correlated with NFE (r = 0.52), plant height (r = 0.53), and CP (r = -0.53). The NFE content was negatively correlated to CP (r = -0.92). Concerning phenology parameters, flowering duration was negatively correlated to the start of flowering (r = -0.57) and positively to FRAP (r = 0.49) and pod setting (r = 0.50, $p < 0.05$). Days to start flowering was negatively correlated to grain yield (r = -0.50). The ME was positively correlated to ash (r = 0.53), EE (r = 0.60), and OM digestibility (r = 0.72).

Principal component analysis. The principal component analysis (PCA) was calculated for 38 quantitative agro-morphological and bromatological parameters of the grains. The first three components explained 60.01% of the variability between bitter vetch ecotypes. Figure 19 represents the distribution of variables and individuals in the first two dimensions. The first dimension (Dim1) explained 26.9% of the total variation with a maximal contribution of plant height (0.78), leaves length (0.77), leaf (0.60) and leaflet number (0.60), stem diameter (0.60), and grain yield (0.71). The second dimension (Dim2) accounted for 19.8% of the total variation, and the traits with the greatest weight on this component were pod number (-0.76), grains per plant (-0.62), FRAP (0.57) and, phenol (0.63) and ash (0.62) contents.

Heatmap analysis. A heatmap was conducted to cluster the ecotypes based on the agro-morpho-phenological and bromatological parameters (Figure 20). The heatmap analysis structured the dendrogram on the left side of the figure according to bitter vetch ecotypes, and the second dendrogram at the top side showed the parameters that contributed to the clustering. The Heatmap described five clusters represented in Figure 3 by the five colored circles. The variables were divided into four groups. The first group was related to yield parameters (grain yield, harvest index, grains per plant, pod number and length) and stem diameter, the second one to vegetative parameters (leaf length, leaf and leaflet number, and plant height) and thousand seed weight, the third one to some bromatological parameters (phenols, FRAP, CT, ash, ME), and the fourth one to the ADL.

Mantel test. The Mantel test was conducted with the edapho-climatic, agro-morphological, and bromatological data. It revealed that no significant correlations were found between morpho-pheno-agronomic and bromatological parameters and geographical data of the collection sites of the ecotypes (r= -0.06, P=0.69), nor with environmental data (r=0.16, P=0.14). The geographic distance and environmental data were also not correlated (0.04, P =0.31).

Discussion

The environmental evaluation is an important tool for formulating conservation policies that naturally lead to more effective exploitation and utilization of the targeted ecotypes (Engels & Thormann, 2020). The tolerance to a particular temperature range is one of the most important traits used to explain the geographic distribution of a species (Willi & Van Buskirk, 2022). During the trial, the annual temperature was comparable to the optimal long-term average required for the growth and development of the bitter vetch ecotypes. Isothermality quantifies how large the day-to-night temperatures oscillate relative to the annual oscillations (O'Donnell & Ignizio, 2012). Some species were reported to be influenced by larger or smaller temperature fluctuations within a month relative to the year (Renzi *et al*., 2023). The long-term isothermality was 33.3% lower than the mean isothermality of the two growing seasons. It is obviously due to climate change effects and could influence the cropping of bitter vetch ecotypes in the long term. On the other hand, the second year was 31% wetter than the first, but they both stayed within the long-term precipitation range of the collection sites for the 1950-2000 period. The rainfall recorded in the sampling and cultivation sites is 300 mm higher than the ones reported for some bitter vetch fields in other Mediterranean countries (Abd El Moneim & Ryan, 2004). The collected ecotypes were found on altitudes varying from 219 to 1154 m. Hassanpour & Sahhafi (2020) reported crops reaching an altitude of 2496 m in Iranian landraces. Some authors observed that ecotypes at high altitudes might exhibit frost and cold tolerance (Abd El Moneim, 1993).

The large pH range of the sampling sites (6.5 to 8.7) was similar to results reported for different Vicia species in Algeria (Issolah *et al*., 2022). In the same study, Vicia species were found on saline soils, while in the present study, the mean electrical conductivity shows that bitter vetch can grow on a large scale of salinity ranging from low saline (5.4 mS/m) to highly saline (98.4 mS/m) soils. The otherchemical edaphic parameters were either lower, in the range, or higher than values reported in the literature showing great adaptability of bitter vetch. For mean textural composition, the soil of the collection farms had a predominance of coarse silt (62%). The related distribution of bitter vetch ecotypes to coarse silt could be because it promotes water retention and reduces air circulation (Bünemann *et al*., 2018). The variability between collection sites regarding edapho-climatic parameters provided an opportunity to study the impact of the agro-climatic conditions of the collection sites on the pheno-agro-morphological variability of the ecotypes.

Table 10: Climatic and physico-chemical characteristics of the soils of the 17 collection farms and the experimental cultivation site in Northern Morocco.

	Collection farms							Experimental site	
	Mean	Minimum	Maximum	Standard deviation	1st quartile	Median	3rd quartile	2019	2020
Altitude (m)	526.6	219	1154	281.1	351.3	556.5	611.0	132	127
Edaphic parameters									
Humidity (%)	5.4	3.9	9.2	1.3	5.0	5.9	6.6	8.6	10.4
pH water	8.1	6.5	8.7	0.6	8.0	8.2	8.6	8.0	8.2
pH KCl	7.6	5.3	8.4	0.9	7.5	8.0	8.1	7.1	7.4
Electrical conductivity (mS/m)	46.3	5.4	98.4	32.5	14.2	39.4	74.0	29.0	36.0
Organic matter (%)	3.3	0.9	5.4	1.6	2.0	3.4	4.5	2.2	2.6
Total limestone ($CaCO_3$, %)	2.2	0.5	6.9	2.1	0.8	1.1	2.8	7.4	3.9
Exchangeable potassium (ppm)	189.4	113.0	480.7	89.1	136.9	150.8	213.5	705.3	367.7
Available phosphorus (ppm)	0.7	0.4	1.2	0.3	0.4	0.5	0.8	28.6	26.9
Carbon to nitrogen ratio	11.5	3.6	20.5	5.4	6.6	12.6	15.8	6.4	8.0
Nitrogen (%)	0.2	0.1	0.2	0.0	0.2	0.2	0.2	0.2	0.2
Clay (%)	24.3	17.9	31.8	4.2	20.4	25.8	27.1	45.9	49.8
Coarse sand (%)	1.6	0.5	7.1	1.7	0.9	1.0	1.3	1.6	2
Fine sand (%)	0.7	0.4	1.0	0.2	0.5	0.6	0.7	2.3	1.2
Coarse silt (%)	62.8	50.3	75.2	8.4	58.5	61.2	72.6	37.2	34
Fine silt (%)	10.7	4.8	21.2	5.0	6.1	10.3	11.9	13	13
Bioclimatic parameters									
Average annual temperature (BIO1, °C)	17.1	13.7	19.0	1.4	16.4	17.2	17.9	18.1	18.5
Average diurnal variation (BIO2, °C)	11.4	9.8	12.2	0.6	11.4	11.5	11.8	22.9	20.4
Isothermality (BIO3 = BIO2/BIO7×100, %)	40.3	39.4	41.4	0.6	39.9	40.2	40.6	57.3	51.1
Temperature seasonality (BIO4)	589.0	518.2	618.2	29.0	567.4	595.8	611.7	542.1	520.4
Maximum temperature of the warmest month (BIO5, °C)	33.0	30.2	35.0	1.7	31.0	33.4	34.3	40.7	35.7
Minimum temperature of the coldest month (BIO6, C°)	4.6	1.5	6.8	1.3	4.1	4.9	5.2	0.7	1.6
Temperature annual range (BIO7 = BIO5-BIO6, °C)	28.3	24.7	30.0	1.4	27.8	28.8	29.3	40.0	39.9

Average temperature of the wettest quarter (BIO8, °C)	10.4	6.9	13.3	1.6	9.9	10.6	10.9	16.2	14.6
Average temperature of the driest quarter (BIO9, °C)	24.6	21.3	26.5	1.5	23.8	25.0	25.8	21.8	22.33
Average temperature of the warmest quarter (BIO10, °C)	24.7	21.4	26.5	1.5	23.9	25.0	25.8	24.4	25.3
Average temperature of the coldest quarter (BIO11, °C)	10.2	6.9	12.3	1.4	9.7	10.6	10.9	11.8	12.8
Annual precipitation (BIO12, mm)	701.2	422.0	947.0	168.3	614.0	770.0	837.0	563.4	736.8
Precipitation of the wettest month (BIO13, mm)	117.5	63.0	164.0	34.3	92.0	135.0	144.0	152.0	232.8
Precipitation of the driest month (BIO14, mm)	1.1	0.0	5.0	1.6	0.0	0.0	1.0	0.0	0.0
Precipitation seasonality (BIO15, mm)	71.8	56.4	88.3	9.8	67.0	72.3	74.9	117.8	106.3
Precipitation of the wettest quarter (BIO16, mm)	318.7	175.0	466.0	99.1	257.0	358.0	398.0	129.8	141.4
Precipitation of the driest quarter (BIO17, mm)	16.5	9.0	29.0	5.4	13.0	16.0	18.0	0.1	1.5
Precipitation of the warmest quarter (BIO18, mm)	18.6	9.0	36.0	7.1	15.0	16.0	18.0	1.3	4.5
Precipitation of the coldest quarter (BIO19, mm)	317.2	175.0	466.0	98.2	257.0	358.0	398.0	44.9	51.6

Agro-Morphological characterization. *Qualitative variables.* Evaluating agro-morphological variation in bitter vetch ecotypes is decisive in determining the local ecotypes' adaptation, agronomic potential, and breeding value. About 82% of the cultivated ecotypes in the present study showed high germination percentages (>90%), and half had a semi-erect growth habit (Table 11). Similar results were reported for Greek and Italian landraces (Livanios *et al*., 2018a; Russi *et al*., 2019). The predominance of the erect and semi-erect habit (86%) could be due to the effect of selection pressure for the adaptation to harvesting machines. Nearly 80% of plants did not have a pigmented stem, which could indirectly indicate lower tannin content (Smýkal, 2014). The ecotypes showed a high variability of grain testa colors, with one-third having light brown color and less than 1% showing greenish orange color. Some ecotypes had a brown or black color of the pattern, close to 10% each. Stem pigmentation and grain color were reported as indicators of the presence of some plant secondary components, including tannins (Mirali *et al*., 2016). More than 80% of the ecotypes have no pattern of testa and pyramidal shape of grains. The predominance of a characteristic for most of the parameters could be due to the exchange of seeds between regions or to the farmer selection.

Table 11: Frequencies of the different qualitative parameters considered to describe the 17 cultivated Moroccan bitter vetch ecotypes.

Traits	Classes	Frequencies
Germination percentage	High	82.35
	Moderate	5.88
	Low	11.76
Growth habit	Prostrate	12.55
	Erect	31.76
	Semi-erect	55.69
Stem pigmentation	Absent	78.82
	Present	21.18
Pattern of testa	Absent	83.14
	Spotted	4.71
	Marbled	2.35
	Combined	9.83
Color pattern of testa	Absent	81.57
	Black	8.63
	Brown	9.02
	Gray	0.78
Color of testa	Dark blue	3.53
	brownish red	7.06
	Bluish gray	1.57
	Light gray	17.25
	Dark gray	2.75
	Greenish gray	8.24
	Light brown	32.16
	Dark brown	19.61
	Orange-brown	5.49
	Light orange	1.57
	Greenich orange	0.78
Seed shape	Circular	4.31

	Conical	7.06
	Pyramidal	88.63

Quantitative variables. Exploiting the phenotypic diversity present in a species can lead to genetic improvement by plant breeders to benefit the farmers and enrich gene banks. The present study's mean values for plant height were below those mentioned for some Mediterranean ecotypes cultivated under green house conditions (26.3 vs. 52.6 and 50.4 cm) (El Fatehi *et al*., 2014; Russi *et al*., 2019) (Table 12). Also, the mean stem diameter was below the values reported for Italian ecotypes (2.3 vs. 3.5 mm) (Russi *et al*., 2019), probably for the same reason. However, it was in the range of 2.0 mm values reported for Moroccan ecotypes (El Fatehi *et al*., 2014).

Mean days to the start of flowering were lower than values reported for Syrian and Italian ecotypes (82 vs. 93 and 183 days, respectively) (Larbi *et al*., 2011; Russi *et al*., 2019). Nonetheless, the present ecotypes were late compared to other early Moroccan ecotypes conducted under greenhouse conditions (El Fatehi *et al*., 2014), possibly because higher temperatures for the latter ones could accelerate their start of flowering (Tun *et al*., 2021). Moreover, the present ecotypes had lower GDD to flowering than Spanish bitter vetch germplasms (González-Verdejo *et al*., 2020). It indicates that, possibly due to genetic differences between the ecotypes, they require less heat accumulation to flower.

Moreover, flowering duration was below 48 days recorded for the same study. The increased temperature under greenhouse conditions could also shorten the flowering duration (Nagahama *et al*., 2018). However, the present observations were above 5 to 19 days recorded for 49 populations in Greece (Livanios *et al*., 2018), showing an adaptation of the crop to diverse agroecological environments. The variability in the days to start flowering among the tested ecotypes being low, breeders could rather use the high variability for flowering durations to help them to select cultivars adapted to different regions of Northern Morocco and neighboring Mediterranean regions. Indeed, flowing duration is especially an adaptation to drought parameter (Kumari *et al*., 2021).

In the present study, yield parameters were intermediate between values reported for Moroccan and Greek ecotypes for pod number and number of grains per pod (El Fatehi *et al*., 2014; Livanios *et al*., 2018b). Pods were longer in the second year, seemingly explained by the higher grain yield. The pod number did not change over the two years, but grains per pod were higher in the second year. It corroborates the fact that the genotype of the plants influences pod number, while the environment influences grains per pod. Low annual rainfall in an arid environment can lead to high rates of abortion of pods after fecundation (Larbi *et al*., 2010). Consequently, grain yield, thousand seed weight, and harvest index were higher in the rainier second year than in the first.

Table 12: Descriptive statistics and genetic parameters of agro-morphological traits of 17 cultivated Moroccan bitter vetch ecotypes across phenological stages.

Traits	Mean	Min	Max	SEM	GCV	PCV	H²	GA	GAM	Years (Y)		Phenological stages (PS)			E	Y	E ×Y	PS	E× PS	Y× PS	E×Y ×PS
										2019	2020	SF	FF	PODS							
Number of primary branches	3.4	3.0	3.8	0.04	19.3	19.4	99.1	1.5	39.7	3.4	3.4	3.5	3.4	3.3	**	ns	ns	**	ns	*	ns
Plant height (cm)	26.3	20.4	32.1	0.34	10.9	11.1	97.8	5.9	22.3	24.8	27.8	25.5	28.2	25.3	***	***	***	***	***	***	***
Stem diameter (cm)	2.3	2.0	2.6	0.02	9.8	11.6	71.4	0.0	17.1	0.2	0.2	0.2	0.3	0.2	***	ns	***	***	***	**	***
Internode number	3.4	3.0	3.8	0.03	5.1	5.6	82.2	0.3	9.5	3.4	3.5	3.3	3.6	3.4	***	*	**	***	***	ns	***
Leaf width (cm)	1.9	1.0	2.2	0.02	13.0	13.3	95.2	0.5	26.1	1.9	1.9	1.9	1.9	1.9	***	ns	ns	ns	***	ns	ns
Leaf length (cm)	10.1	9.6	10.9	0.05	4.1	4.2	95.9	0.8	8.2	9.7	10.5	10	10.4	10	***	***	***	***	***	ns	***
Leaflet number	23.9	21.0	26.4	0.14	5.9	5.9	97.6	2.9	12.0	23.6	24.1	22.4	24.9	24.3	***	***	***	***	***	ns	***
Leaf number	35.1	23.9	46.3	0.64	15.3	15.7	95.4	10.8	30.8	36.7	33.5	31.7	40.6	33.1	***	***	***	***	***	***	***
Start of flowering (days)	82.1	77.7	93.3	0.49	3.5	3.6	96.2	5.8	7.1	82.4	81.7				***	*	***				
Full flowering (days)	91.0	86.3	97.7	0.46	3.3	3.4	94.2	6.0	6.6	91.4	90.5				***	*	***				
Pod setting (days)	105.7	98.0	113.5	0.47	4.1	4.1	99.5	9.0	8.5	106.5	104.8				***	***	***				
Flowering duration (days)	23.6	12.9	28.7	0.52	20.2	20.4	98.2	9.7	41.2	24.1	23.1				***	**	***				
Root length (cm)	4.4	2.8	6.4	0.08	15.2	16.8	82.1	1.3	28.4	5.4	3.4				***	***	***				
Pod length (cm)	1.6	1.5	1.8	0.01	5.5	5.6	93.8	0.2	10.9	1.6	1.6				***	*	ns				
Grains per pod	2.8	2.1	3.2	0.02	9.5	10.0	89.8	0.5	18.5	2.7	2.8				***	***	***				
Pod number	21.3	12.8	31.3	0.32	23.7	24.8	91.3	9.9	46.6	21.1	21.4				***	ns	*				
Grains per plant	51.2	33.5	84.7	0.87	24.6	25.8	90.9	24.7	48.3	48.0	55.1				***	***	ns				
Harvest index (%)	33.6	16.3	48.1	1.01	30.2	30.5	97.9	20.7	61.5	32.7	34.5				***	*	ns				
Thousand seed weight (g)	31.6	29.4	35.0	0.20	5.2	4.8	94.6	3.3	10.5	31.1	32.1				***	***	***				
Grain yield (T/ha)	1.002	0.457	1.350	0.028	25.2	25.5	97.4	0.5	51.2	0.934	1.070				***	***	**				

Min, minimum; *Max*, maximum; *SEM*, standard error of the mean; *GCV*, genotypic coefficient of variation (%); *PCV*, phenotypic coefficient of variation (%); *H^2*, broad-sense heritability (%); *GA*, genetic advance; *GAM*, genetic advance as a percentage of the mean (%); SF, start of flowering; FF, full flowering; PODS, pod setting; *E*, ecotype. n.s; *; ** and *** represent non-significant; significant at $P < 0.05$; $P < 0.01$; and $P < 0.001$; respectively.

Except for plant height, which increased by 12% in the second year, the other morphological parameters did not exceed an 8% increase. Similarly, phenological parameters varied little between the first and second years. Moreover, an increase of 15% in grains per plant and 22% in grain yield was recorded in the second year. The slower increase in these yield parameters despite the differences in rainfall (175 mm) is manifested by the higher heritability and could be due to the resistance of the ecotypes to a slight lack of water during the first year and, thus, to a low influence of the environment on these parameters.

PCV and GCV of the studied parameters offer an opportunity to select phenotypes based on these traits. Genotypic and phenotypic coefficients of variation (GCV and PCV) were categorized as low (0-10%), moderate (10-20%), and high (>20%), as indicated by Chithra *et al*. (2022). Flowering duration, root length, and grain yield-related parameters (grains per pod and per plant, pod number, grain yield, and harvest index), were characterized by high GCV and PCV. The wide genetic diversity for these parameters provides an opportunity to improve grain yield potential, which is the main objective of breeding programs. On the other hand, those genetic parameters were low for all the other morphological parameters. This result is most likely due to bitter vetch's self-pollination, which lowers its diversity (Zohary & Hopf, 2000). Moreover, the small differences between GCV and PCV in almost all parameters indicate that phenotypic variability is a reliable measure of genotypic variability and that selection for improvement of all traits is possible and could be effective on a phenotypic basis. Similar results were reported in Iranian ecotypes about lower GCV and PCV of days to start flowering, plant height, and thousand seed weight (Hassanpour & Sahhafi, 2020).

Heritability is categorized as low (0-30%), moderate (30-60%), and high (60% and above) (Chithra *et al*., 2022). The heritability estimates were high for almost all parameters in the present study. High heritability indicates a low influence of the environment on the expression of the characters. Therefore, breeders could select superior genotypes based on phenotypic performance for these traits. However, heritability alone does not indicate the amount of genetic improvement that would result from selecting individual ecotypes. High Heritability and high genetic advance (GA) are considered more accurate in predicting the gains via the selection of the parameters as they may be controlled by additive gene action in their expression (Sardana *et al*., 2007). According to Chithra et al. (2022), genetic advance as a percentage of the mean (GAM) is classified as low (<10%), moderate (10-20%), and high (>20%). Therefore, flowering duration, pod number, grains per plant, grain yield, and harvest index had moderate GAM and are probably governed by additive gene action (Sardana et al., 2007). Hence, simple selection could be effective for improving those characters. Similar results were reported for 210 pea germplasms (Sardana *et al*., 2007). However, the heritability estimates in the present study were higher than the values reported for Iranian ecotypes (Hassanpour & Sahhafi, 2020), presumably due to the lower effect of the environment on the expression of the traits.

Bromatological parameters. Analyzing the nutritive value of grains and straw could enhance the selection process of better ecotypes for animal production. Moreover, describing the influence of genotype and environment on bitter vetch bromatological characteristics is important to help identify the most suitable conditions for accumulating beneficial compounds in the grain. Following descriptive statistics, a decrease in fibers and ash contents and an increase in CP and EE contents, and different digestibilities were reported for grains in the second year. It could be due to the higher rainfall in the second year that promoted the accumulation of carbohydrates instead of fibers (Panozzo & Eagles, 1999). However, the decrease in the ash content could be due to a dilution effect caused by the competition for minerals induced by the higher yield in the second year (Murphy *et al*., 2008). The nutritive value of the straw showed the opposite trend of grains, with an increase in different fibers and a decrease in CP, CF, EE, ash, and NFE contents. It is presumably due to the intensive exportation of organic matter from leaves and stems to grains, as grain yield and harvest index were high in the second year.

According to several studies in the Mediterranean area, anti-nutritional factors can be the first limitation to bitter vetch grain use (Irakli *et al*., 2018; Sadeghi *et al*., 2009). In the present study, the phenols and condensed tannins (CT) in the grains were lower than those reported for Iranian bitter vetch grains (Golchin-Gelehdooni *et al*., 2014) (0.15 vs. 0.20 % DM and 0.14 vs. 0.23% DM, respectively). Due to these very low values, tannins are not the anti-nutritional factors responsible for the non-use of bitter vetch. For the nutritive value of the grains, the ecotypes in the present study had lower CP (22.9% DM) compared to other ecotypes from the Mediterranean area (26.6 - 28.0 % DM) (Larbi *et al*., 2011; Sadeghi *et al*., 2009). However, a maximal value of 26.8% was reported for one ecotype, highlighting the importance of selection. Interestingly, EE, NFE, and ash contents were higher than values reported for Iranian ecotypes (1.4 vs. 0.4, 66.0 vs. 58.9, and 4.3 vs. 3.4 % DM, respectively) (Sadeghi *et al*., 2009). For the studied straw, even if CP content was slightly higher than values reported for Syrian ecotypes (10.7 vs. 9.1 % DM), the OM digestibility was much lower (30.0 vs. 52.3%) (Larbi *et al*., 2011).

Table 13: Descriptive statistics and genetic parameters of bromatological traits grains and straw of 17 cultivated Moroccan bitter vetch ecotypes.

Traits	Mean	Min	Max	SEM	GCV	PCV	H^2	GA	GAM	Year		E	Y	E×Y
										2019	2020			
Grains														
Dry matter (%)	92.8	92.0	94.0	0.12	0.2	0.5	40.1	0.4	92.8	92.6	93.0	ns	ns	ns
Neutral detergent fiber (% DM)	23.0	17.7	27.2	0.40	11.5	11.9	96.4	5.5	23.0	24.2	21.8	***	***	***
Acid detergent fiber (% DM)	12.8	10.7	13.8	0.18	5.3	7.1	74.9	1.4	12.8	12.9	11.7	**	***	***
Acid detergent lignin (% DM)	5.5	4.1	8.4	0.15	17.5	19.7	88.7	2.0	5.5	5.5	5.5	*	ns	ns
Crude fiber (% DM)	5.4	4.1	6.8	0.11	10.7	12.1	88.3	1.2	5.4	5.9	4.9	***	***	***
Crude protein (% DM)	22.9	18.1	26.8	0.33	8.8	9.4	93.6	4.1	22.9	21.5	24.3	***	***	***
Ether extract (% DM)	1.4	1.2	1.8	0.03	9.5	11.8	80.4	0.3	1.4	1.4	1.5	**	*	**
Ash (% DM)	4.3	3.7	5.2	0.13	10.6	10.9	97.7	0.9	4.3	5.4	3.2	***	***	***
Nitrogen-free extract (% DM)	66.0	62.6	69.0	0.30	2.7	3.0	91.3	3.7	66.0	65.9	66.1	***	ns	***
In vitro crude protein digestibility (% DM)	54.4	50.4	61.3	0.28	4.4	3.8	96.7	4.8	8.9	53.7	55.1	***	***	***
In vitro organic matter digestibility (% DM)	89.0	84.7	93.5	0.37	2.5	2.8	89.3	4.5	89.0	88.3	89.7	***	*	**
In vitro true digestibility (% DM)	92.8	88.4	95.6	0.35	2.0	2.2	87.5	3.8	92.8	91.3	94.3	***	***	*
Metabolizable energy (MJ/kg DM)	12.9	12.0	13.9	0.07	2.7	3.5	75.4	0.7	12.9	13.0	12.8	***	*	ns
Phenols (mg TAE/100 g DM)	146.3	111.6	168.6	2.44	8.3	9.4	88.9	25.1	17.1	147.7	144.5	***	ns	***
Total Tannins (mg TAE/100 g DM)	144.7	100.6	179.4	2.61	10.7	10.7	99.8	31.7	22.0	143.5	144.3	***	ns	***
Condensed tannins (mg TAE /100g DM)	137.5	87.6	176.7	2.84	13.7	13.7	99.9	38.8	28.2	136.2	138.7	***	ns	***
DPPH (IC50 µg/mL)	0.324	0.218	0.503	0.016	20.0	20.5	97.4	0.1	41.1	0.352	0.298	***	***	***
FRAP (mg FeSO4/g DM)	0.207	0.094	0.319	0.006	26.1	26.2	99.6	0.1	53.7	0.202	0.208	***	ns	***
Straw														
Dry matter (%)	89.4	88.4	90.8	0.15	0.3	0.7	37.1	0.5	89.4	89.6	89.3	ns	ns	ns
Neutral detergent fiber (% DM)	64.3	58.2	70.7	0.60	4.7	5.3	89.2	6.3	64.3	61.8	66.9	***	***	***
Acid detergent fiber (% DM)	50.5	44.4	56.7	0.63	5.6	6.5	86.5	5.9	50.5	47.1	53.8	***	***	***
Acid detergent lignin (% DM)	18.6	15.8	19.9	0.20	2.5	5.2	47.1	0.9	18.6	18.5	18.6	ns	ns	ns
Crude protein (% DM)	10.7	8.7	16.6	0.29	16.1	17.0	94.6	3.5	10.7	12.5	8.9	***	***	**
Crude fiber (% DM)	51.4	43.3	56.1	0.47	7.2	7.7	93.9	7.7	51.4	49.7	53.1	***	***	***
Ether extract (% DM)	1.2	0.9	1.5	0.02	8.9	12.6	70.7	0.2	1.2	1.3	1.2	*	*	*
Ash (% DM)	6.5	4.1	9.1	0.20	16.5	19.4	85.3	2.2	6.5	6.8	6.3	***	ns	*
Nitrogen-free extract (% DM)	30.2	23.1	40.3	0.62	16.0	17.0	94.2	10.0	30.2	29.8	30.6	***	**	***
In vitro enzymatic Organic matter digestibility (% DM)	30.0	22.7	37.0	0.46	10.8	11.7	92.7	6.7	30.0	30.0	30.1	***	ns	ns
In vitro true digestibility (% DM)	40.9	28.8	47.7	0.68	10.2	11.4	89.2	8.6	40.9	40.9	41.0	***	ns	*

Metabolizable energy (MJ/kg DM)	3.2	2.6	5.0	0.06	14.2	15.4	92.2	0.9	3.2	3.1	3.3	***	ns	ns

Min, minimum; *Max*, maximum; *SEM*, standard error of the mean; *GCV*, genotypic coefficients of variation (%); *PCV*, phenotypic coefficients of variation (%); H^2, broad-sense heritability (%); *GA*, genetic advance; *GAM*, genetic advance as percentage of the mean (%); *DPPH*, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity; *FRAP*, Ferric reducing ability of plasma; *Phenols*, total phenols; *CT*, condensed tannins.

The genetic variability of the bromatological parameters of the grains and straw was lower than that of agro-morphologic parameters. Most of the bromatological parameters of the grains and straw presented medium to low variability. Otherwise, traits with reasonable variations present a wide opportunity for improvement (Olanrewaju *et al*., 2021). Thus, traits exhibiting low GCV and PCV show low variability; hence, they cannot be used to discriminate among the ecotypes for crop improvement. However, considering heritability and genetic advance, almost half of the grain and straw bromatological in this study showed both high heritability and genetic advance, which implies that a direct selection could be recommended because of the highly addictive gene effect.

Correlation analysis. The positive correlation between plant height, leaf length, and leaf number suggests the possible orientation of some ecotypes toward hay and fodder production. Similar correlations were reported for other species as those are photosynthesis-improving parameters (Amitrano *et al*., 2021). Crude protein is the first component looked for in a legume. The positive correlation between leaflet number and CP was also supported by several authors that reported a higher concentration of CP in leaves (Hakl *et al*., 2016). This latter strong correlation showed that future ecotype selection could use leaflet number during preliminary evaluation, as this trait is easier to measure than crude protein, which needs laboratory analysis using expensive chemicals and materials. Concerning phenology parameters, the negative correlation between flowering duration and the start of flowering was also expected, as it implies that late ecotypes tend to shorten their flowering duration, probably to escape drought at grain filling period, as soil humidity help to improve the translocation of organic matter to grains (Murphy *et al*., 2008). The negative correlation between the start of flowering and grain yield makes it clear that this adaptation could not be enough to avoid yield losses and that phenology is an important parameter to consider to choose the suitable ecotype for a particular region. Similarly, in several Mediterranean legumes (Livanios *et al*., 2018a), grain yield was expectedly positively correlated to the harvest index, as ecotypes in Morocco are cultivated solely for grain production (Enneking *et al*., 1995). The ME was positively correlated to EE and OM digestibility. These results are logical, as a more digestible feed results in less energy being lost in feces and more energy being available for use by the animal (Singh *et al*., 2017).

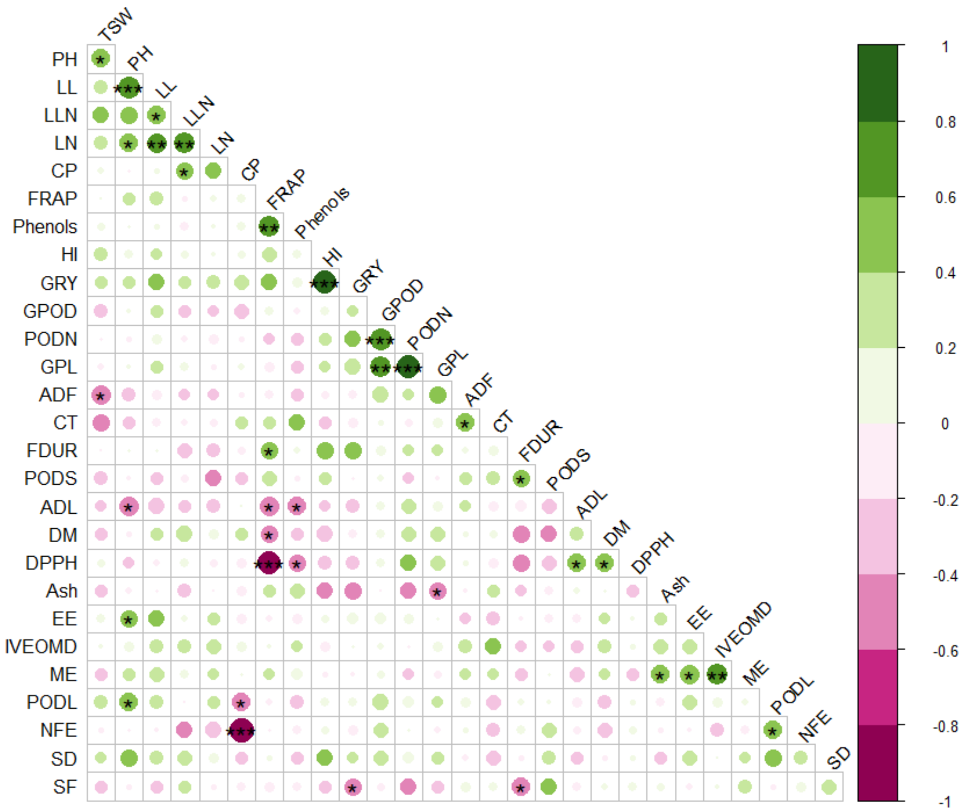

Figure 18: Correlation matrix of agro-morphological and bromatological traits evaluated in 17 Moroccan bitter vetch ecotypes.

The positive correlations are displayed in green and negative correlations in purple color. The color intensity and the size of the circle are proportional to the correlation coefficients. On the right side of the correlogram, the legend color shows the correlation coefficients and the corresponding colors. PH, plant height (cm); SD, stem diameter (mm); LL, leaf length (cm); LLN, leaflet number per leaf; LN, leaf number; FDUR, flowering duration (days); SF, days to start of flowering (days); PODS, days to pod setting (days); GPL, grains per plant; GPOD, grains per pod; PODN, pod number per plant; PODL, pod length (cm); HI, harvest index (%); GRY, grain yield (T/ha); DM, dry matter (%); ADF, acid detergent fiber (% DM); ADL, acid detergent lignin (% DM); CP, crude protein (% DM); EE, ether extract (% DM); Ash, (% DM); NFE, nitrogen-free extract (% DM); IVEOMD, in vitro enzymatic organic matter digestibility (% DM); ME, metabolizable energy (MJ/kg DM). Phenols, total phenols (mg TAE/g DM); FRAP, ferric-reducing antioxidant power (mg FeSO4g DM); DPPH, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity (EC50 µg/mL); CT, condensed tannins (mg/100g DM).

The principal component analysis is a multivariate statistical tool that aims to summarize and analyze the relationships between many variants and reduce the data's dimensionality while retaining all crucial information from the original genotype data set (Price *et al*., 2006). In the present study, 60.01% of the variability was explained by the first three components. In characterizing Greek ecotypes of bitter vetch, the first three components explained only 46.5% of the variability (Livanios *et al*., 2018b). The difference could be possibly due to their collection's lower inter-population levels and higher intra-population diversity levels. In the present study, yield contributing parameters, including grain yield, thousand seed weight, and pod length, are related to the first principal component, as in various legume studies (Hassanpour & Sahhafi, 2020; Livanios *et al.*, 2018b). According to this analysis, the first component concerned agronomical (plant morphology) and grain yield at plot level parameters. The second component was positively related to bromatological parameters (phenols, ash, and FRAP) and negative to grain at plant level dimension (grains per plant and pod number). The distribution of the ecotypes on the axis will contribute to easily choosing the ecotypes for preliminary selection. The ecotypes E2, E3, E4, E6, and E15 placed on the negative side of the first component should be avoided as they combine low vegetative and yield parameters.

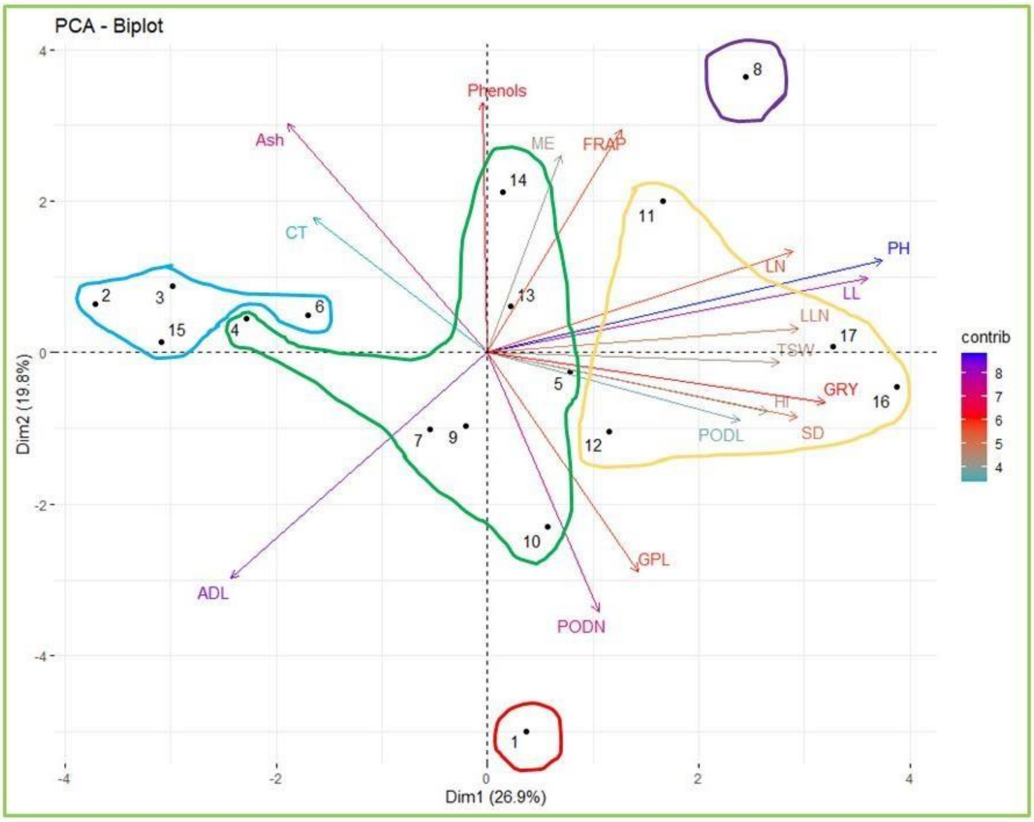

Figure 19: Graph of the variables and individuals of the principal component analysis.

PH, plant height (cm); SD, stem diameter (mm); LL, leaf length (cm); LLN, leaflet number per leaf; LN, leaf number; GPL, grains per plant; PODN, pod number per plant; HI, harvest index (%); TSW, thousand seed weight (g); GRY, grain yield (T/ha); ADL, acid detergent lignin (% DM); Ash, (% DM); ME, metabolizable energy (MJ/kg DM); Phenols, total phenols (mg TAE/g DM); FRAP, ferric-reducing antioxidant power (mg FeSO4/g DM); DPPH, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity (IC50 mg/mL); CT, condensed tannins (mg/100g DM). The arrow colors indicate the importance of the contribution to the two components. Five clusters were determined by the cluster heatmap analysis and represented via the five colored circles (red, green, purple, yellow, and blue) on this figure.

The Heatmap. The Heatmap is a data imagining practice that displays the extent of a phenomenon as color in two dimensions. The color variation gives the reader noticeable visual indications about how the phenomenon is clustered or varies over space. In genetic studies, the Heatmap is used to visualize the relative patterns of high-concentrated parameters against a background of mostly low-concentrated or absent parameters. Heatmap is a multi-information tool that allows plant breeders to develop varieties for specific agroecological zones and different purposes. In the present study, the first cluster composed of one ecotype (E8) was characterized by high vegetative parameters and nutritive value, and low grain yield components. This ecotype could be destined for hay production. Its high thousand seed weight makes it an interesting candidate to improve this parameter. However, its low grain yield and harvest index could hinder the selection. The second cluster had intermediate to high grain yield and vegetative parameters, while except for ecotype E11, they had low bromatological parameters. This interesting grain yield and vegetative parameters could classify this cluster as a dual-purpose group. Ecotype E11 of this group is probably better as it had high ash, metabolizable energy, phenols, and antioxidant activity. It appears to be more promising as it combines high-yield production and high-nutritive value. The third group was only composed of ecotype E1, characterized by a low nutritive value and a medium yield. The four ecotypes of the fourth cluster (E15, E3, E2, and E6) were the least interesting according to all the variable groups, except E2, which had high ME. Intermediate values for all the parameters characterized the fifth cluster, which was subclustered into two groups. Ecotypes E4, E13, and E14 were characterized by high grain yield, harvest index, phenols, and FRAP and low grain-yield components parameters, making them also important for selection. The opposite characterized the rest of the ecotypes.

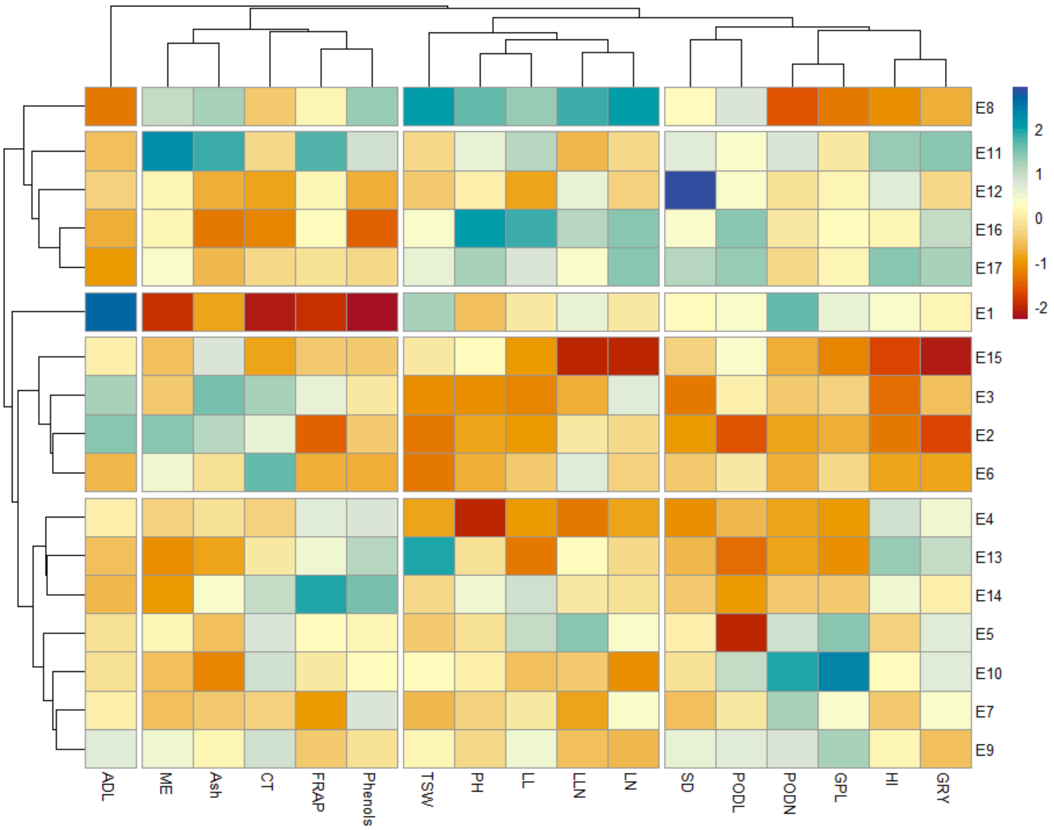

Figure 20: Heatmap and two-dimensional dendrogram of 17 Moroccan bitter vetch ecotypes.

The heatmap plot describes the relative abundance of each bitter vetch ecotype (rows) within each trait (column). The color code (blue to dark red) displays the values of the parameters: blue color indicates high values, while red indicates low values. The dendrogram (on the left) shows the hierarchical clustering of bitter vetch ecotypes based on the Euclidian distance and Ward's clustering method. Phenols, total phenols (mg TAE/ 100g DM); FRAP, ferric-reducing ability of plasma (mg FeSO4/g DM); CT, condensed tannins (mg TAE/100g DM); Ash (% DM); ME, metabolizable energy (MJ/kg DM); GRY, grain yield (T/ha); HI, harvest index (%); GPL, grains per plant; PODN, pod number per plant; PODL, pod length (cm); SD, stem diameter (mm); LN, leaf number; LLN, leaflet number per leaf; LL, leaf length (cm); PH, plant height (cm); TSW, thousand seed weight (g); ADL, acid detergent lignin (%).

Mantel test. The absence of correlation between morpho-pheno-agronomic and bromatological parameters, geographical data of the collection sites of the ecotypes, and environmental data could be due to the highly variable altitudes in the Rif Mountains and neighboring regions where the ecotypes were sampled. Moreover, the clustering of the ecotypes was independent of the region from which they were collected; it could be explained by the free exchange of seed materials between farmers, which led to gene flow among different regions. Scientists have discussed genetic drift, selection pressure, and environment as other major factors that could cause greater diversity than geographical distance (Star & Spencer, 2013). Therefore, more emphasis must be directed to the ecotypes level rather than the geographical level as a source of diversity in this collection.

Conclusion. The results of the present study showed that the cultivated ecotypes of bitter vetch showed higher variability for grain yield components compared to morphological and bromatological parameters. Despite the lower protein content, the grains presented more interesting digestibility and metabolizable energy than ecotypes from other Mediterranean countries. The calculated genetic parameters emphasized the possibility of selecting highly productive and nutritive cultivars. However, interannual variations were also detected, making the selection of the ecotypes harder. Despite the low variability, the multivariate analyses (principal component analysis and Heatmap) allowed the clustering of interesting ecotypes for animal production and bitter vetch selection programs. The multivariate analyses allowed to prefer the second cluster showing interesting grain yield and vegetative parameters, particularly the ecotype E11 with a better nutritive value. In addition to agro-morphological and bromatological characterization, future molecular analysis of this ecotype collection could help to discern similarities and therefore prevent duplication of similarly genetical ecotypes during the selection program. This study was repeated in two years. Therefore, multi-locational and multi-environmental trials should be used to evaluate and give precise locational responses for bitter vetch better crop improvement for the environment-dependent traits.

Materials and methods

All experiment and analysis methods followed relevant regulations and guidelines of Tangier's Regional Agricultural Research Center (INRA—Tangier, Morocco).

Plant material, study site, and experimental set-up. The plant material used was constituted of seventeen ecotypes of bitter vetch collected in July and September 2018, using a simple random sampling from seventeen farms in the north of Morocco. The sites were chosen to cover a maximum cultivation area. The seeds were collected after the harvest. In each farm, 3 kg of seeds were collected from the grain stock (Figure 21). The seeds were sown in 2019 and 2020 in the El Menzla experimental field (35°31’53’’N; 5°42’36’’W; 135 m), a related research station to INRA of Tangier, Morocco.

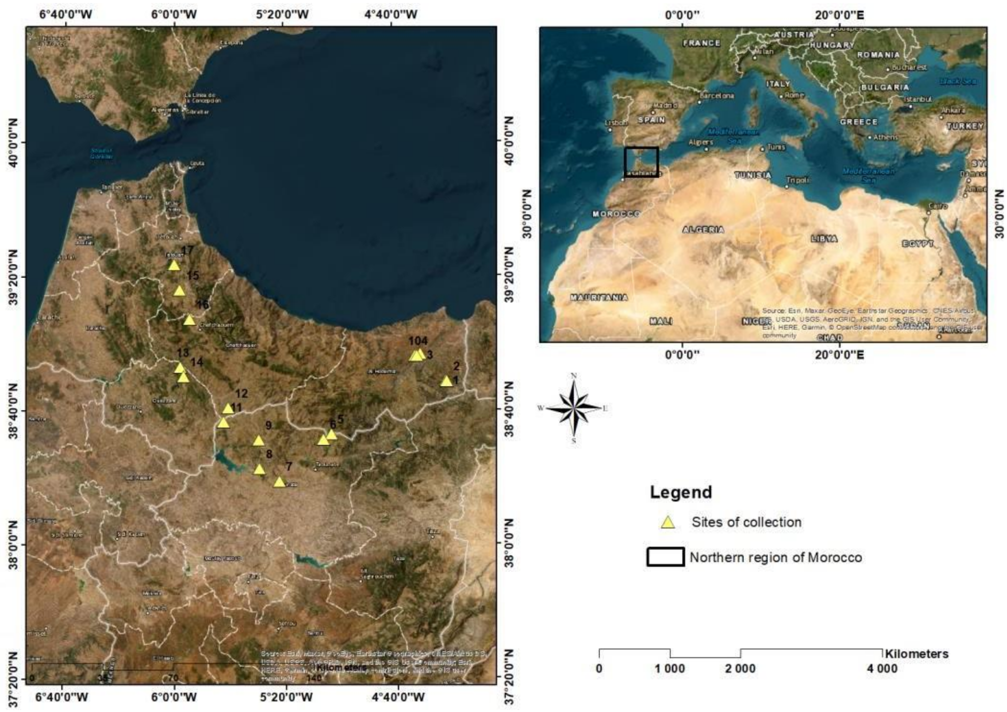

Figure 21: Collection sites of the 17 bitter vetch ecotypes in Northern Morocco (This map was generated using ArcGIS Desktop version 10.4.1 - Esri, Redlands, CA, USA, https://en.freedownloadmanager.org/Windows-PC/Portal-for-ArcGIS.html)

Ecological characterization. At the collection and experimental sites, soil samples were collected at the 0-20 and 20-40 cm horizons for physico-chemical analysis (Table 10). Soil samples were oven dried at 60°C until constant weight to determine soil humidity (%), then grounded and passed through a 2 mm sieve to remove larger particles. The pH (water and KCl) was read with a standard calibrated pH meter in 2:1 distilled water and dry soil ratio (Jackson, 1958). The nitrogen was determined by mineralization and distillation using the Kjeldahl method. A flame photometer analyzed exchangeable potassium in 1 N ammonium acetate extract (Toth & Prince, 1949). Available phosphorus was analyzed by the colorimetric method (Olsen *et al.*, 1954). The Electrical Conductivity (EC) was measured in the soil extract collected from the saturated soil paste by a conductivity meter (Wilcox, 1950). Total limestone ($CaCO3$) was measured by treating the sample with HCl (Nelson, 1983). Carbon was measured through dichromate oxidation and converted to Organic Matter (OM) by multiplying by a factor of 1.72 (Walkley & Black, 1947). Soil texture was determined by using the standard Pipette method and wet sieving (Robinson, 1922).

Farm collection site coordinates (latitude, longitude) were used to extract nineteen long-term bioclimatic data (BIO 1-19) for the 1950-2000 period from major climate databases (www.worldclim.org). The data were extracted from satellite images via ArcGIS Desktop version 9.3 (Esri, Redlands, CA, USA) and were interpolated with a resolution of approximately 1 km (Hijmans *et al*., 2005). The climatic data (minimum, maximum, and mean temperatures (°C), rainfall (mm) of each month, and total rainfall (mm)) of the 2019 and 2020 agronomical years at the experimental sites were collected from a climatic station 10 km nearer. The 2019 and 2020 bioclimatic variables (BIO 1-19) were determined based on these climatic data.

Experimental design. The study was set up under a randomized complete block design with three replicates. Ecotypes were sown on 17 January 2019 and 19 January 2020 in 2×3 m^2 plots with a density of 63 plants/m^2 with a spacing of 15 cm between lines and 10 cm between plants (i.e., 189 plants per ecotype a year). One meter was the distance between plots and between blocks. The trial was conducted under rainfed conditions on a fallow plot; NPK (10-30-10) fertilizer was applied at 50 kg/ha on the day of sowing. No irrigation was needed at sowing because rainfall occurred immediately after sowing. Natural weeds were removed manually during the growing season.

Agro-morphological characterization. *Phenological assessment.* On a whole plot basis, phenological observations were recorded every three days to determine days to start flowering (SF), days to full flowering (FF), and days to pod setting (PODS). Flowering duration (FDUR) was calculated through the difference between SF and PODS. The phenological stages were expressed in days and growing degree days (GDD) by considering the base temperature of 10°C (McMaster & Wilhelm, 1997). They were determined by the following formula:

$$\text{Accumulated GDD} = \Sigma\ [(T_{max} + T_{min})/2] - T_b\ (°C),$$

where Tmax is the daily maximum temperature (°C), Tmin is the daily minimum temperature (°C), and T_b is the base temperature (4°C as for *Vicia sativa* L. (Huang *et al*., 2021)).

Morphological assessment. Five plants in each plot were randomly chosen for the agro-morphological characterization at each phenological stage. Plant height (PH) was evaluated from the base to the tip of the plant. Stem diameter (SD) was measured at the third internode of each plant. Leaves number (LN), internodes number, and number of primary branches were counted on each plant. Leaflets number per leaf (LLN), leaves length (LL), and leaves width were measured from ten randomly selected leaves per plant.

Five plants in each plot were randomly chosen at the maturity stage to characterize the number of pods and grains per plant (PODN and GPL) and the root length. The grains per pod (GPOD) and the pod length (PODL) were assessed from ten randomly selected pods per plant.

Agro-Morphological assessment. All the remaining plants were harvested to determine grain yield (GRY) on a dry matter (DM) basis. Thousand seed weight (TSW) was recorded as the weight of one thousand seeds sampled thrice from bulked seeds in each plot. The straw was measured as the dry weight of the remaining above-ground plant parts. The harvest index (HI) was calculated as the ratio of GRY to straw.

Bromatological analysis. *Chemical composition.* The threshed grains and straw at maturity were dried, grounded, and sieved through a 1 mm sieve. They were evaluated according to AOAC (1990) to determine the content in DM by drying 5 g of the sample at 102°C until constant weight (method 934.01), ash by incinerating 5g of the sample at 550°C for 12h (method 942.05), and ether extract (EE) by extraction with diethyl ether in Soxhlet apparatus (method 963.15). The crude protein (CP) content was determined by multiplying the nitrogen content by 6.25, obtained after mineralization with H2SO4 and distillation with NaOH using the Kjeldahl method (method 977.02). Fiber content (crude fiber (CF), neutral detergent fiber (NDF); acid detergent fiber (ADF), and acid detergent lignin (ADL)) were analyzed using an ANKOM® 200 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA), following the method of AOAC (1990) and Van Soest *et al*. (1991). The nitrogen-free extract (NFE) was estimated using the following formula:

$$\text{NFE (\% DM)} = 100 - (\text{EE} + \text{CP} + \text{CF} + \text{Ash}).$$

Digestibility. *In vitro* enzymatic DM and OM digestibilities (IVEDMD and IVEOMD) were determined by the enzymatic method in a two-step method with first incubation of a 2% pepsin solution diluted in 0.1 N hydrochloric for 24h and second with solubilization in a buffer solution containing 1 g/L cellulase, both at 40°C (Aufrère & Michalet-Doreau, 1983). The metabolizable energy (ME; MJ/kg DM) was calculated using the equation (AOAC, 1990):

$$\text{ME} = 0.17 \times \text{IVEDMD} - 2,$$

where IVEDMD is the enzymatic dry matter digestibility in % DM.

In vitro true digestibility (IVTD) was determined by incubation of feed samples in filter bags in a Daisy II incubator ® (ANKOM Technology, Fairport, NY, USA) (Kowalski *et al*., 2014). Artificial saliva in a 1:5 ratio was mixed with collected rumen fluid from a slaughterhouse and added to heat-sealed ANKOM F57 filter bags containing samples and incubated at 39.5°C for 48h.

The in vitro enzymatic crude protein digestibility (IVECPD) was determined according to the procedure described by Aufrère & Cartailler (1988). Briefly, 1.0 g of ground sample was added to 50 mL of enzyme solution (0.1 g protease per 1L of borate–phosphate buffer; pH 6.8). Then, the tubes were sealed and incubated at 40 °C for 24 h under permanent stirring. Subsequently, samples were filtered, and residual N content was analyzed. The IVECPD was calculated according to the following equation:

$$\text{IVECPD (\%)} = \frac{(\text{Nsample} - \text{Nresidue})}{\text{Nsample}} \times 100,$$

where N_{sample} represents the sample's nitrogen content, and $N_{residue}$ represents the nitrogen remaining after digestion.

Antioxidant activity. One gram of flour from ground grains was extracted with 70 mL of 70% ethanol. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined according to the procedure mentioned by (Chaieb *et al*., 2011). Briefly, 100 µl of the extract was mixed with 100 µl of 0.1 mM DPPH in ethanol. The absorbance was read after a 30 min incubation in the dark against a blank at 517 nm at room temperature. The DPPH radical scavenging in percent (%) was calculated in the following way:

$$\text{Inhibition (\%)} = [A_{blank}-(A_{extract}-A_{sample})]/A_{blank} \times 100,$$

where A_{blank} was the absorbance of the control solution (containing only DPPH), $A_{extract}$ was the absorbance for the plant extract in the presence of the DPPH solution, and A_{sample} was the absorbance for the plant extract solution without DPPH solution. The IC50% value representing extract concentration providing 50% inhibition (IC50) was calculated from the graph plotted of inhibition percentage against sample extract concentration. The ferric-reducing ability of plasma (FRAP) of the bitter vetch grain extract was determined according to the method described by Chaieb *et al*. (2011). Samples (20 µL of extract) in a 96-well microplate were mixed with 1.2 mL of freshly prepared FRAP reagent (10 mmol/L TPTZ [2,4,6-tri (2-pyridyl)-triazine] in 40 mmol/L HCl, 300 mmol/L acetate buffer, and 20 mmol/L $FeCl_3 \cdot 6H_2O$, pH 3.6 in a:1:10:1 ratio). The solution was incubated in darkness at 25°C for 30 min. Absorbance was measured against a blank at 595 nm in a microplate reader. An aqueous solution of known ferrous sulfate concentrations was used for calibration, and the results were expressed in mg FeSO4/g of dry sample. The quantification of total phenols was performed according to the procedure described by Makkar *et al*. (1993) using the Folin–Ciocalteu reagent. Briefly, 0.25 mL of the ethanolic extract was mixed with 0.25 mL Folin-Ciocalteu reagent, 19.5 mL of distilled water, 1 mL of Na_2CO_3 (5%) was added. After vertexing the solution, the absorbance was read at 720 nm against a blank after one hour. Tannic acid (0-1 mg/mL) was used to obtain the standard curve. Results were expressed as mg TAE/g DM of grains. In another centrifuge tube, non-tannic phenols were determined by mixing 3 mL of the extract with 300 mg of polyvinylpolypyrrolidone and 3 mL of demineralized water. The tube was vortexed, kept at 4°C for 15 min, centrifuged at 4°C at 3500 rpm for 10 minutes, and then read at 725 nm. Total tannins were calculated as the difference between non-tannic phenols and total phenols. Condensed tannins (CT) were assayed by Porter *et al*. (1985) method using butanol-HCl and reading the absorbance at 550 nm.

Statistical analysis. Analysis of variance was carried out to test the years, ecotypes, and their interactions on agro-morphological and bromatological variation. Frequency distribution for different classes of qualitative traits was obtained using Excel. The variance components were estimated using a general linear model (GLM) by using SAS 9.4 version. To summarize and visualize the relationship between morphological, phenological, and agronomic parameters, a multivariate analysis was conducted using R software version 4.2.1. Principal component analysis (PCA) was performed using "FactoMineR and Factoextra" packages. A heatmap was created using the "Pheatmap" package, with Euclidean distance as the similarity measure and hierarchical clustering with complete linkage. Mantel test using "Tidyverse", "Vegan", and "Geosphere" packages to estimate the gene flow by correlating the genetic distance between ecotypes with the geographic and environmental one.

Phenotypic coefficients of variation (PCV), genotypic coefficients of variation (GCV), broad-sense heritability (H^2), genetic advance, genetic advance as a percentage of the mean (GAM), and genotypic and phenotypic correlation matrix were estimated following the formula given by Shariatipour *et al*. (2022). All genetic parameters were estimated using the following formulas:

$$\sigma^2 g = \frac{(MSg - MSe)}{r},$$

$$\sigma^2 p = \frac{MSg}{r},$$

$$GCV\ (\%) = \frac{\sqrt{\sigma^2 g}}{\bar{x}} \times 100,$$

$$PCV\ (\%) = \frac{\sqrt{\sigma^2 p}}{\bar{x}} \times 100,$$

$$H^2(\%) = \frac{\sigma^2 g}{\sigma^2 p} \times 100,$$

$$GA = H^2 \times k \times \sqrt{\sigma^2 p},$$

$$\text{GAM} = \frac{\text{GA}}{\bar{x}} \times 100,$$

where σ^2g was the genotypic variance, MSg was the mean square of genotypes, MSe was the mean square of error, r was the number of replications, σ^2p was the phenotypic variance, $\bar{x}$ was the general mean of the trait, and k was the selection differential, which is equal to 2.06 at 5% intensity of selection.

Data availability statement

The datasets presented in this study can be sent by a request to the authors.

Author contributions

C.M., C.J.-F., and C.A. received project funding. B.S., N.A., C.M., C.J.-F., and C.A. conceived and designed the experiment. B.S., N.A., and C.M. collected and provided germplasm for evaluation. B.S., N.A., and C.M. managed field experiments. B.S. and M.N. analyzed data. B.S. wrote manuscript. B.S. J.-F. C., M.C., J.-L. H., C.A., N.M., and A.N., read and reviewed the manuscript for final publication.

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Section expérimentale

Etude 4 :

Effects of Sulla Flexuosa Hay as Alternative Feed Resource on Goat's Milk Production and Quality Morocco

Chapitre 6. Etude 4 : Effects of Sulla Flexuosa Hay as Alternative Feed Resource on Goat's Milk Production and Quality Morocco

Préambule

La première étude dans le cadre de cette thèse, a montré le potentiel intéressant des écotypes de Sulla flexuosa en termes de rendements et de valeur nutritive, comparables à ceux de l'espère Sulla coronaria qui est déjà valorisée en production animale dans la région Méditerranéenne, et a conclu en la possibilité de son incorporation comme foin dans la ration des animaux. Avant d'introduire cette nouvelle ressource alimentaire dans la ration des caprins, il est indispensable de déterminer ses effets sur les performances de production et la qualité des produits animaux. Pour cela, l'objectif de cette étude a été de déterminer l'effet de l'incorporation du foin de Sulla flexuosa sur la production laitière et la qualité du lait des chèvres allaitantes. Pour atteindre cet objectif, 30 chèvres ont été réparties en 3 groupes homogènes de 10 animaux. Le foin a été introduit à deux niveaux, soit 35 ou 70 % (SF70), sur la base de la matière sèche ; il a remplacé partiellement ou totalement le foin de luzerne de la ration témoin. L'incorporation de Sulla flexuosa n'a pas affecté la production de lait ou sa composition physico-chimique. Cependant, la teneur en acides gras du lait a varié en fonction du pourcentage d'incorporation du Sulla flexuosa. Le régime SF70 était associé à une augmentation de la proportion des acides gras, C18:1n-9, C18:2n-6, C18:3n-3 et C22:6n-3 et des acides gras monoinsaturés, polyinsaturés et n-3 totaux. Par conséquent, les indices athérogènes et thrombogènes ont été améliorés. En outre, une meilleure capacité antioxydante dans le lait a été observée avec la ration SF70.

Les hypothèses étaient donc : Le Sulla flexuosa peut remplacer la luzerne sans effet négatif sur :

- La production ?
- La composition chimique ?
- Le profil des acides gras ?
- L'activité antioxydante ?

Section expérimentale

Etude 4 :

Animals, 2023, 13, 709.

Effects of Sulla Flexuosa Hay as Alternative Feed Resource on Goat's Milk Production and Quality Morocco

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Abstract

Sulla flexuosa (Hedysarum flexuosum L.) is an endemic legume growing in some Mediterranean areas in rainfed and cold mountainous conditions. It could be used in goat diets as an alternative protein source instead of alfalfa to supplement forest rangeland. This study aimed to test the effects of incorporating Sulla flexuosa (SF) hay in the diet of Beni Arouss goats on their milk production and quality. The hay was introduced at two levels, i.e., 35 or 70% (SF70), on a DM basis; it partially or totally replaced the alfalfa hay of the control diet. Sulla flexuosa incorporation did not affect milk production or physicochemical composition. However, milk FA content varied in proportion to the percentage of SF incorporation. The SF70 diet was associated with increased milk levels in C18:1n-9, C18:2n-6, C18:3n-3, and C22:6n-3 and total monounsaturated, polyunsaturated, and n-3 fatty acids. As a consequence, atherogenic and thrombogenic indices were improved. Additionally, better antioxidant capacity was observed in SF70.

Introduction

In Northern Morocco, in rural areas, the population generally lives under precarious conditions. Goat breeding is the main occupation of many people, since ancient times, and goat milk is always a fundamental part of their income, diet, and cultural heritage (Godber *et al*., 2016). In Morocco, goat milk reached 46953 metric tons in 2018 (FAOSTAT, 2021).

Nowadays, consumer interest in food with important nutritional and health value is increasing. Regarding dairy products, the demand for goat milk is rising, especially for its richness in calcium, phosphorus, and vitamins, which are necessary for bone mineralization (Diaz-Castro et al., 2017). Moreover, goat milk is characterised by low allergenicity due to its better digestibility relative to bovine milk (Verruck *et al*., 2019). Furthermore, goat milk presents a composition similar to breast milk, making it a better recommendation for breastfeeding newborns (Getaneh *et al*., 2016).

The goat diet in the Mediterranean region consists mainly of forest pastures (Chebli, *et al*., 2021b). In Northern Morocco, due to the overuse of silvopastoral resources by the local population (Chebli, *et al*., 2021b) and due to the recurrent drought period, (Lionello & Scarascia, 2018), rangelands are characterised by limited forage availability and seasonal and annual fluctuations (Chebli, *et al*., 2021b). Farmers have thus to supplement pastoral resources with conventional concentrates to meet the goat nutritional requirements. However, some concentrates compete with human food, present price volatility, and are expensive for the breeders. Using cultivated forage could reduce the amount of used concentrates up to the suitable forage-to-concentrate ratio, while ensuring animal productivity and could alleviate the pressure on the forest to sustain it.

In some Mediterranean areas such as Italy and Spain, the legume Sulla coronaria (*Hedysarum coronarium* L.) is typical of cereal-based crop rotations and is used as fresh forage, hay, and silage, and for grazing. It showed interesting results for milk production and cheese and their quality (Di Trana *et al*., 2015; Gannuscio *et al*., 2022; Ponte *et al*., 2022). In the region of Northern Morocco, Sulla flexuosa (Hedysarum flexuosum L.) is an endemic legume (Boukrouh *et al*., 2020) growing under mountainous rainfed and colder conditions. Few studies about its nutritive value were reported (Kadi *et al*., 2011). They showed promising results in crude protein content, adaptability and productivity and tannic and non-tannic phenol content.

To the authors' knowledge, the effect of Sulla flexuosa on goat milk production and quality has not yet been studied. What about its digestibility, the impacts of its tannins, and its fatty acid profile? This study aimed to analyse the effects of incorporating Sulla flexuosa as forage and as a local alternative feed resource into the goat diet on milk production and quality. Promising results could allow the spreading of this plant in the Mediterranean basin.

Results

Diets. The diets were statistically isoenergetic and isoproteinic (Table 14). The phenol and tannin contents increased with the rising incorporation of SF in the diets. The FA profile differed in the three diets.

Table 14: Chemical composition of the offered diets (n = 6 for each group).

	Co	SF35	SF70	SEM	p-value
Nutrient composition					
Dry matter (DM; g/kg)	904	901	900	1.043	0.597
Ash (g/kg DM)	87.2 [c]	103.8 [b]	112.1 [a]	0.362	<0.001
Crude protein (g/kg DM)	159	155	167	0.341	0.101
Ether extract (g/kg DM)	42.3	42.2	44.3	0.075	0.216
Neutral detergent fibre (g/kg DM)	491	489	479	1.621	0.356
Acid detergent fibre (g/kg DM)	331	346	365	2.534	0.486
Acid detergent lignin (g/kg DM)	97.5	103	106	0.255	0.250
Crude fiber (g/kg DM)	30.5	29.8	29.8	0.146	0.583
Nitrogen-free extract (g/kg DM)	207	225	197	0.313	0.112
Metabolisable energy (MJ/kg DM)	9.60	9.50	9.80	0.049	0.190
FMU (unit/kg DM)	0.90	0.90	0.89	0.009	0.070
PDIE (g/kg DM)	96.0	89.9	102	0.145	0.280
PDIN (g/kg DM)	101	104	108	0.264	0.110
RuProBal	-6	0	7		-
Total Phenols (g/kg DM)	15.8 [c]	25.6 [b]	40.5 [a]	0.467	<0.001
Hydrolysable tannins (g/kg DM)	1.09 [c]	5.94 [b]	12.4 [a]	0.109	<0.001
Condensed tannins (g/kg DM)	1.53 [c]	8.39 [b]	15.2 [a]	0.224	<0.001
Fatty acid composition of the diet (g/ 100g FA) (calculated from feedstuff fatty acid profile)					
C4:0	0.044	0.102	0.154		
C10:0	0.028	0.053	0.073		
C12:0	0.633	0.432	0.248		
C14:0	1.432	0.895	0.404		
C14:1	0.476	0.255	0.054		
C15:0	1.102	0.686	0.307		
C16:0	19.911	19.095	18.360		
C16:1	1.478	0.857	0.290		
C17:0	0.574	0.333	0.112		
C17:1	0.046	0.041	0.037		
C18:0	5.963	3.990	2.191		
9t-C18:1	0.346	0.199	0.065		
C18:1n-9	12.629	12.093	11.611		
C18:2n-6	30.060	27.798	25.746		
C18:3n-3	17.620	28.203	37.883		
C18:3n-6	0.351	0.251	0.159		
C20:0	0.921	0.633	0.371		
C20:1	0.538	0.354	0.187		
C20:2	0.388	0.264	0.127		
C20:3n-3	0.471	0.264	0.076		
C20:4n-6	0.333	0.238	0.127		
C20:5n-3	0.499	0.344	0.203		
C22:0	1.718	1.058	0.456		
C22:1n-9	0.416	0.344	0.253		
C24:0	1.496	0.899	0.355		
C24:1	0.527	0.317	0.152		

Co: control diet; SF35: diet with 35% Sulla flexuosa hay; SF70: diet with 70% Sulla flexuosa hay; FMU: Forage unit for milk production; PDIE: Digestible proteins in the intestines allowed by energy; RuProBal: Rumen Protein Balance.

Milk production and composition. Table 15 shows that the daily milk composition, production, and acidity results were not affected by SF incorporation into the diet. The effect of SF incorporation was nonlinear for all parameters. However, as expected, the effect of the lactation period was significant ($p<0.05$) on all composition parameters except for lactose, total solids and ash for the milk yield, and fat for milk production.

Table 9: Least square means of production, composition, acidity, and Net Energy of goat milk according to diet (n = 80 for each group).

	Co	SF35	SF70	SEM	*p*-value			
					Linear	Diet	Week	Diet × Week
Milk yield (kg/lactation)	62.2	64.2	68.1	0.202		0.788	-	-
Chemical composition (%)								
Fat	2.24	2.32	2.16	0.101	0.792	0.876	0.006	0.855
Proteins	3.46	3.30	3.42	0.079	0.866	0.781	<0.001	0.218
Lactose	5.11	5.14	5.06	0.126	0.850	0.960	0.116	0.034
Ash	0.840	0.836	0.775	0.003	0.306	0.547	0.089	0.830
Total solids	11.7	11.6	11.9	0.147	0.636	0.648	0.300	0.033
Milk production (g/day)								
Milk	663	654	590	17.5	0.474	0.742	<0.001	0.054
Energy corrected milk	532	497	456	0.083	0.296	0.667	0.003	0.094
Fat corrected milk	494	472	419	0.021	0.376	0.664	<0.001	0.033
Fat	15.3	14.0	12.2	0.224	0.356	0.656	0.073	0.100
Proteins	21.5	19.5	17.9	0.092	0.226	0.474	0.003	0.050
Lactose	34.1	34.7	31.7	0.208	0.675	0.865	<0.001	0.193
Total solids	77.3	74.6	60.6	0.301	0.361	0.428	<0.001	0.050
Ash	5.21	5.14	4.36	0.043	0.351	0.594	0.029	0.306
Net energy of milk (MJ/kg)	2.49	2.49	2.45	0.034	0.817	0.959	<0.001	0.409
pH	6.44	6.50	6.48	0.033	0.615	0.722	0.148	0.200
Acidity (°D)	16.8	15.7	15.8	0.021	0.161	0.216	<0.001	0.067

(1) Due to the unsynchronised kiddings, some goats could not be sampled at the beginning of the trial.

Milk fatty acid. The individual profiles, summaries, ratios, and indexes of milk FA according to the diet are given in Table 16. The gradual incorporation of SF into the diets was not systematically correlated to the results; the SF35 diet showed results either intermediate to the extreme diets or similar to one of them.

Table 16: Least square means and standard error of the mean of fatty acid profile (g/100 g FA) and summaries (g/100 g FA), ratios, and indexes in goat milk fat according to diet (n = 40 for each group).

	Co	SF35	SF70	SEM	*p*-value			
					Linear	Diet	Week	Diet × Week
Fatty acid profile								
C4:0	1.41	1.41	1.19	0.048	0.108	0.218	0.682	0.308
C6:0	2.11	1.95	1.99	0.074	0.606	0.742	0.277	0.232
C8:0	1.60	1.46	1.29	0.069	0.154	0.359	0.009	0.839
C10:0	5.83	5.37	5.92	0.125	0.821	0.330	0.084	0.456
C11:0	0.183	0.148	0.146	0.008	0.081	0.119	0.022	0.497
C12:0	2.56	2.74	2.38	0.093	0.462	0.501	0.251	0.083
C13:0	0.117	0.136	0.094	0.006	0.223	0.111	0.852	0.994
C14:0	9.52[a]	8.29[ab]	7.67[b]	0.186	0.007	0.020	0.416	0.498
C15:0	1.69[ab]	2.14[a]	1.41[b]	0.097	0.318	0.044	0.177	0.477
C16:0	21.2	21.0	22.3	0.221	0.159	0.211	0.335	0.030
C17:0	1.55[a]	1.10[b]	1.06[b]	0.064	0.006	0.006	0.366	0.466
C18:0	12.8[a]	12.3[a]	8.06[b]	0.289	<0.001	<0.001	0.179	0.112
C20:0	0.432[a]	0.433[a]	0.305[b]	0.016	0.004	0.007	0.708	0.476
C21:0	0.315[b]	0.423[a]	0.343[b]	0.012	0.395	0.005	0.963	0.775
C22:0	0.372	0.370	0.399	0.005	0.046	0.075	0.253	0.887
C23:0	0.131	0.136	0.129	0.002	0.735	0.572	0.838	0.384
C24:0	0.071	0.062	0.058	0.004	0.268	0.513	0.482	0.876
Total SFA	62.0[a]	59.4[b]	54.5[c]	0.408	<0.001	<0.001	0.343	0.336
C14:1	0.365[b]	0.410[b]	0.481[a]	0.012	<0.001	0.002	0.019	0.495
C15:1	1.32	1.53	1.44	0.067	0.582	0.569	0.230	0.022
C16:1	1.03	1.02	1.13	0.034	0.350	0.556	0.827	0.359
C17:1	1.56[a]	1.74[a]	1.12[b]	0.061	0.010	0.003	0.693	0.588
9t-C18:1	0.288	0.323	0.243	0.010	0.236	0.121	0.065	0.380
C18:1n-9	26.3[c]	28.7[b]	31.6[a]	0.342	<0.001	<0.001	0.014	0.187
C20:1	0.223	0.227	0.202	0.011	0.593	0.805	0.805	0.847
C22:1n-9	0.447[b]	0.431[b]	0.657[a]	0.020	<0.001	<0.001	0.741	0.474
C24:1	0.313	0.337	0.326	0.008	0.569	0.564	0.146	0.538
Total MUFA	31.8[b]	34.7[a]	37.1[a]	0.345	<0.001	<0.001	0.018	0.318
6t-C18:2	0.786[a]	0.392[b]	0.336[b]	0.067	0.012	0.017	0.150	0.050
C18:2n-6	1.81[b]	1.91[b]	2.85[a]	0.094	<0.001	0.001	0.240	0.059
C18:3n-3	1.34[b]	1.44[b]	2.73[a]	0.080	<0.001	<0.001	0.002	0.060
C18:3n-6	0.232	0.238	0.179	0.012	0.183	0.292	0.991	0.283
C20:2	0.403	0.380	0.393	0.011	0.752	0.743	0.442	0.473
C20:3n-3	0.255[b]	0.328[a]	0.372[a]	0.011	<0.001	<0.001	0.151	0.117
C20:3n-6	0.465[a]	0.350[b]	0.356[b]	0.011	<0.001	<0.001	0.090	0.202
C20:4n-6 (ARA)	0.334[a]	0.203[b]	0.202[b]	0.009	<0.001	<0.001	0.982	0.996
C20:5n-3 (EPA)	0.360	0.412	0.461	0.012	0.019	0.060	0.934	0.752
C22:2	0.188	0.175	0.230	0.007	0.072	0.068	0.734	0.630
C22:6n-3 (DHA)	0.054[b]	0.048[b]	0.075[a]	0.002	<0.001	<0.001	0.183	0.182
Total PUFA	6.19b	5.87b	8.10a	0.155	<0.001	<0.001	0.054	0.363
Summaries								
SCFA	10.4	10.2	10.4	0.165	0.260	0.284	0.233	0.261
MCFA	12.8[a]	11.7[ab]	10.8[b]	0.205	0.008	0.029	0.291	0.471
LCFA	76.2[b]	78.0[a]	78.7[a]	0.265	0.007	0.019	0.812	0.266
DFA	50.8	52.8	53.3	0.310	0.032	0.069	0.517	0.771
EPA+DHA	0.414[b]	0.461[a]	0.537[a]	0.012	0.006	0.023	0.968	0.840
n-3	2.01[b]	2.23[b]	3.64[a]	0.089	<0.001	<0.001	0.003	0.092

n-6	2.84^{b}	2.70^{b}	3.59^{a}	0.094	0.013	0.011	0.166	0.061
n-9	27.0^{c}	29.5^{b}	32.5^{a}	0.347	<0.001	<0.001	0.012	0.162
Ratios								
PUFA/SFA	0.100^{b}	0.099^{b}	0.149^{a}	0.003	<0.001	<0.001	0.152	0.292
MUFA/PUFA	5.51^{a}	6.12^{a}	4.69^{b}	0.135	0.038	0.004	0.004	0.698
UFA/SFA	0.619^{b}	0.687^{b}	0.838^{a}	0.012	<0.001	<0.001	0.444	0.428
LA/ALA	1.40	1.53	1.26	0.073	0.558	0.571	0.420	0.016
n-6/n-3	1.44	1.29	1.10	0.129	0.036	0.109	0.528	0.016
Indexes								
AI	1.65^{a}	1.41^{b}	1.23^{b}	0.030	<0.001	<0.001	0.281	0.310
TI	1.83^{a}	1.59^{b}	1.19^{c}	0.032	<0.001	<0.001	0.431	0.051
(C18:0+C18:1)/C16:0	1.88	1.99	1.82	0.030	0.608	0.305	0.339	0.047
Δ9C14	0.040^{b}	0.050^{b}	0.061^{a}	0.002	<0.001	<0.001	0.094	0.347
Δ9C16	0.046	0.046	0.048	0.002	0.564	0.837	0.840	0.286
Δ9C18	0.674^{b}	0.703^{b}	0.799^{a}	0.007	<0.001	<0.001	0.039	0.061

Co: control diet; SF35: diet with 35% Sulla flexuosa hay; SF70: diet with 70% Sulla flexuosa hay; SCFA: short-chain fatty acids; MCFA: medium-chain fatty acids; LCFA: long-chain fatty acids; MUFA: mono-unsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; DFA: desirable fatty acids; EPA: eicosapentaenoic acid (20:5n-3); DHA: docosahexaenoic (C22:6n-3); UFA: unsaturated fatty acids; AI: Atherogenicity index; TI: Thrombogenic index; Δ9C14 = (C14:1)/(C14:1 + C14:0) activity of Δ9 desaturase enzyme to convert C14:0 into C14:1; Δ9C16 = (C16:1)/(C16:1 + C16:0) activity of Δ9 desaturase enzyme to convert C16:0 into C16:1; Δ9C18 = (C18:1)/(C18:1 + C18:0) activity of Δ9 desaturase enzyme to convert C18:0 into C18:1; a,b,c: values followed by different letters in the same row differ statistically by Tukey's test at (p<0.05).

Myristic (C14:0), myristoleic (C14:1), pentadecanoic (C15:0), margaric (C17:0), margaroleic (C17:1), stearic (C18:0), oleic (C18:1n-9), linoleic (LA, C18:2n-6), trans-linoleic (6t-C18:2n-6), α-linolenic (ALA, C18:3n-3), arachidic (C20:0), eicosatrienoic (C20:3n-3), dihomo-gamma-linolenic (C20:3n-6), arachidonic (ARA, C20:4n-6), heneicosylic (C21:0), erucic (C22:1n-9), docosahexaenoic (DHA, C22:6n-3) acids were significantly affected by the diet (p<0.05). The SF70 diet significantly increased C14:1, C18:2n-6, C18:3n-3, C22:1n-9 and C22:6n-3 and decreased C14:0, C17:1, C18:0 and C20:0 proportions in milk fat compared to the Co and SF35 diets. The SF35 and SF70 diets increased C20:3n-3 and C21:0 and decreased C17:0, 6t-C18:2n-6, C20:3n-6 and C20:4n-6 proportions in milk fat compared to the Co diet. The lactation period also had a significant effect on some FA.

Concerning FA summaries, the SF diet significantly increased LCFA, MUFA, EPA + DHA, and n-9 and decreased SFA proportions compared to the Co diet. In addition, the SF70 diet significantly increased PUFA, n-3 and n-6 proportions compared to SF35 and Co diets.

For FA ratios, the SF70 diet significantly increased PUFA/SFA and UFA/SFA and decreased MUFA/PUFA compared to the Co and SF35 diets. The SF incorporation in diets did not affect the LA/ALA and n-6/n-3 ratios.

For indexes, the SF70 diet significantly increased Δ9C14 and Δ9C18 activities compared to the two other diets. The experimental diets decreased AI and TI indexes compared to the Co diet, with an SF70 higher effect for TI.

For linearity, a significant and nonlinear effect of SF hay incorporation level on FA profile was observed for C15:0 and C21:0. Besides, EPA, DFA and n-6/n-3 had non significant but linear effect of SF incorporation.

Milk antioxidant activity. For the antioxidant activity (Table 17), regarding TP and FRAP, SF70 showed higher values than Co and SF35 diets ($p<0.05$). Contemporary DPPH values were higher with both SF integrations (SF35 and SF70) than Co diet ($p<0.05$). The effects of SF incorporation were nonlinear.

Table 17: Least square means and standard error of the mean of total phenols and antioxidant activities of goat milk according to diet (n = 80 for each group).

	Co	SF35	SF70	SEM	*p*-value			
					Linear	Diet	Week	Diet × Week
Total phenols (mg GAE/L)	29.5^{b}	30.9^{b}	39.8^{a}	0.320	0.111	<0.001	0.030	0.064
FRAP (mmol $FeSO_4$/L)	0.758^{b}	0.762^{b}	1.103^{a}	0.002	0.164	<0.001	0.499	0.466
DPPH (%)	30.1^{b}	34.5^{a}	35.6^{a}	0.600	0.129	0.003	0.106	0.025

Co: control diet; SF35: diet with 35% Sulla flexuosa hay; SF70: diet with 70% Sulla flexuosa hay; FRAP: ferric reducing ability of plasma; GAE: gallic acid equivalent; DPPH: 2,2-diphényl 1-picrylhydrazyle inhibition.

Discussion

This study aimed to evaluate SF hay effects on milk production parameters in Beni Arouss goat, a dual-purpose (milk and meat) North Moroccan indigenous breed well known for its rusticity.

Milk production and physico-chemical composition. Overall, the daily and total milk yields of the Beni Arouss goat, which is a double-purpose breed (meat and milk), were lower than those of dairy goat breeds but close to those reported in the literature for local Beni Arouss and Draa goats (Boujenane *et al*., 2010; El Otmani *et al*., 2021a). The present results were nevertheless higher than values reported by El Otmani *et al*., (2021a) for the same breed and could be explained by higher dietary energy and protein proportions in this trial. Compared to the control diet, the experimental diets contained higher condensed tannin (CT) concentrations but below 3-5 % DM. Below this threshold, ruminal activity and feed digestion would not be impaired due to CT-protein-binding in the rumen (Rufino-Moya *et al*., 2019). Indeed, De Lucena *et al*., (2018) reported a general depressive effect in milk yield with high polyphenol concentrations in sheep and goat diets. The SF hay could thus be distributed to goats without such adverse effects. In the present trial, due to diet similarities regarding CP, metabolisable energy and all types of fibres, close data were observed for milk production and quality in all groups. The observed fat milk percentages (2.16 – 2.32%) were lower than values reported in the literature for goats (4.0% - 4.5%) (Lad *et al*., 2017) but similar to those of El Otmani *et al*., (2021a) with the same breed but very different diets. It seems thus that a low-fat proportion in milk characterises the Beni Arouss goat breed. The observed protein, lactose and ash proportions were similar to the averaged values reported by Lad *et al*., (2017) for goats. As expected, the milk composition varied according to the lactation stage. Thus, hay of SF did not negatively affect milk composition and production in Beni Arouss goats.

Milk fatty acids. Unlike milk production and composition, the milk fatty acid profile was significantly affected by the SF incorporation. Milk C14:0 is synthesised de novo in the mammary gland and milk C14:1 originates exclusively from the desaturation of C14:0 at this site (Lock & Garnsworthy, 2003). The Δ9C14 ratio is considered as the marker for delta-9 desaturase activity in the udder (de Lucena *et al*., 2018). The lower C14:1 proportion observed with the Co and SF35 diets was confirmed by the lower Δ9C14 ratio observed in the Co and SF35 groups compared to SF70, with respectively 0.045 vs. 0.061. Similar to the present results, Purba *et al*., (2020) and Buccioni *et al*., (2015) suggested that the dietary tannins can increase the expression of Δ9 desaturase activity, as observed in the SF70 group. Although the lack of significant difference between SF35 and Co groups, the significant and linear effect of the level of SF incorporation suggests that SF could have a dose dependent impact.

The present results showed a lower C17:0 proportion for the SF35 and SF70 compared to the Co diet (1.08 vs. 1.55 g/100g FA), respectively, for the SF35 and SF70 diets and the Co diet). The ruminal microorganisms synthesise C17:0 by elongation of propionate. Cabiddu *et al*., (2009) reported an increase of C17:0 percentage in sheep's milk receiving a PolyEthylen Glycol supplementation in the diet, this molecule having the ability to neutralise tannins. This lower C17:0 proportion in milk could be due to a ruminal microbiota modification, impairing the saturation process in the rumen (Dias Junior *et al*., 2023).

The smaller C18:0 milk proportion with SF70 (8.06 vs. 12.55 g/100 g FA, respectively, for the SF70 diets and both the Co and SF35 diets). The observation could be explained by different hypotheses: the lower C18:0 proportion in the SF70 diet, the modification of the ruminal microbiota and thus the biohydrogenation (as for C17:0) and the higher desaturation of C18:0 in C18:1 in the mammary gland as shown by the higher Δ9C18 activity.

The modification of the ruminal biohydrogenation process could also explain the higher C18:2n-6 and C18:3n-3 proportion in SF70 milk (Buccioni *et al*., 2015). Usually, around 90% of C18:3n-3 and C18:2n-6 are biohydrogenated in the rumen (Bernard *et al*., 2009). These two FA represented 51%, 57% and 69% of the observed PUFA proportion, respectively, for the Co, SF35 and SF70 diets. The highest level of SF was thus required to observe an effect. The C20:3n-6 and C20:4n-6 FA result from the C18:2n-6 elongations and desaturations. A lower proportion – not SF dose dependant - of these two FA in the SF35 and SF70 milk, despite a higher C18:2n-6 proportion in SF70 milk, is hard to explain. Sulla flexuosa could limit the activities of some enzymes. The capacity of conversion of C18:3n-3 to health-promoting n-3 Long Chain-PUFA (eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) is limited in human metabolism, which strengthens its dietary supply's importance. Like C18:2n-6, the C18:3n-3 milk proportion was greater in the SF70 group than in the two other groups (respectively, 2.73 vs. 1.39 g/100g FA). Although the milk concentration of DHA was also significantly higher in the SF70 group, the EPA milk proportions did not differ between the three groups. An absence of a parallel increase in n-3 and n-6 LCFA between groups was thus observed; tannin's impact on enzyme competition between n-3 et n-6 metabolism is not currently understood.

Fatty acids summaries, ratios, and indexes. The calculated FA summaries, ratios, and indexes of a foodstuff are usually considered indicators to the consumer for choosing a healthy diet. So, the long-chain n-3 and n-6 PUFA are considered as bio-regulators of important cellular processes and are associated with immune system functionality and development. The MUFA improve cardiovascular health by decreasing inflammation and reducing total and low-density lipoprotein (LDL)-cholesterol. The SFA have negative consequences on human health by increasing the danger of cardiovascular disorders and the level of blood plasma cholesterol (Chen & Liu, 2020). In the present trial, on average, the PUFA and MUFA proportions observed in the three groups were higher, and consequently, SFA lower than the values reported for goat milk, with respectively 3.7, 24.5 and 68.8% (Markiewicz-Kęszycka *et al*., 2013). The increased proportion of SF in the diets increased MUFA and decreased SFA proportions in milk. The significantly greater PUFA level with SF70 was because of the higher C18:2n-6 and C18:3n-3 levels (and cumulative overall higher n-3 FA). The difference in the MUFA milk proportions according to the group was mainly caused by the differences observed in the C18:1n-9 proportions; this last one has beneficial effects on human health (Chen & Liu, 2020). Similar to the current study, a greater unsaturated fatty acid (UFA) proportion and a lower SFA proportion in milk from goats and ewes supplemented with tannin-rich diets were reported and explained by a change of the ruminal microbial population activity, manifested by a decrease in FA biohydrogenation (De Lucena *et al*., 2018).

In goat milk, the PUFA/SFA ratio ranges from 0.04 to 0.18, the n-6/n-3 ratio from 1.49 to 6.60, the AI ratio from 1.89 to 2.77, the TI ratio from 2.04 to 3.20 and the LA/ALA ratio, from 1.15 to 10.67 (Chen & Liu, 2020; Bodnár *et al*., 2021; El Otmani *et al*., 2021a; Kasapidou *et al*., 2022). The PUFA/SFA and LA/ALA ratios detected in our study were in the range, whereas the other indicators were lower. The incorporation of 70% SF significantly enhanced the PUFA/SFA ratio and the AI and TI indexes. De Lucena *et al*., (2018) reported decreased AI and TI in Saanen goat milk with up to 28 g tannins per kg DM diet; the SF70 diet containing 15.2 g/kg DM.

The SF incorporation in diets did not affect the n-6/n-3 ratio. The recommended ratio ranges around 1-2:1 (Mariamenatu & Abdu, 2021a), which we observed in the three groups. Some authors reported ratios up to 6.6 in goat milk (see above). An optimal n-6/n-3 ratio could have some anti-inflammatory and anti-carcinogenic effects. On the other hand, LA, an n-6 FA, could protect against cardiovascular diseases (Liput *et al*., 2021). Some authors (Chen & Liu, 2020) question thus the usefulness of some of these indicators, arguing that they do not reflect the actual effect of each FA present in a FA group or an indicator. So, for example, all n-3 FA do not positively impact human/animal metabolism in the same way. In the present trial, the most interesting FA, like C18:1n-9, C18:2n-6, C18:3n-3, and DHA, were observed in higher concentrations in SF70 milk.

Antioxidant activity. It is well known that animals at early lactation may face a mobilisation of lipids from their adipose tissue and produce high amounts of reactive oxygen species, which requires a more intensive antioxidant defence of the body (Sordillo *et al*., 2014). The SF70 milk presented the highest TP content, which is explained by this diet's highest TP content and its transfer to the milk via the bloodstream (Di Trana *et al*., 2015). The TP playing an antioxidant role, FRAP and DPPH, which measure the antioxidant activity (Delgadillo-Puga *et al*., 2019), showed obviously the highest values in SF70 milk. Di Trana *et al*., (2015) also reported a positive correlation between goat plasma antioxidant capacity and Sulla coronaria CT intake.

DPPH values in the present study were lower than the values of 71% reported by Lakram *et al*., (2019) for the effects of detoxified Argania spinosa pressed cake on antioxidant activity in Alpine goat milk. Alyaqoubi *et al*., (2014) also reported higher values, but probably due to their extraction method with HCl instead of direct analysis. Other authors (Feng *et al*., 2019; Tian *et al*., 2019) also reported values (18-36%) close to the ones observed in the present trial, with either jujube pulp or purple corn (Zea mays L.) rich in anthocyanins as antioxidant. For the FRAP activity, the observed values (0.760 – 1.103 mmol FeSO4/L) were higher than 0.19 mmol FeSO4/L considered as the critical value to limit oxidative damage to milk (Amamcharla & Metzger, 2014). The antioxidant activity in the SF35 milk was different for these two parameters, not significantly different with SF70 for DPPH and not significantly different with Co for FRAP; it was probably due to the used methods. It is thus important to analyse DPPH and FRAP activities in order to well qualify the antioxidant activity.

The higher proportions of PUFA, MUFA, n-3, n-6 and n-9 in SF70 milk could, therefore, also be due to the protection of the UFA against biohydrogenation and/or oxidation because of the higher TP and TC contents in milk and diet. It was, however, surprising to observe the non-linearity of the effect of SF diet incorporation on these previous parameters.

Conclusion. Sulla flexuosa hay in the diet of dairy goats had no adverse effects on animal production and raw milk characteristics. Tannins present in Sulla flexuosa hay probably had a protective effect on fatty acid biohydrogenation in rumen and an impact on fatty acid desaturating enzymes in mammary gland. The data of this study suggest that Sulla flexuosa hay should be incorporated with no less than half of dry matter intake to show noticeable effects. Therefore, Sulla flexuosa should be suggested as an available alternative forage and protein resource in the lactating goat diet. Further studies are recommended to test its use as fresh or silage forage and to explore its effect on ruminal microbiota and different goat milk product characteristics.

Materials and Methods

Experimental animals and diets. The study was conducted at the Regional Agricultural Research Center of Tangier (INRA—Morocco), exactly at the Bougdour experimental station (35°67' N and 5°85' W). G*Power 3.1.4.9 was used to estimate the sample size (number of goats). Assuming a statistical power of 0.95 and a significance level of $p<0.05$, the required sample for our study was estimated as "24" goats to expect a statistically significant difference. To remedy any surprise, we decided to analyse thirty lactating goats.

The thirty multiparous local "Beni Arouss" goats were divided into three homogeneous groups based on body weight (38.25 ± 0.50 kg), parity (2.5 ± 0.21) and milk production in the previous lactations (527 ± 63 g/day). In each group, the animals (ten) were housed in the same pen, with metal barriers separating the experimental groups. The thermal conditions and humidity were controlled. Each group was randomly allocated to a treatment. The animals of the control group (Co, n =10) were fed a diet containing alfalfa hay, wheat straw and barley and oat grains. The tested animals received a diet where the alfalfa hay and the wheat straw were either partially (SF35, n =10) or totally (SF70, n =10) replaced by Sulla flexuosa (SF) hay. The forage to concentrate ratio was 70:30; the diets were formulated to meet the requirements of lactating multiparous goats (Agabriel, 2010) and were isoenergetic and isonitrogenous. The diet transition period began one week before the parturition and lasted for two weeks. Sulla flexuosa was cultivated at the experimental station and cut at the beginning of the flowering stage; it was dried in an open, naturally ventilated building to make hay. The other feedstuffs were bought from the local market. One representative sample of each raw material was collected at the beginning of the experiment and of each diet twice a week during the first three weeks of the trial. The diets, ingredient chemical composition and diet chemical composition are shown respectively in Tables 13, 17 and 18. The forages were minced in a forage chopper with a 2.5 cm screen and mixed manually with the concentrate in the feeder. The goats were not fed individually but in their groups. They were fed twice a day, at 08h00 and 18h00; the amount of diet distributed was adjusted to obtain about 10% refusal. The animals had ad libitum access to fresh and clean water. All the study procedures were approved by the Regional Center of Agricultural Research of Tangier (number: 01/CRRAT/2021).

Table 18: Chemical composition of the diet ingredients.

	Sulla hay	Alfalfa hay	Wheat straw	Oats grains	Barley grains
Dry matter (DM; g/kg)	907	911	880	917	903
Ash (g/kg DM)	130	102	74.7	29.6	28.1
Crude protein (g/kg DM)	162	215	27.8	118	88.7
Ether extract (g/kg DM)	33.2	39.5	10.5	33.5	79.8
Neutral detergent fibre (g/kg DM)	520	394	800	121	181
Acid detergent fibre (g/kg DM)	412	263	506	94.8	136
Acid detergent lignin (g/kg DM)	118	76.3	150	27.1	38.2
Crude fiber (g/kg DM)	265	202	420	42.6	67.8
Nitrogen-free extract (g/kg DM)	386	441	467	776	736
IVOMD (g/kg DM)	563	676	220	843	803
Metabolisable energy (MJ/kg DM)	7.44	9.61	2.22	11.2	11.9
FMU (unit/kg DM)	0.67	0.82	0.42	1.05	1.07
PDIE (g/kg DM)	93	142	46	85	73
RuProBal	19	22	-41	-17	-27
Total phenols (g/kg DM)	45.3	13.3	0.20	28.8	21.7
Hydrolysable tannins (g/kg DM)	16.9	0.98	0.17	3.2	1.26
Condensed tannins (g/kg DM)	17.5	1.31	0.15	4.01	1.16

IVOMD: In vitro organic matter digestibility; FMU: Forage unit for milk production; PDIE: Digestible proteins in the intestines allowed by energy; RuProBal: Rumen Protein Balance.

Table 19: Diet composition

	Co	SF35	SF70
Diet ingredients (on DM basis)			
Sulla hay (g/kg DM)	0	350	700
Alfalfa hay (g/kg DM)	450	225	0
Wheat straw (g/kg DM)	250	125	0
Barley grains (g/kg DM)	137	137	137
Oat grains (g/kg DM)	140	140	140
Vitamin-Mineral Supplement* (g/kg DM)	23	23	23

*Per kg of diet Vitamin-Mineral Supplement: 8050 IU retinol, 1840 IU cholecalciferol, 34.5 mg α-tocopherol, 92 mg Manganese, 115 mg Zinc, 5.52 g Calcium, 1.15 g Magnesium.

Feed analysis. In order to formulate the diets, a representative sample of each feedstuff was collected at the beginning of the experiment. The diets were sampled twice a week during the first three weeks of the trial. All samples were analysed at the INRA-Tangier laboratory. They were dried and ground using a Wiley mill with a 1-mm screen and stored in Kraft bags in a desiccator. The AOAC, (1990) methods were used for analyses. In brief, DM was determined by drying 100 g of fresh samples in a ventilated oven at 105 ± 1.0°C until constant weight (method 934.01). Ash content was determined by incinerating 2 g DM in a muffle furnace at 550°C for 12 h (method 942.05). Ether extract (EE) was extracted using diethyl ether as a solvent in the Soxhlet apparatus (method 963.15). After using the Kjeldahl method (mineralisation, distillation, and titration) (method 977.02), the CP content was determined by multiplying the nitrogen content by 6.25. The crude fiber (CF), ADL, ADF and NDF and contents were analysed using an ANKOM® 200 Fiber Analyser (ANKOM Technology, Macedon, NY, USA); CF was determined according to (method 962.09) and ADL, ADF and NDF were analysed following the method of Van Soest *et al*., (1991).

Determination of NDF was performed using α-amylase and sodium sulphite. The nitrogen-free extract (NFE) content was estimated according to the formula (all in g/kg DM):

$$NFE = 1000 - (EE + CP + CF + Ash) \quad (1)$$

The in vitro enzymatic dry matter (IVDMD) and organic matter (IVOMD) digestibility was determined using the method of Aufrère & Michalet-Doreau (1983) (Supplementary Material S1). The metabolisable energy (ME) of the experimental diets was estimated based on IVDMD (%) according to the formula given by AOAC (1990):

$$ME\ (MJ/kg\ DM) = 0.17 \times IVDMD - 2 \quad (2)$$

In vitro enzymatic CP degradability was determined as described by Aufrère & Michalet-Doreau, (1983) (Supplementary Material S2).

The forage unit for milk production (FMU; 1 FMU = 1700 kcal or 7.12 MJ), the digestible proteins in the intestines allowed by nitrogen (PDIN) and the digestible proteins in the intestines allowed by energy (PDIE) were calculated by using the INRAtion® software (INRAE, Paris, France). The Rumen Protein Balance (RuProBal) was obtained according to the formula:

$$(PDIN-PDIE) / 0.64 \quad (3)$$

Quantification of total phenols (TP) and total tannins (TT) was performed according to the procedure described by Makkar *et al*., (1993). Condensed tannins (CT) were assayed by Porter *et al*., (1985) method (Supplementary Material S3).

The fatty acid (FA) profile of the feedstuffs was extracted using sulfuric acid and was determined by gas chromatography as described by O'Fallon *et al*., (2007). (Supplementary Material S4). The FA profiles of the feedstuffs were used to calculate the FA profiles of the three diets.

Milk production and composition. The data collection began one week after the parturition and lasted ten weeks (70 days). Milk production was measured and sampled every Friday. The goats were milked twice, at 08h00 and 18h00. After mixing the evening and morning milking yields, a 200 mL sample was collected, covered with aluminium foil to protect it from light and stored at −80°C before analysis. The suckling kids were separated from their mothers the day before sampling. After the milk sample collection, the rest of the milk was given to kids using clean nursing bottles.

Milk production per lactation was estimated by the Fleischmann method by using the simplified formula of El Otmani *et al*., (2021a) (Supplementary Material S5).

Milk's chemical composition and antioxidant capacity were analysed on each sample (n = 80 samples per group); the FA profile was determined on the samples of the odd-numbered weeks (weeks 1, 3, 5, 7 and 9) (n = 40 samples per group).

The lactose, non-fat solids, fat, and protein contents were determined by infra-red using MilkoScan™Minor adapted for goats (FOSS, Hilleroed, Denmark). The total solids and ash contents were determined by the AOAC (1990) methods. The ash was analysed by incinerating 5 mL of milk in a muffle furnace for 3 h at 550°C (945.46 method). Total solids were estimated by drying 5 mL of milk in a ventilated oven at 105 ± 1.0°C until constant weight (925.23 method).

Titratable acidity was determined by 10 mL milk titration using 0.1 N sodium hydroxide, as described by Almeida *et al*., (2006). Milk pH was measured using a pH-meter pen (HANNA HI 98120, Lingolsheim, France). The energy-corrected milk (ECM), fat-corrected milk (FCM) and net energy of milk (NEmilk) were calculated from the daily milk yield and milk composition. The 4% FCM was calculated according to NRC (2001) using the equation:

$$\text{FCM (4\%) (g/day)} = 0.4 \times \text{milk production (g/day)} + 15 \times \text{fat production (g/day)} \quad (4)$$

The ECM was calculated using the formula described by NRC (2001):

$$\text{ECM (g/day)} = \text{Milk production (g/day)} \times (0.38 \times \text{fat (\%)} + 0.24 \times \text{protein (\%)} + 0.17 \times \text{lactose (\%)}/3.14 \quad (5)$$

The NEmilk was calculated based on the gross energy per kilogram in fat, protein, and lactose using the NRC (2001) equation:

$$\text{NEmilk (Mcal/kg)} = 0.0929 \times \text{fat (\%)} + 0.0563 \times \text{protein (\%)} + 0.0395 \times \text{lactose (\%)} \quad (6)$$

Milk antioxidant capacity. All samples were prepared at room temperature and in low-light conditions. The milk TP compounds were extracted and subsequently quantified using the Folin-Ciocalteu method (Vázquez *et al*., 2015). The 2,2-diphényl 1-picrylhydrazyle inhibition (DPPH) scavenging activity was quantified according to the method described by Alyaqoubi *et al*., (2014). The ferric-reducing ability of plasma (FRAP) in milk was determined using the method of Benzie & Strain, (1996). All these methods are described in Supplementary Material S6.

Milk fatty acid profile. The fatty acids profile of milk was also determined by gas chromatography following the same methodology used for the feedstuffs. The observed FA were grouped into different categories (Mierlita, 2016) (Supplementary Material S4 and S7).

Statistical analysis. The experimental data were analysed using the SAS software version 9.4 (SAS Inst. Inc., Cary, NC, USA). The normality of the data was verified. Production, chemical composition, FA proportion, and antioxidant capacity of milk were analysed using the PROC MIXED function, including the random effect of goats and the fixed effects of the diet (Co, SF35, SF70) and the sampling week (j=10 for production, composition and antioxidant capacity and j=5 for fatty acid profile), and their interaction according to the model:

$$Yijk = \mu + Di + Pj + (D \times P)ij + Gijk + eijkn, \quad (7)$$

where Yijk is the dependent variable, μ is the general mean, Di is the effect of diet, Pj is the effect of the sampling week, (D × P)ij is the interaction between diet and sampling week, Gijk is the goat's random effect associated to AR1 covariance structure, and eijkn is the random residual effect. The analysis has been performed considering repeated measurements on goats. CONTRAST statement was used in SAS to estimate "contrasts" values in order to study the linearity of the results. Post hoc analyses were performed using the Tukey test when the results for a parameter were significantly different according to the diets. The significance was set at $p<0.05$.

Author Contributions

Conceptualisation, S.B., A.N., N.M., C.A., J.-L.H., M.C., and J.-F.C.; methodology, S.B., A.N., C.A., J.-L.H., M.C., and J.-F.C.; software, S.B., N.M., and J.-L.H.; validation, S.B., N.M., C.A., J.-L.H., M.C., and J.-F.C.; formal analysis, S.B., N.M., C.A., J.-L.H., and J.-F.C.; investigation, S.B., A.N., and M.C.; resources, A.N., and M.C.; data curation, S.B., N.M., J.-L.H., and J.-F.C.; writing—original draft preparation, S.B., N.M., and J.-F.C.; writing—review and editing, S.B., N.M., C.A., J.-L.H., M.C., and J.-F.C.; visualisation, S.B., N.M., J.-L.H., M.C., and J.-F.C.; supervision, A.N., N.M., C.A., J.-L.H., M.C., and J.-F.C.; project administration, M.C., and J.-F.C.; funding acquisition, M.C., and J.-F.C.; All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

All the study procedures and Guidelines for Experimental Animals were approved by the Regional Center of Agricultural Research of Tangier (number: 01/CRRAT/2021).

Data Availability Statement

The data that support the findings of this study are available upon request from the authors.

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Conflicts of Interest

The authors declare no conflict of interest.

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Supplemental material

Supplementary Material S1

In vitro enzymatic dry matter (IVDMD) and organic matter (IVOMD) digestibility. The in vitro enzymatic dry matter (IVDMD) and organic matter (IVOMD) digestibility was determined using the method of Aufrère & Michalet-Doreau (1983). In brief, 0.5 g of dried sample was incubated at 40°C for 24h with 20 mL of a 2% pepsin solution diluted in 0.1 N hydrochloric acid. Then, the sample was solubilized in 50 mL of a buffer solution containing 1 g.l-1 cellulase, shaken and incubated at 40°C for 24 h. After incubation, the sample was rinsed with hot distilled water and then placed in an oven at 60°C until constant weight. It was weighed to determine the IVDMD. The sample was incinerated in the muffle furnace at 550°C for 12 hours to determine IVOMD.

Supplementary Material S2

In vitro enzymatic CP degradability.

In vitro enzymatic CP degradability was determined by enzymatic hydrolysis for 1 h by protease extracted from Streptomyces griseus in a borate phosphate buffer at pH 8 as described by Aufrère & Cartailler (1988). Briefly, in a centrifuge tube, 0.5 g of the sample was mixed with 25 mL of a protease solution and was incubated at 40°C for one hour. The sample was centrifuged for 5 minutes at 3500 rpm and then filtered. The liquid phase was mineralised for one hour at 120°C and for 2 hours at 350°C using sulfuric acid. The tube containing the mineralised sample was connected to the distiller. Finally, the contents of the Erlenmeyer flask were titrated with 0.1 N HCL to determine the digested nitrogenous matter.

Supplementary Material S3

Total phenols and tannins.

Quantification of total phenols (TP) and total tannins (TT) was performed according to the procedure described by Makkar *et al.* (1993). Briefly, the extraction was done by mixing 200 mg of finely ground dry sample with 10 mL of 70% acetone in a 25 mL glass beaker. The beaker was subject to ultrasonic treatment for 20 minutes at room temperature, and then the content of the beaker was transferred to centrifuge tubes and subjected to centrifugation for 10 minutes at 3500 rpm at 4°C. In a tube, 0.05 mL of the extract was mixed with 0.5 mL of distilled water, 0.25 mL of Folin Ciocalteu 1N reagent, and 1.25 of 20% sodium carbonate solution. The solution was then vortexed and left in the dark for 40 minutes; the absorbance was read at 725 nm to determine total phenols. In a centrifuge tube, 300 mg of polyvinylpolypyrrolidone was mixed with 3 mL of demineralised water and 3 mL of the extract. The tube was vortexed, kept at 4°C for 15 min, centrifuged at 4°C at 3500 rpm for 10 minutes, and then read at 725 nm to determine non-tannic phenols. Total tannins were calculated as the difference between total phenols and non-tannic phenols. Condensed tannins (CT) were assayed by Porter *et al.* (1985) method. Briefly, in a glass tube, 0.5 mL of the extract was mixed with 3 mL of Butanol-HCl reagent and 0.1 mL of ferric reagent. The tubes were vortexed and put in a water bath at 97°C for one hour. After cooling, the absorbance was read at 550 nm. The difference between TT and CT deduced hydrolysable tannins (HT).

Supplementary Material S4

Dietary fat extraction and methylesters preparation

Fatty acid profile in feed samples was determined according to the method of O'Fallon *et al* (2007) Samples of ground feeds (0.5 g) were mixed with 0.35 ml of 10 (N) KOH and 2.65 ml of methanol. The tubes containing these solutions were incubated at 55 °C for 1.5 h with vigorous hand shaking. Then, 0.29 ml of 24 (N) H_2SO_4 was added. The tube was mixed thoroughly and incubated again at 55 °C for 1.5 h. After FA methyl ester (FAME) synthesis, the tube was cooled in a cold tap water bath, 1.5 ml of hexane was added, and the tube was mixed for 5 min on a vortex. The tubes were centrifuged for 5 min at 2500 rpm and the hexane layer containing FAME was transferred into a vial. The vial was capped and placed at −20 °C until analysis.

Milk fat extraction and methylesters preparation. Milk fat extraction and methylesters preparation was processed according to Barbano *et al.* (1998). Briefly, milk fat was obtained by a sequence of 3 successive extractions from 10 mL of milk. The first extraction was the addition of 10 mL of 95% alcohol, 25 mL of ethyl ether and 25 mL of petroleum ether, then the decanting of the ether layer. The second extraction was the addition of 5 mL of 95% alcohol, and 15 mL of ethyl and petroleum ether. The third extraction was the addition of only 15 mL of ethyl and petroleum ether. The three solutions were combined, dried, and put in hexane. The FA methyl esters (FAMEs) were prepared using 0.5 mL of sodium methoxide in methanol 0.5 N and 1 mL of hexane. The vial was capped and placed at −20 °C until analysis.

Gas-chromatographic procedure the separation and the identification of the FAME from feeds and milk.

The fatty acid (FA) profile of the feedstuffs was determined by gas chromatography (GC) with a Varian GC CP 3800 device (Agilent Technologies, Santa Clara, CA, USA) equipped with a split/split less injector, flame ionisation detector, fitted with a capillary column type CP- SIL88

(100m×0.25mm×0.2µm) and helium carrier gas at a flow of 1 mL/min. The oven temperature in the GC was programmed at 50°C and held for 4 min, then increased to 120°C at 10°C/min, held for 1 min, then increased up to 180°C at 5°C/min, held for 18 min, then increased up to 200°C at 2°C/min, and finally increased up to 230°C at 2°C/min and held for 19 min. The injector and detector temperatures were at 270°C and 300°C, respectively. The FA were identified by comparing their retention times with a standard analytical mixture of C4 to C24 FA (Sigma-Aldrich, Darmstadt, Germany), referring to 37 FA.

Supplementary Material S5

Milk production per lactation was estimated by the Fleischmann method by using the simplified formula of El Otmani *et al.* (2021a):

$$Milk\ yield\ = I_1 \times X_1 + \sum_{i=2}^{n}(I_i - I_{i-1}) \times \frac{X_i - X_{i-1}}{2} + I_m \times I_n$$

Where:

I1: number of days between parturition and the first milk recording;

X1: milk quantity produced at the first milk recording;

Ii: number of days between the parturition and the i milk recording (i = 2,...,n);

Xi: milk quantity produced at the i milk recording (i = 2,...,n);

Im: number of days between the last milk recording and dry-off date;

Xn: milk quantity produced at the last milk recording;

n = 10.

Supplementary Material S6

Milk total phenolic compounds. The milk TP compounds were extracted and subsequently quantified using the Folin-Ciocalteu method (Vázquez *et al.* 2015). Briefly, after defrosting the samples in a water bath at 35–40°C, they were homogenized by vortexing for 1 min. Then, in a 25 mL volumetric flask, 8 mL of milk were mixed with 10 mL of methanol–water (1:1, v/v), 500 µl of Carrez I solution containing potassium hexacyanoferrate (II) trihydrate, 500 µl of Carrez II solution and 5 mL of acetonitrile. Following the addition of each solution, the mixture was vortexed. The solution was complemented to 25 mL with methanol–water (1:1, v/v) and the mixture was then allowed to stand for

25 min. The resulting solution was centrifuged at 7800 rpm at 5 C for 15 min. Total phenol compounds in the liquid extract were subsequently quantified using the Folin–Ciocalteu method. The 100 μL of methanol extract was mixed with 1 mL of Folin-Ciocalteu reagent (diluted with H20; 1:1, v/v) and 3 mL of 20% Na_2CO_3 and vortexed. The absorbance of the mixtures was measured at 765 nm after 30 min of incubation in darkness at room temperature. The standard solution of gallic acid (0.5 mg mL-1) dissolved in methanol was used for preparing a calibration curve (0.0–0.10 mg mL−1); its coefficient of variation (r2) was 0.99. All TP concentrations have been expressed in Gallic Acid Equivalents (GAE)/L of milk.

DPPH scavenging activity. The 2,2-diphényl 1-picrylhydrazyle inhibition (DPPH) was estimated directly into samples without any extraction according to the method described by Alyaqoubi, *et al.* (2014). The DPPH solution was prepared by 40 dissolving mg of DPPH in 100 ml methanol. By mixing 100 mL of the 1 $mmol.L^{-1}$ DPPH prepared solution with 100 mL methanol. An absorbance of 1 unit was obtained at 517 nm wavelength. Briefly, 0.1 mL milk was mixed with 1 mL freshly prepared 1 $mmol.L^{-1}$ DPPH solution. The mixture was incubated in the dark for 30 min. A spectrophotometer determined the absorbance at 517 nm. The percentage of DPPH scavenging activity was calculated as follows:

DPPH scavenging activity (%) = [(A blank – A sample) / A blank] × 100,

where A is the absorbance.

FRAP in milk. The ferric-reducing ability of plasma (FRAP) in milk was determined using the method described by Benzie & Strain (1996). The FRAP solution was prepared by mixing 300 mmol L^{-1} acetate buffer pH 3.6, 10 mmol L^{-1} TPTZ (2,4,6-tripyridyl-s-triazine) and 20 mmol L^{-1} FeCl3 in a ratio of 10:1:1. Then, 2 mL of FRAP reagent was mixed with 200 μl of milk extract. This mixture was vortex mixed and incubated in an oven at 37°C for 30 min. The absorbance was then measured at 595 nm. A calibration curve was prepared using an aqueous solution of $FeSO_4$ (0.1–1 mmol. L-1); FRAP wasexpressed as mmol $FeSO_4$ L-1 of milk.

Supplementary Material S7

Summaries, ratios, and indexes of the fatty acids. The fatty acids profile of milk was used to calculate FA summaries, indexes, and ratios.

Short-chain fatty acids (SCFA) = C4:0 + C6:0 + C8:0 + C10:0,

Medium-chain fatty acids (MCFA) = C11:0 + C12:0 + C13:0 + C14:0 + C14:1, Long-chain fatty acids (LCFA) = C15:0 + C15:1 + C16:0 + C16:1 + C17:0 + C17:1 + C18:0 + 9t-C18:1 + C18:1n-9 + 6t-C18:2 + C18:2n-6 + C18:3n-6 + C18:3n-3 + C20:0 + C20:1 + C20:2 + C20:3n-6 + C20:3n-3 + C20:4n-6+ C21:0 + C22:1n-9 + C22:0 + C22:2 + C22:5n-3 + C22:6n-3 + C23:0 + C24:0 + C24:1,

Saturated fatty acids (SFA) = C4:0 + C6:0 + C8:0 + C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0+ C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C23:0 + C22:0 + C24:0,

Mono-unsaturated fatty acids (MUFA) = C14:1 + C15:1 + C16:1 + C17:1 +9t-C18:1 + C18:1n-9 + C20:1 + C22:1n-9 + C24:1,

Polyunsaturated fatty acids (PUFA) = 6t-C18:2 + C18:2n-6 + C18:3n-6 + C18:3n-3 + C20:2 + C20:3n-6 + C20:3n-3 + C20:4n-6 + C22:2 + C22:5n-3 + C22:6n-3,

Desirable fatty acids (DFA) = C18:0 + PUFA + MUFA,

EPA + DHA = C22:6n-3 + 20:5n-3,

n-3 = C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:6n-3,

n-6 = 6t-C18:2 + C18:2n-6 + C18:3n-6 + C20:3n-6 + C20:4n-6,

n-9 = 9t-C18:1 + C18:1n-9 + C22:1n-9,

Atherogenicity index (AI) = (C12:0 + (4 x C14:0) + C16:0)/(MUFA + PUFA),

Thrombogenic index (TI) = (C14:0 + C16:0 + C18:0)/(0.5 x MUFA + 0.5 x n-6PUFA + 3 x n-3PUFA + n-3PUFA/n-6PUFA),

Δ9C14 = (C14:1)/(C14:1 + C14:0),

Δ9C16 = (C16:1)/(C16:1 + C16:0),

Δ9C18 = (C18:1)/(C18:1 + C18:0),

Section expérimentale

Etude 5 :

Growth performance, carcass characteristics, and meat quality of male goat kids supplemented by bitter vetch and sorghum grains.

Chapitre 7. Etude 5: Growth performance, carcass characteristics, and meat quality of male goat kids supplemented by bitter vetch and sorghum grains

Préambule

Les plantes d'orobe et de sorgho sont des ressources alimentaires locales cultivées principalement pour leurs grains, mais sont sous-utilisées dans le sud de la Méditerranée. Les études 2 et 3 ont montré la possibilité d'introduire les écotypes Marocains dans l'alimentation des animaux. L'étude actuelle a été menée pour concrétiser cette probabilité. Généralement, en nutrition animale, les grains de sorgho peuvent être comparés aux grains de maïs, avec une différence en fibres, énergie et en dégradabilité de l'amidon. L'intérêt pour le sorgho vient de ses caractéristiques environnementales, notamment sa plus grande résistance à la sécheresse, son adaptabilité à différents environnements et types de sol, ses faibles besoins en fertilisants et surtout sa courte saison de croissance. L'orobe a été utilisée dans la nutrition des ovins et des monogastriques, après avoir subi des traitements thermiques et chimiques. Dans la ration des caprins, l'incorporation des grains d'orobe a été réalisée la première fois dans notre essai. En effet, les grains d'orobe et de sorgho ont été incorporés dans la ration des chevreaux locaux pour évaluer leur effet sur les performances de croissance, les caractéristiques de la carcasse et la qualité de la viande. Vingt-quatre chevreaux ont été répartis en trois groupes. Le groupe témoin a reçu une alimentation conventionnelle à base de foin d'avoine, d'orge et de fève. Dans le premier groupe testé, la fèverole a été remplacée par l'orobe, et dans le deuxième groupe, l'orge a été remplacé par le sorgho. À la fin de l'essai, les animaux ont été abattus, et les caractéristiques de la carcasse et la qualité de la viande des muscles *longissimus dorsi* et *semimembranosus* ont été déterminées. Les résultats ont montré que la ration a affecté le gain moyen quotidien et le taux de matière sèche ingérée, mais pas le poids corporel à l'abattage, le poids de la carcasse chaude et le rendement de la carcasse (15,0 kg, 6,8 kg et 44,6 %, respectivement). Les régimes ont influencé plusieurs paramètres, mais pas de la même manière sur chaque muscle. En ce qui concerne la qualité de la viande, les régimes ont influencé la teneur en protéines musculaires avec moins de protéines dans la viande des animaux ayant reçu les grains d'orobe. Les grains d'orobe et de sorgho peuvent être incorporés en toute sécurité dans l'alimentation des chevreaux locaux.

Les hypothèses sont donc : Les grains de l'orobe et du sorgho peuvent respectivement remplacer les grains de féverole et d'orge de la ration conventionnelle sans avoir d'effet sur :

- ✓ Les paramètres de croissance ?
- ✓ Les caractéristiques de la carcasse ?

- ✓ Les caractéristiques physiques et chimiques de la viande ?
- ✓ Le profil des acide gras de la viande ?

Section expérimentale

Etude 5 :

Growth performance, carcass characteristics, and meat quality of male goat kids supplemented by bitter vetch and sorghum grains.

À soumettre

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Abstract

Bitter vetch and sorghum grains are underused alternative local feed resources in the southern Mediterranean. The current study was carried out to evaluate the effect of their incorporation into the diet on growth performance, carcass characteristics, and meat quality of local goat kids. Twenty-four goat kids were divided into three groups. The control group received a conventional diet based on oat hay, barley, and fava bean. In the first tested group, fava bean was replaced by bitter vetch, and in the second, barley by sorghum. At the end of the trial, the animals were slaughtered, and carcass characteristics and meat quality of *longissimus dorsi* and *semimembranosus* muscles were determined. The diet affected average daily gain and DM intake but not slaughter body weight, hot carcass weight, and dressing percentage (15.0 kg, 6.8 kg, and 44.6 %, respectively). The diets influenced several parameters but not in the same way on each muscle. Regarding meat quality, the diets influenced muscle protein content with less protein in the chevon from animals receiving bitter vetch. Bitter vetch and sorghum grains can be safely incorporated into the fattening diet of goat kids.

Introduction

Goat breeding (Capra hircus) has been a prevalent occupation for lots of people in Northern Morocco since ancient times. It plays an important socio-economic role, providing food and contributing to more than 70% of income in rural mountain communities. The goat herds are mainly made up of local indigenous populations well adapted to their environment. Part of the goat females born are used for herd replacement, whereas the remaining animals are destined for meat consumption (Godber *et al*., 2020).

Nowadays, consumers have become more nutrition- and health-conscious. The interest in lean chevon meat with less cholesterol and saturated fatty acids has increased. Lean meat is demonstrated to have fewer negative impacts on health (Mazhangara *et al*., 2019). Chevon meat has a favorable chemicalnutrient composition with comparable arginine, isoleucine, lysine, methionine, threonine, and tryptophan concentration to beef, pork, and lamb meat (Mazhangara *et al*., 2019). Moreover, goat meat was reported as a major source of micronutrients, especially iron, potassium, and B12 vitamin. These essential elements are reported to prevent anemia, a real threat to women of childbearing age, especially in rural areas (Mwangi *et al*., 2017). It is also an important source of magnesium and potassium (Osman & Mahgoub, 2012), which are important for enzymatic reactions in the body and energy metabolism (Fiorentini *et al*., 2021), maintaining healthy blood pressure, and the functioning of the nervous system (Stone Michael & Weaver, 2021).

Goat breeding is conducted in traditional silvopastoral and agro-silvopastoral systems (Chebli *et al*., 2018; 2020). However, those areas have undergone serious degradation and decreased biodiversity (Chebli *et al*., 2021b). Due to climate change, farmers tend to reduce the size of herds during drought to maintain only the number of animals for which they can provide adequate feed. Reduced herd sizes also lower pressure on the degraded rangelands (Godber *et al*., 2020). Therefore, the need for new resources to sustain goat production and alleviate rangeland degradation has emerged.

Bitter vetch (*Vicia ervilia*) and sorghum (*Sorghum bicolor* (L.) Moench) are underused crops in Northern Morocco (Boukrouh et al., 2021, 2022). Bitter vetch is an ancient legume of the Mediterranean region, which is harvested for its fodder and grains, the latter ones as a source of proteins (270 g/kg DM) and energy (13 MJ metabolizable energy/kg DM) (Boukrouh *et al*., 2021; Sadeghi *et al*., 2009). This interesting grain nutritive value guaranteed the continuous cultivation of *Vicia ervilia* as ruminant feed in Morocco, Spain, and Turkey. However, the presence of antinutritional factors limited the amount of bitter vetch that can be incorporated into the diet of non-ruminants due to low utilization efficiency (Sadeghi *et al*., 2004). The effect of incorporating bitter vetch grains on the growth and meat quality of poultry and sheep is documented (Abdullah *et al*., 2010; Sadeghi *et al*., 2009).

Corn grain is the most common concentrated feedstuff in ruminant diets because of its highly energizing starch content (Gómez *et al*., 2016). While corn grain has some similarities with sorghum in its chemical composition, sorghum is generally higher in fiber and lower in starch than corn (Johnston & Moreau, 2017). Sorghum also tends to have higher fat, protein, and minerals, such as calcium and iron levels, than corn (Tuna & Bressani, 1992). Sorghum bicolor is also characterized by higher resource use efficiency, i.e., a higher drought resistance, a wider adaptability to different environments and soil conditions, and a low fertilizer requirement (Assefa *et al*., 2013; Safian *et al*., 2022) making it an interesting candidate for its replacement. Phenologically, differences between corn and sorghum concerning growth and development patterns are manifested by a shorter growing season for sorghum (Assefa *et al*., 2013).

To the best of the authors' knowledge, the use of bitter vetch and sorghum grains in goat-kid diets has not yet been studied. Promising results could reinforce using those local plants as alternative feed concentrates. It could also lead to more sustainable goat farming by decreasing goat pressure on silvopastoral rangelands and using plants more resistant to drought and climate change. Thus, this trial aimed to analyze the effects of incorporating bitter vetch and sorghum grains on animal performance, carcass and meat quality of goat-kid.

Result

Diets. The diets were statistically isoenergetic and isoproteinic with a close RuProBal (Table 24). The total phenol presented a higher content in the SGR diet, and the CT had the lowest content in the BV diet.

Animal performance and, slaughter and carcass characteristics. Initial, final, and slaughter body weight, hot and cold carcass weight, and dressing percentage were not statistically different between the three groups (9.4 kg, 15.5 kg, 15.0 kg, 6.8 kg, 6.5 kg, and 44.6%, respectively) (Table 20). However, the Co group showed a higher ADG than the two other groups (65.7 vs. 48.5 g/d). The animals receiving the BV diet had lower DMI (1346 vs. 1413 g/d in the other groups). The test groups increased the feed conversion ratio compared to the Co group (28.6 vs. 21.7 kg of DM/kg of gain).

Although full and empty GIT weights were similar for the three groups, the ratio of empty GIT to SBW was higher for the SRG group. Except for the skin rate that was lower for BV and SRG diets compared to the Co group, the proportion and weight of pluck, anterior and posterior paws, and head were similar between the three groups. The SRG Diet significantly reduced mesenteric fat weight but did not affect the perirenal weight and rate. Carcass measurements (carcass length, thigh length, width, and thickness, shoulder length, and rib cage length and width) did not vary according to diet, but shoulder perimeter was significantly ($p < 0.01$) lower in the SRG group. Also, muscle and conformity indexes varied significantly ($p < 0.05$) according to diet and were lower for BV and SRG groups.

Table 20: Growth, rumen pH and carcass characteristics of goat kids according to the diet.

	Co	BV	SRG	SEM	*p*-value
Dry matter intake (g/day)	1414	1346	1411		
Feed conversion ratio (kg of DM/kg of gain)	21.7	27.6	29.6		
Initial body weight (kg)	10.3	9.0	9.0	2.610	0.610
Final body weight (kg)	17.1	15.1	14.3	3.385	0.310
Average daily gain (g/d)	65.7 [a]	48.7 [b]	48.3 [b]	2.89	0.019
Slaughter body weight (kg)	16.5	14.6	13.8	3.703	0.319
Hot carcass weight (kg)	7.6	6.8	5.9	1.838	0.230
Dressing percentage (%)	46.1	45.5	42.3	3.091	0.321
Cold carcass weight (kg)	7.4	6.5	5.6	1.783	0.224
Gastrointestinal tract					
Weight of full GIT (g)	3567	3646	3684	290.6	0.963
Weight of empty GIT (g)	567	467	726	228.4	0.100
Rate of empty GIT (%)	3.34 [b]	3.07 [b]	5.05 [a]	0.346	0.025
Rumen pH	5.55 [b]	6.08 [ab]	6.18 [a]	0.11	0.025
Skin					
Weight (g)	1249	998	917	254.3	0.070
Rate (%)	7.28 [a]	6.53 [b]	6.46 [b]	0.423	0.004
Pluck					
Weight (g)	863	809	729	170.5	0.354

Rate (%)		5.08	5.32	5.21	0.618	0.769
Anterior paws						
Weight (g)		323	258	265	62.6	0.148
Rate (%)		1.91	1.70	1.87	0.243	0.238
Posterior paws						
Weight (g)		257	217	188	52.2	0.072
Rate (%)		1.52	1.43	1.31	0.149	0.054
Head						
Weight (g)		1299	1082	1021	232.3	0.100
Rate (%)		7.68	7.12	7.31	0.917	0.532
Perirenal fat (g)						
Weight (g)		234	218	172	63.1	0.176
Rate (%)		1.42	1.45	1.24	0.471	0.650
Mesenteric fat						
Weight (g)		437 ^{a}	425 ^{a}	266 ^{b}	138.9	0.048
Rate (%)		2.53	2.78	1.90	1.621	0.089
Carcass length (cm)		50.0	47.2	46.9	4.287	0.376
Thigh length (cm)		26.1	27.8	26.1	1.921	0.182
Tight width (cm)		11.7	10.4	10.1	1.157	0.060
Tight thickness (cm)		23.4	21.9	20.8	2.566	0.198
Shoulder perimeter (cm)		20.8 ^{a}	20.0 ^{a}	16.0 ^{b}	2.331	0.002
Shoulder length (cm)		25.7	24.1	23.9	1.656	0.132
Rib cage length (cm)		27.7	26.2	24.6	2.485	0.101
Rib cage width (cm)		15.6	15.8	16.1	1.362	0.860
Compactness index (g/cm)		14.6	13.7	11.9	3.069	0.238
Muscle index		44.7 ^{a}	37.6 ^{b}	39.0 ^{ab}	0.043	0.018
Conformity index (g/cm)		59.4 ^{a}	51.3 ^{ab}	50.9 ^{b}	0.061	0.036
Color parameters						
Belly	L*	56.5	58.8	58.9	3.530	0.382
	a*	13.7	13.3	12.6	2.936	0.774
	b*	3.95	4.46	2.87	2.741	0.508
Back	L*	51.3 ^{b}	59.1 ^{a}	55.1 ^{a}	4.290	0.012
	a*	9.74 ^{ab}	8.81 ^{b}	11.4 ^{a}	1.682	0.022
	b*	2.22 ^{a}	0.723 ^{ab}	-1.29 ^{b}	2.324	0.034
Saddle	L*	51.8 ^{b}	60.9 ^{ab}	64.6 ^{a}	7.095	0.012
	a*	13.7 ^{a}	11.2 ^{ab}	9.55 ^{b}	2.336	0.015
	b*	3.85	1.95	1.96	1.114	0.200
Tail outline	L*	50.0	50.6	52.1	4.851	0.713
	a*	18.3	20.0	18.3	3.102	0.469
	b*	6.73	7.95	6.37	2.994	0.558

Co: control diet; BV: diet with bitter vetch replacing fava bean; SRG: diet with sorghum replacing barley; Pluck: liver, lung, pancreas, heart, spleen, and trachea; L*: lightness index; a*: redness index; b*: yellowness index; $^{a, b, c}$: values followed by different letters in the same row differ statistically by Tukey's test at ($p < 0.05$).

The incorporation of bitter vetch and sorghum grains in diets had significant effects on carcass color. The back and saddle of the BV and SRG goat-kid carcass were lighter compared to the control group ($p < 0.05$). Redness and yellowness of the back and redness of the saddle varied significantly ($p < 0.05$) according to diet, with especially lower values in SRG when compared to the Co. No effect was observed on the belly and tail outline color.

Meat quality. The meat's physical characteristics and chemical composition are reported in Table 21. *Longissimus dorsi* showed higher water loss and Shear force parameters. Although a diet effect was observed on pH 0, no difference was observed on pH 24. The diet type never affected the meat's color, tenderness, and water loss.

Strong interactions Diet x Muscle were observed, especially for the SRG diet that provided SM darker, more red, and more yellow than in the other groups, while opposite effects were observed on LD.

Lower meat ash content was observed in the LD of the SRG group and protein content in both muscles from BV (202 vs. 218 g/kg in the LD muscle and 200 vs. 216 g/kg in the SM muscle, respectively).

Fatty acid profile. The type of muscle and diet significantly affected the FA profile, families, ratios, and indexes of meat (Table 22). Generally, lower levels of short-chain saturated FA but higher of very long-chain FA were observed in LD compared to SM. The behavior was variable for the UFA. As expected, the n-3 FA were less abundant in LD than the n-6. Overall, the family sums (SFA, MUFA, PUFA) were not influenced by the type of muscle.

The effects of the diet on the different FA were highly variable with few interactions so that the overall family sums were not affected or to a low extent for the PUFA, i.e., the SRG diet led to lower values compared to the others.

The elongase activity, thrombogenic index (TI), health-promoting index (HPI), and nutritive value index (NVI) were also modified by the diet in both muscles but in variable ways.

Table 101: Physical characteristics and chemical composition of longissimus dorsi and semimembranosus muscles of goat kids according to the diet.

	Longissimus dorsi			***Semimembranosus***			**SEM**	***p*-value**		
	Co	**BV**	**SRG**	**Co**	**BV**	**SRG**		**Diet**	**Muscle**	**Muscle*Diet**
Physical characteristics										
pH0	7.05	7.08	7.07	7.08 [b]	7.00 [c]	7.17 [a]	0.025	0.014	0.630	0.002
pH24	6.06	6.03	6.05	6.08	5.96	6.30	0.036	0.068	0.993	0.221
Meat color										
L*	46.8	48.9	52.6	54.5	56.3	44.5	2.551	0.572	0.045	0.005
a*	21.65	22.8	18.84	18.4	18.0	22.3	1.291	0.918	0.095	0.002
b*	6.31	6.68	4.72	3.70	3.36	6.11	0.762	0.821	0.016	0.005
Hue angle (H°)	16.1	15.9	13.3	9.45	8.60	15.0	1.873	0.579	0.011	0.030
Chroma	7.45	7.65	6.74	5.47	5.51	7.70	0.385	0.128	0.001	<0.001
Water loss (g/kg)	147	174	169	86.4	88.8	116	13.6	0.209	<0.001	0.264
Raw meat Shear force (N)	109	101	102	94.2	90.2	81.0	10.323	0.661	0.047	0.836
Cooked meat Shear force (N)	66.2	67.6	66.5	61.3	57.2	52.1	3.888	0.549	0.014	0.586
Chemical composition										
Moisture (g/kg)	748.5	773.0	753.2	756.0	764.3	748.0	11.36	0.453	0.887	0.904
Ash (g/kg)	16.1 [a]	15.5 [a]	14.4 [b]	16.1	16.3	15.7	0.321	0.013	0.007	0.124
Protein (g/kg)	220 [a]	202 [b]	215 [a]	214 [a]	200 [b]	217 [a]	3.288	<0.001	0.579	0.741
Fat (g/kg)	19.9	20.1	20.3	20.8	20.9	22.1	1.360	0.717	0.178	0.870

Co: control diet; BV: diet with bitter vetch replacing fava bean; SRG: diet with sorghum replacing barley; L*: lightness index; a*: redness index; b*: yellowness index; [a, b, c]: values within muscle followed by different letters in the same row differ statistically by Tukey's test at ($p < 0.05$).

Table 22: Fatty acid profile and summaries (g/100 g fatty acids), ratios, and indexes of *longissimus dorsi* and *semimembranosus* muscles of goat kids according to the diet.

	Longissimus dorsi			*Semimembranosus*			SEM	*p*-value		
	Co	BV	SRG	Co	BV	SRG		Diet	Muscle	Muscle* Diet
C4:0	0.42	0.47	0.51	0.56	0.58	0.57	0.044	0.514	0.011	0.705
C6:0	0.37	0.38	0.51	0.38	0.38	0.36	0.047	0.424	0.103	0.043
C8:0	0.38	0.47	0.40	0.53	0.51	0.46	0.068	0.803	0.616	0.127
C10:0	0.17	0.28	0.23	0.44	0.29	0.49	0.061	0.434	0.008	0.150
C11:0	0.06	0.06	0.07	0.42	0.32	0.40	0.031	0.179	<0.001	0.247
C12:0	0.23	0.15	0.21	0.37	0.35	0.33	0.056	0.678	<0.001	0.480
C13:0	0.51	0.61	0.61	0.58	0.59	0.52	0.042	0.467	0.681	0.294
C14:0	1.23	0.93	1.04	1.42	1.06	1.10	0.046	0.013	0.167	0.837
C15:0	4.61[ab]	5.53[a]	3.63[b]	5.34[a]	4.88[ab]	3.94[b]	0.361	0.001	0.663	0.176
C16:0	13.0[ab]	11.6[b]	14.3[a]	13.9[ab]	14.5[b]	16.5[a]	0.530	<0.001	<0.001	0.089
C17:0	3.27[a]	3.22[a]	2.13[b]	3.46	3.26	2.61	0.345	0.017	0.403	0.785
C18:0	17.5	18.3	18.0	16.7[b]	18.0[ab]	18.6[a]	0.342	0.005	0.683	0.539
C20:0	0.50	0.57	0.54	0.56	0.53	0.80	0.095	0.294	0.614	0.396
C21:0	0.37	0.46	0.45	0.79	0.80	0.76	0.055	0.680	<0.001	0.400
C22:0	1.25	1.24	1.22	0.60	0.69	0.61	0.032	0.297	<0.001	0.487
C23:0	5.74	5.65	6.24	3.82	3.82	3.96	0.308	0.432	<0.001	0.729
C24:0	2.10	2.42	2.48	1.63	1.68	1.66	0.082	0.052	<0.001	0.181
SFA	51.6	52.4	52.6	51.0	52.0	53.6	0.712	0.118	0.774	0.558
C14:1	0.26[b]	0.21[b]	0.21[b]	0.61[a]	0.13[b]	0.55[a]	0.034	<0.001	<0.001	<0.001
C15:1	1.25	1.08	0.86	1.07	1.06	0.98	0.051	0.201	0.773	0.520
C16:1	1.35[ab]	0.95[b]	1.66[a]	1.38[a]	0.87[b]	1.25[ab]	0.139	0.001	0.319	0.467
C17:1	1.00	0.96	0.74	0.93	0.88	0.83	0.098	0.190	0.747	0.526
9t-C18:1	1.03	1.05	1.13	0.445	1.06	1.13	0.201	0.169	0.238	0.259
C18:1n-9	27.5	27.5	27.5	28.0	27.5	25.9	0.704	0.305	0.527	0.270
C20:1	0.56	0.63	0.83	0.68	0.58	0.63	0.094	0.354	0.583	0.350
C22:1n-9	0.39	0.39	0.40	0.26	0.36	0.31	0.036	0.359	0.004	0.247
C24:1	1.15	1.26	1.33	1.10	1.25	1.28	0.141	0.467	0.643	0.973
MUFA	34.5	34.0	34.7	34.5	33.7	32.8	0.777	0.608	0.290	0.464
6t-C18:2	1.73	1.44	1.69	2.95	3.26	2.99	0.194	0.997	<0.001	0.102
C18:2n-6	7.30[a]	6.99[ab]	6.26[b]	4.97	5.05	4.63	0.226	0.013	<0.001	0.562
C18:3n-3	0.34[b]	0.43[a]	0.22[c]	0.31[a]	0.34[a]	0.20[b]	0.026	<0.001	0.058	0.484
C18:3n-6	0.26	0.27	0.25	0.20	0.22	0.21	0.012	0.420	<0.001	0.617
C20:2	0.61	0.59	0.56	0.32	0.24	0.23	0.052	0.409	<0.001	0.774
C20:3n-3	0.58	0.70	0.35	0.90	0.92	0.99	0.148	0.606	0.005	0.333
C20:3n-6	0.44	0.59	0.60	0.43	0.39	0.41	0.046	0.348	0.004	0.175
C20:4n-6	0.45	0.50	0.49	0.49	0.52	0.57	0.024	0.074	0.016	0.390
C20:5n-3	1.31	1.40	1.43	1.77	1.77	1.70	0.045	0.905	<0.001	0.628
C22:2	0.49	0.41	0.50	0.36	0.35	0.36	0.060	0.677	0.055	0.813
C22:6n-3	0.32	0.36	0.34	1.34	1.23	1.26	0.079	0.894	<0.001	0.715
PUFA	13.8[a]	13.7[ab]	12.7[b]	14.0	14.3	13.5	0.325	0.022	0.087	0.700
Summaries										
DFA	65.8	66.0	65.4	65.2	67.0	64.9	0.592	0.403	0.272	0.681
n-3	2.56	2.88	2.34	4.32	4.26	4.15	0.148	0.096	<0.001	0.511
n-6	10.2	9.79	9.29	9.04	9.44	8.80	0.292	0.099	0.025	0.494
n-9	29.0	28.9	29.1	28.7	31.0	27.3	0.739	0.543	0.297	0.391
EPA+DHA	1.64	1.76	1.77	3.11	2.99	2.97	0.121	0.998	<0.001	0.643
Ratios										

PUFA/SFA	0.27[a]	0.26[ab]	0.24[b]	0.27[ab]	0.28[a]	0.25[b]	0.007	0.006	0.101	0.757
MUFA/PUFA	2.51	2.50	2.74	2.47	2.38	2.44	0.095	0.268	0.103	0.471
EPA/AA	2.97	2.83	2.96	3.65	3.44	3.03	0.197	0.311	0.010	0.241
DHA/AA	0.73	0.72	0.71	2.78	2.38	2.28	0.173	0.341	<0.001	0.499
n-6/n-3	3.99	3.48	4.03	2.12	2.25	2.16	0.158	0.302	<0.001	0.177
Indexes										
Δ9C16	0.10[ab]	0.08[b]	0.11[a]	0.09[a]	0.05[b]	0.07[ab]	0.008	0.006	0.039	0.444
Δ9C18	0.62	0.61	0.61	0.63[a]	0.61[ab]	0.59[b]	0.009	0.049	0.890	0.395
AA/EPA+DHA	0.66	0.72	0.69	1.62	1.52	1.62	0.082	0.930	<0.001	0.607
Elongase activity	0.75[b]	0.79[a]	0.74[b]	0.75[a]	0.75[a]	0.72[b]	0.009	0.001	<0.001	0.227
HPI	0.38[b]	0.33[c]	0.40[b]	0.41[b]	0.40[b]	0.46[a]	0.016	0.002	<0.001	0.431
TI	1.05[ab]	1.00[b]	1.14[a]	0.91[b]	0.97[ab]	1.08[a]	0.031	<0.001	0.010	0.320
NVI	3.57[b]	4.07[a]	3.31[b]	3.25[a]	3.24[a]	2.79[b]	0.160	0.003	<0.001	0.152

Co: control diet; BV: diet with bitter vetch replacing fava bean; SRG: diet with sorghum replacing barley. MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; DFA: Desirable fatty acids; EPA: eicosapentaenoic acid (20:5n-3); DHA: docosahexaenoic (C22:6n-3); AA: arachidonic acid (C20:4n-6); LA: linolenic acid (C18:2n-6); ALA: α-linolenic acid (C18:3n-3); Δ9C16 = (C16:1)/(C16:1 + C16:0) activity of Δ 9 desaturase enzyme to convert C16:0 into C16:1; Δ 9C18 = (C18:1)/(C18:1 + C18:0) activity of Δ9 desaturase enzyme to convert C18:0 into C18:1; TI: Thrombogenic index; HPI:health-promoting index; NVI: nutritive value index; [a, b, c]: values within muscle followed by different letters in the same row differ statistically by Tukey's test at ($p < 0.05$).

Discussion

Growth and carcass parameters. Several factors influence the carcass composition of goats, such as concentrate level and type, forage type, and protein level and source (Pophiwa *et al*., 2020). The dry matter intake is a key driver of animal performance for fattening purposes. In the present study, DMI was apparently lower in the group that received bitter vetch as a protein resource. Several antinutritional components, including protease inhibitors and canavanine, confer a bitter taste or negative metabolic effects to the bitter vetch grains (Sadeghi *et al*., 2009). Canavanine is a non-protein AA analogous to arginine, leading to the production of non-functional proteins (Sadeghi *et al*., 2009). Noteworthy, the meat protein content in this group was lower than in the other groups. However, nutrient intake was not influenced in another study where soybean meal was substituted with bitter vetch grains in a lamb diet (Haddad, 2006). Bitter vetch ecotype characteristics or incorporation level of bitter vetch grains (15% for the lamb diet vs. about 30% for the present diet) could explain such a difference.

A lower BV percentage in the diet could ameliorate DMI. Interestingly, DMI was similar in the SRG group as that of the Co, while ADG was lower. The lower ADG observed in the tested groups thus was due to lower DMI and/or lower diet efficiency. Consequently, the feed conversion ratio was higher for the two test diets. This parameter was higher than values reported in the Mediterranean area (Titi *et al*., 2008) but was in the range of calculated values for the same breed goat kids receiving olive cake and cactus cladodes as feed alternatives (El Otmani *et al*., 2021). However, the observed ADGs were higher than the 35g/day reported by the same authors (El Otmani *et al*., 2021). Final body weight, hot and cold carcass weight, and dressing percentage were similar between the groups and in the range of values reported by El Otmani *et al*. (2021).

Starch in barley grains is more fermentable than in sorghum grains; it is the same for starch in fava beans compared to bitter vetch grains (Crépon *et al*., 2010; Taghavi *et al*., 2023). It could explain the lower Co, intermediate BV, and higher SRG ruminal pH observed in the trial. However, the three values were in the same normal pH range (5.5 -7.5) but with values slightly lower than the optimal ruminal pH (6.5).

Diets had very few effects on the non-carcass components. Surprisingly, the rate of empty GIT was higher in the SRG group. It could be associated with lower ADG associated with unchanged feed intake, but the reason is unclear. The BV and SRG skin proportion was lower than the one of the control group. Methionine and cysteine are the first limiting factor in bitter vetch grains (Sadeghi *et al.*, 2009). Also, sorghum grains contain less methionine than barley (Al-Marzooqi, 2020). Cysteine and methionine are usually the limiting amino acids in keratin synthesis and among the limiting ones for meat production (Cao *et al*., 2021). Moreover, these limiting AA are prioritized for muscle growth rather than tegument growth. Presumed differences in those AA contents in the diets could explain the differences observed for the skin rate (lowest in the SRG and BV groups) and the protein content of the muscles (lowest in the two BV muscles).

The carcass compactness index is an important economic indicator since the meat market prefers more compact carcasses (Nascimento *et al*., 2018). The distributed diets did not affect this parameter. However, the lower muscle and conformity indexes for goat kids receiving BV or sorghum grains could negatively affect their incorporation and, thus, their use. However, the present muscle and conformity indexes were higher than those reported by Lahkim Bennani *et al*., (2022) and El Otmani *et al*., (2021b) with goat kids from the Beni Arouss breed.

Meat quality. Immediately after slaughter, SRG s*emimembranosus* muscle presented a higher pH value, and the one from BV presented a lower value than the Co group. After the completion of glycolysis, the pH remained unchanged. Also, the water holding capacity did not change according to the diet.

Meat color is the first criterion used by consumers to judge meat quality. The red color is influenced by the level and state of myoglobin (Zhu *et al*., 2022). Vioque *et al*., (2020) reported that polyphenols exhibit antioxidant properties (Vioque *et al*., 2020) that prolong the red color stability of meat indirectly by delaying the oxidation in metmyoglobin (Zhu *et al*., 2022). The absence of difference in meat color could be due to the low concentration of polyphenols. Indeed, despite the highest total polyphenol content observed in the SRG diet, the TP contents in the three diets were lower than 20 – 30 g/kg DM, reported to affect meat antioxidative stability (García *et al*., 2019).

Tenderness is the most important organoleptic trait contributing to consumer acceptance and eating satisfaction with meat (Garmyn, 2020). The shear force test is generally used to measure meat tenderness and is positively affected by increased collagen content (Florek *et al*., 2022). The absence of differences between diets for the shear force values in the two muscles was expected due to low differences in ultimate pH between diets. The current cooked LD was rather tender (61.8 N) when compared to values reported in the literature (47 – 94 N) (Cao *et al*., 2021; Hwang & Joo, 2017; Mwangi *et al*., 2017).

The tested diets were complemented with urea to be isoproteic. However, the BV meat showed lower protein content, possibly due to the lower content of bitter vetch in essential and limiting amino acids (methionine and cysteine) (Sadeghi *et al*., 2009), as explained above, and could also be due to the presence of canavanine. Also, meat protein results were close to 23 % DM reported by Mazhangara *et al*., (2019) for chevon.

Fatty acids. The fatty acid profile has a crucial impact on meat quality (Mazhangara *et al*., 2019). In the present study, the meat FA profiles are not easy to interpret due to unclear trends. It is translated in the lack of differences in families sums of FA (SFA, MUFA, PUFA). However, overall, SM showed higher amounts of SCSFA and lower ones of LCSFA. It could be due to the difference in FA metabolism between the two muscles.

Diet effects were strongly variable and hard to interpret, as stated above. However, it is interesting to consider the family of PUFA frequently represented in the feed from the vegetal origin, i.e., C18:2 n-6 and C18:3 n-3, because they are important to improve meat quality in ruminants. There was less C18:2 n-6 in the SM muscle. It could be explained by the fact that this muscle contained more fat, which diluted the C18:2n-6, mainly originating from the membrane cells. By contrast, the lack of significant muscle effect on C18:3 n-3 is probably because the diets had low amounts of fat. However, the lower level of this FA in the muscles of the SRG group – and consequently the lower PUFA/SFA ratio - is in line with its lower amounts measured in the corresponding diet (see Table 19). The diets did not appear to affect the sequence of saturation-elongation of the VLCPUFA. Considering the low-fat levels in the diets, there was no reason to expect such an effect.

Incorporating bitter vetch and sorghum grains did not affect the n-6/n-3 ratio. However, the muscle type impacted this index with 3.83 and 2.17 for the LD and the SM muscles, respectively. The desirable range for this index is 1 4:1 to help maintain a balanced and healthy life (Ponnampalam *et al*., 2021). Sorghum grain incorporation increased the health-promoting index in SM muscle. It is probably of little signification. The HPI was in the range of 0.16 to 0.68 values reported for dairy products (Chen & Liu, 2020). The SRG diet increased the thrombogenic index and decreased the nutritive value index. Recommendations to consume food with reduced TI and high NVI have also been suggested to improve human health (Chen & Liu, 2020). However, the NVI of 3.51 was still higher than the values reported for goats, while the TI of 1.14 was intermediate (Taboada *et al*., 2022; Yang *et al*., 2022).

Materials and methods

Experimental Animals and Diets. The study was conducted at the Bougdour experimental station (35°67' N and 5°85' W) of Tangier's Regional Agricultural Research Center (INRA—Tangier, Morocco). The sample size (number of goats) was estimated using G*Power 3.1.4.9 software. Assuming a statistical power of 0.95 and a significance level of $p < 0.05$, twenty-four goat kids were required to compose the sample size for expecting a statistically significant difference according to an effect size close to 0.25.

The goat kids from the local "Beni Arouss" breed were divided into three homogeneous groups based on initial body weight (9.43 ± 0.1 kg), age (86.5 ± 12.5 d), and casual twinship. The animals of each group were housed in the same pen, with metal barriers separating the experimental groups. Thermal conditions and humidity were controlled. Each group was randomly allocated to one treatment (Table 22). The animals of the control group (Co, n = 8) were fed a conventional diet, as distributed by regional farmers for fattening. It was made of oat hay, barley, and fava bean. The tested groups received either bitter vetch replacing fava bean grains (BV, n = 8) or sorghum replacing barley grains (SRG, n = 8). The forage-to-concentrate ratio was 50:50.

The diets were isoenergetic, isonitrogenous, and formulated to meet the requirements of growing goat kids. The kids were weaned at about 2.5-3 months; after an adaptation period of 15 days, the experiment lasted 90 days. All the feedstuffs were bought from a local market. In order to formulate the diets, a representative sample of each feedstuff was collected at the beginning of the experiment; each diet was sampled twice a week during the first three weeks of the trial. The ingredient chemical composition, diets, and diet chemical composition are shown in Tables 19, 23 and 24, respectively. Oathay was minced in a forage chopper with a 2.5 cm screen and mixed manually with the concentrate in the feeder. The kids were fed collectively. The animals also had ad libitum access to fresh and clean water. The total mixed ration was provided *ad libitum* twice a day at 8:00 and 18:00. The offered rations were adapted to allow 10 % refusal.

Table 113: Diet composition.

	Co	BV	SRG
Diet ingredients (on a DM basis)			
Oat hay (g/kg DM)	491	492	496
Bitter vetch grains (g/kg DM)	0	298	0
Sorghum grains (g/kg DM)	0	0	190
Barley grains (g/kg DM)	186	192	0
Fava bean grains (g/kg DM)	312	0	300
Urea (g/kg DM)	4.4	11.6	7.4
Vitamin-Mineral Supplement* (g/kg DM)	6.6	6.4	6.6

Co: control diet; BV: diet with bitter vetch replacing fava bean; SRG: diet with sorghum replacing barley.

* Per kg vitamin-mineral supplement: 2,310 IU retinol, 528 IU cholecalciferol, 9.9 mg α-tocopherol, 26.4 mg manganese, 33 mg zinc, 1.58 g calcium, 330 mg magnesium.

All experiment and analysis methods were performed following relevant regulations and guidelines. The National Center of Agricultural Research approved all the study procedures.

Chemical analysis. All feed/diet samples were analyzed at the INRA-Tangier laboratory using AOAC (1990) methods (Table 24 and 25). Samples were dried at 55°C until constant weight, then ground and sieved at 1 mm. Dry matter was obtained by drying 100 g of samples at 105 ± 1°C until constant weight (method 934.01). Ash content was determined by the incineration of a 2 g sample in a muffle furnace at 550 °C for 12 h (method 942.05). Ether extract (EE) was extracted in a Soxhlet apparatus using diethyl ether as a solvent (method 963.15). After nitrogen determination by the Kjeldahl method (mineralization, distillation, and titration) (method 977.02), the CP content was determined by multiplying the nitrogen content by 6.25. The ADL, ADF, NDF, and CF fiber contents were analyzed using an ANKOM® 200 Fiber Analyser (ANKOM Technology, Macedon, NY, USA) and following the method of Van Soest *et al*., (1991) for ADL, ADF, and NDF, and the method 962.09 for CF. The NDF determination was carried out using α-amylase and sodium sulfite. The nitrogen-free extract (NFE) content was estimated according to the formula (all in g/kg DM):

$$\text{NFE} = 100 - (\text{EE} + \text{CP} + \text{CF} + \text{Ash}) \quad (1)$$

The in vitro enzymatic dry matter (IVDMD) and organic matter (IVOMD) digestibility were determined using the method of Aufrère & Michalet-Doreau (1983) (Supplementary Material S8). The metabolizable energy (ME) of the experimental diets was estimated based on IVDMD (%) according to the formula given by AOAC (1990):

$$\text{ME (MJ/kg DM)} = 0.17 \times \text{IVDMD} - 2 \quad (2)$$

Table 24: Chemical composition of the diet ingredients.

Items	**Oat hay**	**Bitter vetch grains**	**Fava bean grains**	**Sorghum grains**	**Barley grains**
Dry matter (g/kg)	899.9	907.7	904.6	906.9	902.4
Crude protein (g/kg DM)	62.1	234.0	262.6	88.7	111.8
Ether extract (g/kg DM)	11.8	10.9	19.7	40.7	31.9
Ash (g/kg DM)	77.1	31.0	49.0	13.1	24.7
Neutral detergent fiber (g/kg DM)	571.4	160.2	141.5	110.2	181.4
Acid detergent fiber (g/kg DM)	432.9	74.9	107.5	60.8	87.9
Acid detergent lignin (g/kg DM)	61.2	8.59	26.7	6.70	17.6
Crude fiber (g/kg DM)	324.0	44.1	65.3	17.7	57.6
Nitrogen-free extract (g/kg DM)	525.0	680.0	603.5	839.8	774.0
In vitro Enzymatic Organic matter digestibility (g/kg DM)	483.3	892.9	695.7	753.9	825.7
Metabolizable energy (MJ/kg DM)	5.4	11.2	9.7	10.3	11.0
Forage unit for meat (FUMeat/kg DM)	0.48	0.92	0.78	0.91	0.95
PDIE (g/kg DM)	74.0	115.0	71.0	85.0	67.0
RuProBal	-46.9	57.8	132.8	-40.6	-12.5
Total phenols (g/kg)	6.42	1.46	5.43	37.1	0.92
Hydrolysable tannins (g/kg DM)	0.44	0.26	1.09	0.11	0.09
Condensed tannins (g/kg DM)	0.41	1.06	4.07	0.38	0.71

PDIE: digestible proteins in the intestines allowed by energy; RuProBal: rumen protein balance

Table 25: Chemical composition of the offered diets (n = 6 for each diet).

	Co	BV	SRG	SEM	*p*-Value
Nutrient composition					
Dry matter (g/kg)	913	907	912	2.025	0.337
Ash (g/kg DM)	63.7	53.2	60.1	0.476	0.098
Protein (g/kg DM)	160	160	160	0.134	0.889
Ether extract (g/kg DM)	19.7	15.7	21.2	0.111	0.184
Neutral detergent fiber (g/kg DM)	477	453	457	1.428	0.643
Acid detergent fiber (g/kg DM)	264	267	249	0.274	0.335
Acid detergent lignin (g/kg DM)	46.0	36.2	43.0	0.084	0.224
Crude fiber (g/kg DM)	215	195	202	0.103	0.117
Nitrogen-free extract (g/kg DM)	651	631	650	1.041	0.215
In vitro enzymatic organic matter digestibility (g/ kg DM)	671	689	642	0.745	0.279
Metabolizable energy (ME; MJ/kg DM)	8.52	8.42	8.18	0.089	0.723
Forage unit for meat (FUMeat/kg DM)	0.70	0.70	0.71	0.042	0.523
PDIE (g/kg DM)	80.0	75.9	81.5	0.234	0.191
RuProBal	29.2	22.7	29.8	-	-
Total phenols (g/kg DM)	5.21 [b]	3.86 [b]	12.44 [a]	0.108	0.021
Condensed tannins (g/kg DM)	1.71 [a]	0.61 [b]	1.57 [a]	0.071	0.034
Hydrolyzable tannins (g/kg DM)	0.60	0.31	0.59	0.002	0.091
Urea (g/kg DM)	4.27	11.70	7.43		
Fatty acid profile of the diet (g/100g FA) (calculated from feedstuff fatty acid profile)					
C12:0	0.000	0.292	0.074		
C14:0	3.761	3.774	2.847		
C14:1	0.000	0.249	0.000		
C16:0	42.162	41.810	37.812		
C16:1	0.710	0.679	1.129		
C15:0	0.000	0.124	0.000		
C17:0	0.952	1.016	0.797		
C18:0	10.276	12.428	8.750		
C18:1n-9	8.632	7.435	14.713		
C18:2n-6	14.673	12.227	19.022		
C18:3n-3	16.426	16.642	12.201		
C18:3n-6	0.000	0.013	0.031		
9t-C18:1	0.091	0.127	0.811		
C20:0	1.126	0.638	0.753		
C20:1	0.587	1.917	0.584		
C22:0	0.604	0.480	0.221		
C23:0	0.000	0.150	0.254		

Co: control diet; BV: diet with bitter vetch replacing fava bean; SRG: diet with sorghum replacing barley; PDIE: digestible proteins in the intestines allowed by energy; RuProBal: rumen protein balance; [a, b, c]: values followed by different letters in the same row differ statistically by Tukey's test at ($p < 0.05$).

In vitro enzymatic CP degradability was determined as described by Aufrère & Cartailler (1988) (Supplementary Material S9) to calculate the digestible proteins in the intestines allowed by nitrogen (PDIN), and the digestible proteins in the intestines allowed by energy (PDIE). The forage unit for meat production (FUMeat; 1 FUMeat = 1700 kcal or 7.12 MJ), PDIN, and PDIE were calculated using the

INRAtion® software (INRAE, Paris, France). The rumen protein balance (RuProBal) was obtained according to the formula:

$$(PDIN-PDIE)/0.64 \qquad (3)$$

Quantification of total phenols (TP) and total tannins (TT) was performed according to the procedure described by Makkar *et al*., (1993). Condensed tannins (CTs) were assayed by Porter *et al*., (1985) method (Supplementary Material S10).

The fatty acid (FA) profile of the feedstuffs was extracted using sulfuric acid and was determined by gas chromatography as described by O'Fallon *et al*., (2007) (Supplementary Material S11). The FA profiles of the feedstuffs were used to calculate the FA profiles of the three diets. Only the FA detected were presented in Table 19.

Slaughtering procedures and carcass traits measurements. At the experiment's beginning and end, goat kids were weighed to obtain the initial and final body weights. The average daily gain (ADG) was calculated based on the initial and final body weight difference divided by the number of days in the trial period (i.e., 90 d). The feed conversion ratio was calculated by dividing DMI on ADG.

After a 24-h fasting period with free access to water, the animals were weighed for slaughter body weight (SBW) determination and slaughtered following the guidelines of the ethical standards of the Regional Center of Agricultural Research of Tangier (License N° 01/CRRAT/2017). Hot carcass weight (HCW) was determined just after the removal of non-carcass components (anterior and posterior paws, head, skin, pluck (trachea, lungs, trachea, heart, pancreas, liver, kidneys, testes), gastrointestinal tract (GIT), and perirenal and mesenteric fat). Dressing percentage (DP) was determined as the ratio between HCW and SBW. After the carcasses were chilled for 24 h at 4°C, cold carcass weight (CCW) was recorded. Non-carcass components were weighed; the GIT was weighed full and empty after hand-wash cleaning. A pH meter measured rumen pH directly after GIT removal (HANNA HI98120, HANNA instruments). Carcass measurements included carcass length (CL), thigh length (TL), thigh width, thigh thickness (TT), shoulder perimeter, shoulder length, rib cage length, and rib cage width. Compactness index (CI) and muscle index (MI) were calculated based on the ratios between CCW and CL, and between TL and TT, respectively. The conformation index was calculated as the sum of CI and MI.

Evaluation of lightness (*L**), redness (*a**), and yellowness (*b**) indexes was carried out 24 hours post-mortem on the belly, back, saddle, and tail outline regions of the carcass. L* is the index related to luminosity (L* = 0 black, 100 white); a* is the index that ranges from green (-) to red (+); and b* is the index that ranges from blue (-) to yellow (+). The reading was performed with a Konica- Minolta colorimeter CR-400 with measurement and illumination area of Ø 8 mm and Ø 11 mm, and 0° viewing angle, calibrated using white calibration plate CR-A43. Measurements were performed three times for each region, and the average of each triplicate was calculated for each animal.

Meat analysis. A piece of *longissimus dorsi* (LD) and *semimembranosus* (SM) muscles of each carcass was excised 24h after slaughter in the middle of each muscle. The samples were then divided into two halves. The first was directly used to determine the physical characteristics, and the second was ground for homogenization to determine the chemical composition. Meat samples used for chemical composition were then sealed in vacuum bags and conserved at -25°C until analyses.

The pH of muscles was determined directly on the hot carcass and after 24h *post-mortem* on the samples with a penetration pH meter (HANNA HI98120, HANNA instruments). Evaluation of lightness (*L**), redness (*a**), and yellowness (*b**) indexes was also carried out on the meat samples. Hue angle and chroma indexes were determined using the a* and b* indexes according to the following formulas proposed by Hunt and King (King et al., 2012):

$$\text{Chroma} = (a^{*2} + b^{*2})^{0.5} \quad (4)$$

$$\text{Hue angle} = \text{arctangent}\,(b^*/a^*) \times [360/(2 \times 3.14)] \quad (5)$$

Muscles' water-holding capacity was determined by cutting each sample into cubes of two grams each, which were placed on filter paper between two acrylic plates and received a load of 10 kg for 5 min (Kauffman *et al*., 1986). Then, the samples were weighed, and the water loss was calculated as the percentage difference in sample weight before and after load exposure. Meat tenderness was determined on the two muscles according to Sarriés and Beriain method (Sarriés & Beriain, 2006). Six pieces with a square shape of 1.27 cm on each side and 2.00 cm of height (oriented parallel to the muscle fibers) were removed from each sample using a cork borer. Three pieces were placed in plastic bags, sealed hermetically, then cooked in a water bath at 75°C for 40 min. At the cooking end, the meat's internal temperature was 70°C. The cooked samples were placed in a refrigerator for 24hs. The tenderness of raw (24h *post-mortem*) and cooked (48h *post-mortem*) samples was measured by a Warner-Bratzler shear force protocol using TA.HDplusC Texture Analyser with a V-shaped cutting blade (Stable Micro Systems Ltd, Godalming, Surrey, UK). Each was then sheared perpendicular to the fiber direction, and values were expressed in N.

Meat chemical composition was determined according to AOAC (1990). Before use, the ground meat samples were thawed in a refrigerator for 12 h at 4°C. A meat sample was put in an oven at 105

°C for 24 h; the difference between the initial and the dried weights allowed to calculate the moisture percentage. Meat ash percentage was obtained by calculating the difference between the initial weight plus 1 ml magnesium acetate and the ashed weight after 5h at 550°C in the muffle furnace. The meat fat was determined by acid hydrolysis by boiling 5 g of the sample in 100 ml of 3 N hydrochloric acid for 1 hour. The mixture was filtered, and fat was extracted using the Soxhlet apparatus. Protein was obtained using the Kjeldahl method, multiplying the total nitrogen content by 6.25. The fatty acid extraction was carried out using the method of Folch *et al*., (1957). The observed FAs were grouped into different categories (Adeyemi *et al*., 2015; Boukrouh *et al*., 2023; Chen & Liu, 2020) (Supplementary Material S11 and S12).

Statistical analysis. The experimental data were processed using SAS software 9.4 (SAS Institute Inc., Cary, NC, USA). The diet effects were tested by generalized linear models (GLM) for growth performance and slaughter and carcass measurements, including the random effect of goats according to the following model:

$$Y_{ij} = \mu + D_i + G_{ij} + e_{ij} \quad (6)$$

where Y_{ijk} was the dependent variable; μ was the mean; D_i was the fixed effect of the *i*th modalityof diet (Co, BV, and SGR); G_{ij} was the goat's random effect; e_{ik} was the residual error.

The meat quality and the FA profile of meat were analyzed using the PROC MIXED function, including the random effect and repeated measurements on goats and the fixed effects of the diet according to the following model:

$$Y_{ijk} = \mu + D_i + M_j + (D\times M)_{ij} + G_{ijk} + e_{ijk} \quad (7)$$

where Y_{ijk} was the dependent variable; μ was the mean; D_i was the fixed effect of the *i*th modalityof diet (Co, BV, and SGR); M_j was the fixed effect of the *j*th modality of muscle (*longissimus dorsi* and *semimembranosus*); $(D\times M)_{ij}$ was the effect of interaction in *ij*th case; G_{ijk} was the goat's random effect associated to a compound symmetry structure; e_{ijk} was the residual error. To compare the tested groups' means to the control one, a Tukey's test was performed when a significant effect of the model was obtained at $p < 0.05$.

Conclusion

Bitter vetch and sorghum grains are two alternative energy and protein resources that can be incorporated into the diet of goat kids without negative impacts on the slaughter characteristics, despite some negative effects on average daily gain and DM intake at the level of incorporation used in this trial. The carcass characteristics, meat quality, and fatty acids profile were variously affected according to the distributed diets. Nevertheless, even if the diet influenced some parameters of the meat quality and fatty acid profile, most of these parameters were rarely influenced in the same way for each studied muscle. Further investigations are recommended to evaluate the effects of bitter vetch and sorghum grains on ruminal microbiota and goat milk quality. Different levels of incorporation or treatments of grains should also be evaluated.

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Supplementary Materials

S8: The in vitro enzymatic dry matter (IVDMD) and organic matter (IVOMD) digestibility using the method of Aufrère & Michalet-Doreau (1983), Supplementary Material S9: In vitro enzymatic CP degradability using the method of Aufrère & Cartailler (1988), Supplementary Material S10: Quantification of total phenols (TPs) and total tannins (TTs) according to the procedure described by Makkar *et al.* (1993) and Condensed tannins (CTs) by Porter *et al.* (1985) Supplementary Material S11: The fatty acid (FA) profile of meat and feedstuffs as described by Folch *et al.* (1957) and O'Fallon *et al.* (2007b) respectively. Supplementary Material S12: The observed FAs were grouped into different categories (Adeyemi *et al*., 2015; Boukrouh *et al*., 2023; Chen & Liu, 2020).

Author Contributions

Conceptualisation, S.B., A.N., N.M., C.A., J.-L.H., M.C., and J.-F.C.; methodology, S.B., A.N., C.A., J.-L.H., M.C., and J.-F.C.; software, S.B., N.M., and J.-L.H.; validation, S.B., N.M., C.A., J.-L.H., M.C., and J.-F.C.; formal analysis, S.B., N.M., C.A., J.-L.H., and J.-F.C.; investigation, S.B., A.N., and M.C.; resources, A.N., and M.C.; data curation, S.B., N.M., J.-L.H., and J.-F.C.; writing—original draft preparation, S.B., N.M., and J.-F.C.; writing—review and editing, S.B., N.M., C.A., J.-L.H., M.C., and J.-F.C.; visualization, S.B., N.M., J.-L.H., M.C., and J.-F.C.; supervision, A.N., N.M., C.A., J.-L.H., M.C., and J.-F.C.; project administration, M.C., and J.-F.C.; funding acquisition, M.C., and J.-F.C.; All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

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Informed Consent Statement

Not applicable.

Data Availability Statement

The data supporting this study's findings are available upon request from the authors.

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Conflicts of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary Material S8. *In vitro enzymatic dry matter (IVDMD) and organic matter (IVOMD) digestibility*. The in vitro enzymatic dry matter (IVDMD) and organic matter (IVOMD) digestibility was determined using the method of Aufrère & Michalet-Doreau (Aufrère & Michalet-Doreau, 1983). In brief, 0.5 g of dried sample was incubated at 40°C for 24h with 20 mL of a 2% pepsin solution diluted in 0.1 N hydrochloric acid. Then, the sample was solubilized in 50 mL of a buffer solution containing 1 g.l-1 cellulase, shaken and incubated at 40°C for 24 h. After incubation, the sample was rinsed with hot distilled water and then placed in an oven at 60°C until constant weight. It was weighed to determine the IVDMD. The sample was incinerated in the muffle furnace at 550°C for 12 hours to determine IVOMD.

Supplementary Material S9. *In vitro enzymatic CP degradability*. In vitro enzymatic CP degradability was determined by enzymatic hydrolysis for 1 h by protease extracted from Streptomyces griseus in a borate phosphate buffer at pH 8 as described by Aufrère & Cartailler (1988). Briefly, in a centrifuge tube, 0.5 g of the sample was mixed with 25 mL of a protease solution and was incubated at 40°C for one hour. The sample was centrifuged for 5 minutes at 3500 rpm and then filtered. The liquid phase was mineralised for one hour at 120°C and for 2 hours at 350°C using sulfuric acid. The tube containing the mineralised sample was connected to the distiller. Finally, the contents of the Erlenmeyer flask were titrated with 0.1 N HCL to determine the digested nitrogenous matter.

Supplementary Material S10. *Total phenols and tannins.* Quantification of total phenols (TP) and total tannins (TT) was performed according to the procedure described by Makkar *et al.*, (1993). Briefly, the extraction was done by mixing 200 mg of finely ground dry sample with 10 mL of 70% acetone in a 25 mL glass beaker. The beaker was subject to ultrasonic treatment for 20 minutes at room temperature, and then the content of the beaker was transferred to centrifuge tubes and subjected to centrifugation for 10 minutes at 3500 rpm at 4°C. In a tube, 0.05 mL of the extract was mixed with 0.5 mL of distilled water, 0.25 mL of Folin Ciocalteu 1N reagent, and 1.25 of 20% sodium carbonate solution. The solution was then vortexed and left in the dark for 40 minutes; the absorbance was read at 725 nm to determine total phenols. In a centrifuge tube, 300 mg of polyvinylpolypyrrolidone was mixed with 3 mL of demineralised water and 3 mL of the extract. The tube was vortexed, kept at 4°C for 15 min, centrifuged at 4°C at 3500 rpm for 10 minutes, and then read at 725 nm to determine non-tannic phenols. Total tannins were calculated as the difference between total phenols and non-tannic phenols. Condensed tannins (CT) were assayed by Porter *et al.*, (1985) method. Briefly, in a glass tube, 0.5 mL of the extract was mixed with 3 mL of Butanol-HCl reagent and 0.1 mL of ferric reagent. The tubes were vortexed and put in a water bath at 97°C for one hour. After cooling, the absorbance was read at 550 nm. The difference between TT and CT deduced hydrolysable tannins (HT).

Supplementary Material S11. *Dietary fat extraction, methylesters preparation and fatty acids profile determination of feedstuffs.* Fatty acid profile in feed samples was determined according to the method of O'Fallon *et al.*, (2007). Samples of ground feeds (0.5 g) were mixed with 0.35 ml of 10 (N) KOH and 2.65 ml of methanol. The tubes containing these solutions were incubated at 55 °C for 1.5 h with vigorous hand shaking. Then, 0.29 ml of 24 (N) H_2SO_4 was added. The tube was mixed thoroughly and incubated again at 55 °C for 1.5 h. The tube was cooled in a cold tap water bath, 1.5 ml of hexane was added, and the tube was mixed for 5 min on a vortex. The tubes were centrifuged for 5 min at 2500 rpm and the hexane layer containing FAME was transferred into a vial. The vial was capped and placed at −20 °C until analysis.

The fatty acid methyl esters FAME were analyzed by gas chromatography (GC) with a Varian GC CP 3800 device (Agilent Technologies, Santa Clara, CA, USA) equipped with a split/split less injector, flame ionization detector, fitted with a capillary column type CP- SIL88 (100m×0.25mm×0.2µm) and helium carrier gas at a flow of 1 mL/min. The oven temperature was programmed at 50°C and held for 4 min, then increased to 120°C at 10°C/min, held for 1 min, then increased up to 180°C at 5°C/min, held for 18 min, then increased up to 200°C at 2°C/min, and finally increased up to 230°C at 2°C/min and held for 19 min. The injector and detector temperatures were kept at 270°C and 300°C, respectively.

Meat fat extraction, methylesters preparation and fatty acids profile determination of meat. Fat extraction of meat samples was carried out using method of Folch *et al.*, (1957). Briefly, 10 g of meat samples were homogenized using chloroform/methanol (2:1, v/v) solution and centrifuged at 3000 rpm for 10 minutes. After sample filtration, 5 mL of distilled water was added, and the samples were shaken vigorously. Once again, the sample was centrifuged (3000 rpm, 10 min) to allow the layer to separate. The upper aqueous layer was discarded and the lower chloroformic layer containing FAME was transferred into a vial. The vial was capped and placed at −20 °C until analysis.

The fatty acid methyl esters (FAME) of meat were also analyzed using gas chromatography (GC) with similar characteristics as for feedstuffs. The conditions were an injector temperature of 255°C and an initial oven temperature of 75°C, which was increased to 165°C at 8°C/min (held for 35 min), then increased to 210°C at 5.5°C/min (held for 2 min), and finally, increased to 240°C at 15°C/min (held for 3 min). The temperature of the detector was 280°C. Nitrogen was used as the carrier gas (18 mL/min) with a pressure of 38 psi.

The FA were identified by comparing their retention times with a standard analytical mixture of C4 to C24 FA (Sigma-Aldrich, Darmstadt, Germany), referring to 37 FA.

Supplementary Material S12. *Summaries, ratios, and indexes of the fatty acids.* The fatty acids profile of milk was used to calculate FA summaries, indexes, and ratios (Adeyemi *et al.*, 2015; Chen & Liu, 2020; Boukrouh *et al.*, 2023):

Saturated fatty acids (SFA) = C4:0 + C6:0 + C8:0 + C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0+ C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C23:0 + C22:0 + C24:0, (1)

Mono-unsaturated fatty acids (MUFA) = C14:1 + C15:1 + C16:1 + C17:1 +9t-C18:1 + C18:1n-9 + C20:1 + C22:1n-9 + C24:1, (2)

Polyunsaturated fatty acids (PUFA) = 6t-C18:2 + C18:2n-6 + C18:3n-6 + C18:3n-3 + C20:2 + C20:3n-6+ C20:3n-3 + C20:4n-6 + C22:2 + C22:5n-3 + C22:6n-3, (3)

Desirable fatty acids (DFA) = C18:0 + PUFA + MUFA, (4)

n-3 = C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:6n-3, (5)

n-6 = 6t-C18:2 + C18:2n-6 + C18:3n-6 + C20:3n-6 + C20:4n-6, (6)

n-9 = 9t-C18:1 + C18:1n-9 + C22:1n-9, (7)

UFA/SFA, (8)

PUFA/SFA ratio, (9)

MUFA/PUFA ratio, (10)

n-6/n-3 ratio, (11)

(C18:0 + C18:1)/C16:0, (12)

Thrombogenic index (TI) = (C14:0 + C16:0 + C18:0)/[0.5 (MUFA) + 0.5 (n-6PUFA) + 3 (n-3PUFA) + (n-3PUFA/n-6PUFA)], (13)

Health promoting index (HPI) = Σ UFA/(C12:0+4xC14:0+C16:0), (14)

Nutritive value index (NVI) = (C18:0+C18:1)/C16:0, (15)

Thrombogenic index (TI) = (C14:0+C16:0+C18:0)/[(0.5 x MUFA)+(0.5 x Σ n-6)+(3 x Σn-3)+(Σn-3/Σn-6)], (16)

$$\Delta 9C16 = C16{:}1/(C16{:}1 + C16{:}0), \quad (17)$$

$$\Delta 9C18 = C18{:}1n\text{-}9/(C18{:}1n\text{-}9 + C18{:}0), \quad (18)$$

$$\text{Elongase activity} = (C18{:}1n\text{-}9 + C18{:}0)/(C18{:}1n\text{-}9 + C18{:}0 + C16{:}0 + C16{:}1), \quad (19)$$

Discussion générale

Chapitre 8. Discussion générale

Dans la région du Nord du Maroc, l'élevage caprin est conduit en système d'élevage extensif basé sur la production de viande et en système d'élevage semi-extensif basé sur la production mixte (lait et viande). L'alimentation des animaux est basée principalement sur les parcours forestiers. Ces derniers sont caractérisés par une dégradation ainsi qu'une variation temporelle des disponibilités fourragères et de leur valeur nutritive. La productivité des animaux est donc instable, ce qui a un impact direct sur les revenus des éleveurs. L'utilisation de nouvelles ressources alternatives s'impose afin d'alléger la dégradation des parcours, exploiter de nouvelles sources de protéines et/ou d'énergie, éviter la compétition avec l'alimentation humaine et valoriser les ressources génétiques locales. Le Sulla flexuosa est une légumineuse endémique à la région du Nord du Maroc. L'orobe et le sorgho sont cultivés par les agriculteurs principalement pour leurs grains. La paille est aussi valorisée. Pour leur utilisation comme alternatives dans la région du Nord du Maroc, il est primordial de collecter ces écotypes ainsi que d'évaluer leurs caractéristiques agro-morphologiques, leur valeur nutritive ainsi que leurs effets sur les performances zootechniques et la qualité des produits caprins. Cette étude a été entreprise pour la caractérisation écologique, morpho-agronomique et bromatologique des écotypes (i) du Sulla flexuosa, (ii) du sorgho, (iii) et de l'orobe, (iv) la caractérisation des effets de l'incorporation du foin de Sulla flexuosa en replacement de la luzerne dans la ration de la chèvre de la race locale Beni Arouss sur la production et la qualité du lait, et, (v) la détermination des effets des grains de l'orobe et du sorgho en remplacement respectivement de la féverole et de l'orge, sur les performances et sur la qualité de la carcasse et de la viande de chevreaux Beni Arouss.

Caractérisation écologique des écotypes de Sulla flexuosa, orobe et sorgho. La caractérisation édapho-climatique des endroits de collecte des écotypes donnera des informations sur la possibilité de leur semis dans d'autres régions Méditerranéennes. Les sites de collecte du Sulla flexuosa n'ont pas dépassé 358 m en altitude, alors que le sorgho et l'orobe ont été trouvés dans des sites avec des altitudes allant de 940 à 1155 m respectivement. Ces altitudes élevées ont été rencontrées dans la région du Rif. Plusieurs études ont souligné la biodiversité élevée de cette zone (Ater & Hmimsa, 2006). Les trois plantes ont été rencontrées majoritairement dans des sols alcalins. La conductivité électrique moyenne montre que l'orobe et le sorgho peuvent pousser sur des sols peu salins à très salins alors que le Sulla flexuosa a été trouvé seulement sur des sols peu salins (Richard, 1954). Le pH et la salinité des sols peuvent être des contraintes lors du semis du Sulla flexuosa dans d'autres régions du Maroc. L'orobe et le sorgho ont été rencontrés sur des sols ayant une matière organique élevée par rapport à l'habitat naturel du Sulla flexuosa. Ceci peut être dû au fait que les deux plantes ont été collectées à partir des parcelles agricoles alors que le Sulla flexuosa a été collecté sur des sols non cultivés.

Les sols où le sorgho a été collecté sont riches en phosphore par rapport aux sols de collecte du Sulla flexuosa et de l'orobe, alors que pour le potassium, c'était l'inverse. Ces différences peuvent être dues aux besoins des plantes en ces éléments. L'azote dans les sols de prélèvement du Sulla flexuosa a été plus faible par rapport à l'azote dans les sols de collecte du sorgho et de l'orobe, bien que le sorgho ne soit pas une légumineuse. Ce résultat montre que la fixation de l'azote par le Sulla flexuosa a été plus faible que la fertilisation azotée apportée par les agriculteurs. Concernant la texture des sols, les 3 plantes ont été trouvées dans des sols avec des teneurs faibles en sable fin et grossier. Concernant le Sulla flexuosa, une autre espèce du genre *Hedysarum* a été rapportée aussi sur des sols argileux, a toléré la texture limoneuse et n'a pas été trouvée sur des sols à texture sableuse (Issolah *et al*., 2012). Les sols avec des teneurs élevées en sable fin et grossier peuvent être limitants pour la multiplication de ces 3 plantes.

Sur le long terme, les données climatiques des sites de collecte des trois plantes étaient proches. Les précipitations annuelles moyennes ont été supérieures à 700 mm, ce qui a expliqué la distribution du Sulla flexuosa et de l'orobe majoritairement dans la région du Nord du Maroc. Cependant, le sorgho a été trouvé dans d'autres régions du Maroc (Farré & Faci, 2006). La multiplication des écotypes performants dans des régions avec des précipitations inférieures à cette marge sera conditionnée par la résistance des écotypes à la sécheresse.

Caractérisation agro-morphologique des écotypes. *Variabilité des paramètres agro-morphologiques.* La variabilité au sein des écotypes de Sulla flexuosa a été estimée par le coefficient de variation. La grande variabilité des paramètres morphologiques (avec 12,6 % pour le diamètre de la tige et 35,9 % pour le nombre d'inflorescences par plante) entre les écotypes reflète le faible niveau d'intervention humaine pour la sélection. La faible variabilité de la phénologie (avec 0,24 % pour le début de floraison et 7,91 % pour le nombre de jours jusqu'à la levée) (Figure 22) a également été rapportée pour d'autres espèces de légumineuses (Yohannes *et al*., 2020).

Figure 22 : Plantes de Sulla flexuosa au stade début floraison

Concernant le sorgho (Figure 23) et l'orobe (Figure 24), la variabilité génétique a été estimée à l'aide des coefficients de variation phénotypiques et génotypiques, l'héritabilité et le progrès génétique. Pour le sorgho, la plupart des paramètres y compris la hauteur de la plante, le pourcentage de feuilles, la longueur du pédoncule, la longueur, la largeur et le poids de la panicule, la période de remplissage des grains, les grains par plante et le rendement en grains, l'indice de récolte et le poids de mille graines, avaient des coefficients de variation phénotypiques et génotypiques élevés, ce qui indique qu'une sélection peut être appliquée sur la base de ces caractères pour isoler des cultivars plus prometteurs. Par rapport au sorgho, moins de paramètres ont été caractérisés par une variabilité élevée pour l'orobe, notamment la durée de floraison, la longueur des racines et les paramètres liés au rendement en grain (grains par gousse et par plante, nombre de gousses, rendement en grain et indice de récolte). Cette différence peut être due à l'autopollinisation de la vesce amère qui réduit sa diversité (Zohary & Hopf, 2000). En outre, trente-huit amorces RAPD et quatre amorces ISSR ont été utilisées pour évaluer la variabilité entre les écotypes de sorgho au Nord du Maroc. Un polymorphisme a été trouvé et chaque population représentait une entité de conservation et une importante réserve de gènes à utiliser pour l'amélioration de cette céréale.

Figure 23 : Caractérisation agro-morphologique des plantes de sorgho

Figure 24 : Plantes et grains de l'orobe

La sélection d'écotypes performants est généralement basée sur la caractérisation phénotypique, et le succès dépendra naturellement de la relation entre le phénotype et le génotype. Pour le sorgho et l'orobe, presque tous les paramètres étaient hautement héritables.

L'héritabilité élevée d'un trait génétique indique qu'une grande partie de la variation observée dans ce trait est due à des facteurs génétiques, avec une faible influence de l'environnement sur son expression. En d'autres termes, les différences observées dans le trait sont principalement héritées des parents. Par conséquent, les sélectionneurs peuvent faire une sélection de génotypes supérieurs basée sur les performances phénotypiques pour ces caractères. Le progrès génétique, quant à lui, fait référence à l'amélioration de ce trait au fil des générations grâce à la sélection des individus ayant les caractéristiques souhaitées. Donc, la réussite de la sélection sera basée sur une héritabilité et un progrès génétique élevés. Dans notre étude, le nombre de paramètres qui avaient une héritabilité et progrès génétique élevés était élevé pour le sorgho par rapport à l'orobe. Cela montre que pour le sorgho, par rapport à l'orobe, en sélectionnant les écotypes avec les meilleures valeurs génétiques pour ces caractères, on peut s'attendre à un progrès génétique plus important, car ses caractéristiques souhaitables seront transmises à la génération suivante (Singh *et al*., 2018).

Paramètres agro-morphologiques. L'étude de la variabilité au sein de la collection d'écotypes du Sulla flexuosa, orobe, et sorgho a pour objectif principal de sélectionner les meilleurs écotypes en termes de productivité et de qualité. Tous les écotypes ont été stockés dans la banque de gènes de l'INRA. Ils vont servir pour préserver la biodiversité et enrichir la banque par des gènes codant pour la résistance aux stresses biotiques et abiotiques ; ces stresses pouvant émerger dans les conditions du changement climatiques. Le Sulla flexuosa est utilisé comme fourrage ; le rendement en matière sèche a été comparable à celui de Sulla coronaria, déjà sélectionné dans la zone Méditerranéenne (4.8 vs. 3.5 T MS/ha) (Cordoba *et al*., 2013). Le sorgho et l'orobe sont exploités principalement pour leurs grains et paille. Le rendement moyen en grains du sorgho et de l'orobe a été dans la marge des valeurs rapportées pour des écotypes Méditerranéens (Larbi *et al*., 2011 ; Sugg *et al*., 2017). Certains écotypes avaient donné des rendements assez intéressants arrivant à 7.7 T/ha pour le sorgho et qui peuvent être exploités dans la sélection des écotypes performants.

Les degrés-jours de croissance (GDD) sont une mesure de l'accumulation de chaleur utilisée pour prédire le développement des plantes. Son importance réside dans sa capacité à prévoir avec précision la date à laquelle une culture atteindra un stade phénologique prédéterminé (McMaster & Wilhelm, 1997). Concernant les paramètres phénologiques, le Sulla flexuosa avait besoin de plus d'accumulation de chaleur pour initier la floraison (1224,4 °C), par rapport à l'orobe (813,4 °C) et au sorgho (883,9 °C) (Table 9). Cela est probablement dû à ses caractéristiques génétiques. La période de floraison des écotypes de Sulla flexuosa et de l'orobe était le mois d'avril, alors que le Sulla flexuosa a été semé en novembre, et l'orobe, jusqu'en février.

Au Nord du Maroc, le sorgho est la seule plante qui peut valoriser le sol en période estivale dans les conditions pluviales (Chentouf *et al*., 2015). Puisque les périodes de coupes de Sulla flexuosa coïncident avec les dates de semis du sorgho, cette légumineuse pourrait être un bon précédent cultural et pourrait être utilisée en rotation avec le sorgho. Les légumineuses sont couramment utilisées dans les systèmes de culture comme source d'azote, par la fixation symbiotique de l'azote atmosphérique pour les cultures ultérieures et le maintien des niveaux d'azote du sol (Gathumbi *et al*., 2002).

Plusieurs auteurs ont mentionné une amélioration des rendements dans les systèmes de rotation avec les légumineuses par rapport aux monocultures (Gathumbi *et al*., 2002). Des études sur les dates optimales de semis du sorgho doivent être réalisées, et les écotypes les plus précoces ou les plus tardifs peuvent être choisis pour coïncider avec ces dates. Le Sulla flexuosa et l'orobe peuvent en général être conduits en rotation avec les céréales déjà utilisées dans la région. Ils vont permettre, d'une part, d'améliorer la fertilité des sols, d'autre part, de contribuer à équilibrer les calendriers alimentaires déficitaires en sources protéiques.

Caractérisation bromatologique des écotypes. *Variabilité de la valeur nutritive des écotypes.* A part les tannins hydrolysables, le coefficient de variation des écotypes de Sulla flexuosa a été faible. Le coefficient de variation génotypique et phénotypique a été aussi faible pour le sorgho et l'orobe par rapport aux paramètres agro-morphologiques. Cependant, une héritabilité élevée couplée à un progrès génétique élevé a été trouvée pour la majorité des paramètres des grains et de la paille du sorgho et de l'orobe. Ceci montre que la sélection pour les paramètres bromatologiques est possible, grâce à la transmission et l'amélioration des caractéristiques liées à la bonne valeur nutritive des grains et de la paille à la génération suivante.

Valeur nutritive des écotypes. Le choix des meilleurs écotypes à utiliser dans une région particulière ou un programme de sélection ne dépend pas seulement de leur productivité. La valeur nutritive joue un rôle crucial dans le soutien des programmes de sélection végétale. L'objectif à long terme de la présente étude est de développer des variétés à haute valeur nutritive des grains puisque l'orobe et le sorgho sont principalement utilisés pour leurs grains dans le Nord du Maroc (Hmimsa & Ater, 2008 ; Boukrouh *et al*., 2021). Pour le Sulla flexuosa, l'objectif a été de déterminer les écotypes ayant une valeur nutritive élevée et le stade de coupe pour obtenir cette valeur. Le Sulla flexuosa a montré une teneur moyennement élevée en protéines brutes (19,4% MS) qui est proche des valeurs rapportées pour la même espèce dans la région Méditerranéenne (Zirmi-Zembri & Kadi, 2020). Heureusement, même si cette teneur diminuait depuis le stade bourgeonnement jusqu'au stade pleine floraison, le rendement en protéines augmentait avec l'avancement des stades. Pour les fibres, sauf l'ADL qui a été élevé par rapport à d'autres écotypes (Zirmi-Zembri & Kadi, 2016), la cellulose brute, les NDF et ADF ont été dans la marge de leurs valeurs. Pour les grains de sorgho, la teneur en protéines se situait également dans la fourchette (10,5% MS) des valeurs obtenues dans la région Méditerranéenne.

Cependant, les valeurs obtenues pour l’orobe (22,9% MS) étaient faibles par rapport aux valeurs obtenues dans la région Méditerranéenne (Larbi *et al*., 2011).

Corrélations entre les paramètres. L’étude des corrélations entre différents paramètres est cruciale car elle permet de réaliser la sélection indirecte des paramètres hérités quantitativement et influencés par des effets génétiques. Pour le Sulla flexuosa, la sélection indirecte d’un rendement en matière sèche élevé peut être réalisée par la sélection d’écotypes avec un nombre élevé de feuilles, branches secondaires et totales, et diamètre. Des résultats similaires ont été rapportés pour d’autres légumineuses fourragères dû à l’augmentation de l’activité photosynthétique (Bhattarai *et al*., 2018).

Pour le sorgho, la corrélation positive du rendement en grain avec le poids de la panicule, le nombre de grains par plante et le poids de mille grains montre que la sélection pour des rendements élevés peut être basée sur ces paramètres. Pour l’orobe, le rendement en grain a été corrélé seulement avec l’indice de récolte. Pour l’orobe et le sorgho, la corrélation négative entre le rendement en grains et le nombre de jour pour commencer la floraison montre que la phénologie est un paramètre important à prendre en considération pour choisir l'écotype approprié pour une région particulière. Pour l’orobe et le Sulla flexuosa, certains paramètres végétatifs ont été positivement corrélés, ce qui suggère l'orientation possible de certains écotypes vers la production de foin et de fourrage. Des corrélations similaires ont été signalées pour d'autres espèces, car il s'agit de paramètres améliorant la photosynthèse (Amitrano *et al*., 2021).

Classification des écotypes. Les écotypes des 3 plantes ont été classifiés en un nombre de groupes différents, basés sur des paramètres différents. La classification des écotypes de Sulla flexuosa a donné 4 groupes. Les premier et deuxième groupes étaient caractérisés par de faibles valeurs de fibres, tandis que les troisième et quatrième groupes étaient caractérisés par le contraire. Le deuxième groupe était caractérisé par des paramètres de rendement inférieurs, contrairement aux autres groupes. Les troisième et quatrième groupes étaient opposés selon la phénologie. Les premier et deuxième groupes étaient intermédiaires. Le quatrième groupe était caractérisé par une faible valeur nutritive. À l'exception du premier groupe, les autres groupes avaient de faibles paramètres de reproduction. L'écotype E1 était le meilleur écotype car il avait la productivité et la valeur nutritive les plus élevées (faible NDF et CF, digestibilité élevée, DMY, ME et OM), avec un diamètre de tige élevé, et probablement une protection contre la verse (Gawłowska *et al*., 2021). Cet écotype avait un double avantage car il présentait également des paramètres de reproduction élevés, qui sont importants pour la dissémination des graines. Pour le sorgho, les écotypes ont été classifiés en 5 groupes. Les trois écotypes (E5, E12 et E15) du premier groupe étaient caractérisés par des grains ayant une valeur nutritive et un rendement faible, et une teneur élevée en fibres et en phénols. Le deuxième groupe était similaire au premier à l'exception de la phénologie (écotypes ultérieurs) et de la faible teneur en fibres. Des traits opposés du premier groupe caractérisent le troisième groupe. Des facteurs antioxydants élevés et des valeurs intermédiaires pour les autres composants caractérisent le seul écotype (E6) présent dans le quatrième groupe. Le cinquième groupe se distinguait par sa valeur nutritive plus élevée, des paramètres végétatifs plus intéressants et des valeurs plus faibles pour les autres paramètres.

Concernant l'orobe, la « heatmap » ou la carte thermique a permis de distinguer 5 groupes. Le premier groupe composé de l'écotype (E8) était caractérisé par des paramètres végétatifs élevés et des composantes de la valeur nutritive et de rendement en grain faibles. Cet écotype pourrait être destiné à la production de foin. Son poids élevé de mille graines on en fait un candidat intéressant pour améliorer ce paramètre. Cependant, son faible rendement en grain et son indice de récolte pourraient entraver la sélection. Le deuxième groupe avait un rendement en grains et des paramètres végétatifs intermédiaires à élevés, alors qu'à l'exception de l'écotype (E11), ils avaient des paramètres bromatologiques faibles. E11 semble être un écotype plus prometteur car il combine une production à haut rendement et une valeur nutritive élevée. D'autre part, une faible valeur nutritive malgré le rendement moyen caractérise le seul écotype (E1) regroupé en troisième groupe. Les quatre écotypes du quatrième groupe (E16, E3, E2 et E6) étaient les moins intéressants selon tous les groupes de variables, sauf E2 qui avait une énergie métabolisable élevée. Ces deux derniers groupes devraient être évités par les agriculteurs. Le dernier groupe était caractérisé par des valeurs intermédiaires pour tous les paramètres. Il a été subdivisé en deux groupes. Les écotypes (E4, E13 et E14) étaient caractérisés par un rendement en grain élevé, un indice de récolte, des phénols et des FRAP et des paramètres faibles de composantes du rendement. Les autres écotypes étaient caractérisés par le contraire.

Corrélation entre les distances phénotypiques avec les distances géographiques. Le test de Mantel a été réalisé pour relier les données environnementales et agro-morphologiques et aux valeurs bromatologiques. Cette analyse a montré qu'aucune corrélation significative n'a été trouvée entre les paramètres morpho-phéno-agronomiques et bromatologiques et les données géographiques (latitude et longitude) des sites de collecte des écotypes de l'orobe et du sorgho. Ces plantes déjà cultivées par les éleveurs, n'avaient pas développé des caractéristiques morphologiques pour s'adapter aux conditions environnementales spécifiques de chaque site, probablement dû à l'échange des semences entre régions dans les marchés hebdomadaires (Ater & Hmimsa, 2006). Le contraire était attendu pour le Sulla flexuosa qui est une plante sauvage spontanée dans la région du Nord du Maroc. Les scientifiques ont discuté de la dérive génétique, de la pression de sélection et de l'environnement comme d'autres facteurs majeurs pouvant entraîner une plus grande diversité que la distance géographique (Star & Spencer, 2013). D'autres scientifiques ont encouragé l'utilisation des marqueurs génétiques pour mieux cerner l'adaptabilité des écotypes (Marghali *et al.*, 2016). Par conséquent, le choix des écotypes intéressants devrait être basé sur le niveau des écotypes plutôt que sur la zone géographique.

Effet des plantes sur la production et la qualité du lait. La première étude dans le cadre de cette thèse, a démontré en analyse in vitro l'intérêt des écotypes de Sulla flexuosa comme ressources alternatives pour les caprins dans la région du Nord du Maroc. Avant de les recommander dans l'alimentation des caprins, il est primordial d'étudier leurs effets sur la production et la qualité du lait. Pour ce faire, un essai a été réalisé sur les chèvres locales de race Beni Arouss dans la région du Nord du Maroc durant la période de lactation (Chapitre 6 - Étude 4).

L'étude sur les écotypes locaux a montré une teneur en protéines intéressante. Cependant, cette teneur diminue avec l'avancement des stades phénologiques. Le stade début de floraison a été choisi comme le meilleur compromis entre rendement en matière sèche, teneur en protéines et digestibilités. En raison de l'évolution de la valeur nutritive avec l'avancement des stades et la durée de l'essai qui était de 90 jours, les plantes de Sulla flexuosa ont été coupées au début de floraison et ont été conservées sous forme de foin. Le fanage a été réalisé à moindre coût, nécessitant juste le retournement des plantes pour accélérer le processus du séchage.

Les rations contenaient deux taux d'incorporations du foin de Sulla flexuosa (70 %MS (SF70) et 35%MS (SF35)). Elles ont été comparées à une ration témoin (Co) contenant le foin de luzerne et la paille de blé pour équilibrer les fibres entre les rations. Cette ration est choisie comme témoin afin de convaincre les éleveurs de l'importance des ressources alternatives qui peuvent être comparables aux aliments conventionnels, surtout que le Sulla flexuosa peut être produite à moindre coût par rapport à la luzerne puisqu'elle est cultivée sous des conditions pluvieuses et nécessite moins d'intrants**.**

L'incorporation proportionnelle du foin de Sulla flexuosa a donné des rations avec des teneurs proportionnelles en tannins condensés. Cependant, même si les rations expérimentales contenaient des taux élevés en tannins condensés par rapport à la ration témoin, leur concentration était inférieure à 3 à 5 % de MS, rapporté dans la littérature comme le seuil au-dessus duquel l'activité ruminale et la digestion des aliments sont altérées (Rufino-Moya *et al*., 2019).

Concernant les résultats obtenus, les rendements laitiers journaliers et totaux de la chèvre Beni Arouss, qui est à double usage (viande et lait), étaient inférieurs à ceux des races caprines laitières mais proches de celles rapportées dans la littérature pour les chèvres locales de la race Beni Arouss et Draa (Boujenane *et al*., 2010 ; El Otmani *et al*., 2021a). Les résultats ont appuyé la possibilité de la valorisation du Sulla flexuosa sans effet négatif sur la production laitière ou bien la composition chimique. Ces résultats sont probablement dûs aux rations iso-énergétiques et iso-protéiques et aux teneurs faibles en tannins. Dans d'autres études, un effet dépressif général sur la production laitière des caprins a été rapporté avec des concentrations en tannins supérieures à la présente étude (De Lucena *et al*., 2018).

Les teneurs en matière grasse observées (2,16 à 2,32 %) étaient inférieures aux valeurs rapportées dans la littérature pour les caprins (4,0–4,5 %) (Lad et al., 2017), mais similaires à celles d'El

Otmani *et al*., (2021a) avec la même race mais des régimes très différents. Une faible proportion en matière grasse pourrait être une caractéristique du lait de la race caprine Beni Arouss.

L'absence de différence concernant la production et la composition du lait entre les rations, permettra donc aux éleveurs, surtout dans les systèmes d'élevage semi-extensifs, d'optimiser la production laitière et fromagère en utilisant des ressources alternatives locales à faible coût c'est-à-dire en adoptant le foin de Sulla flexuosa dans l'alimentation de la chèvre en lactation.

Concernant le profil des acides gras du lait, il a été significativement affecté par l'incorporation du foin de Sulla flexuosa. Le C14:0 du lait est synthétisé dans la glande mammaire. Le C14:1 provient exclusivement de la désaturation de C14:0 sur ce site (Lock & Garnsworthy, 2003). Le rapport Δ9C14 est considéré le marqueur de l'activité du delta-9 désaturase dans la mamelle (De Lucena *et al.,* 2018). La proportion inférieure de C14:1 observée avec les rations Co et SF35 a été confirmée par le rapport Δ9C14 plus faible observé dans les groupes Co et SF35 par rapport au SF70, avec respectivement 0,045 vs 0,061. Semblable à nos résultats, Purba et al., (2020) et Buccioni et al., (2015) ont suggéré que les tannins ingérés peuvent augmenter l'expression de l'activité du Δ9 désaturase, comme observé dans le groupe SF70.

L'effet du niveau d'incorporation du foin de Sulla flexuosa suggère qu'elle pourrait avoir un impact dose-dépendant. Les résultats ont montré une proportion C17:0 plus faible pour le SF35 et le SF70 par rapport au régime témoin (1,08 vs 1,55 g/100 g FA, respectivement, pour les régimes SF35 et SF70 et le régime Co). Les microorganismes du rumen synthétisent le C17:0 par élongation du propionate. Cabiddu *et al*., (2009) avaient rapporté une augmentation du pourcentage de C17:0 dans le lait de brebis recevant une supplémentation en Polyéthylène Glycol dans l'alimentation, cette molécule a la capacité de neutraliser les tannins. Cette plus faible proportion de C17:0 dans le lait pourrait être due à la modification due au microbiote ruminal, altérant le processus de saturation dans le rumen (Dias Junior et al., 2023). Les résultats avaient montré également une plus faible proportion de C18:0 du lait avec SF70 (8,06 contre 12,55 g/100 g FA, respectivement pour les rations SF70 et les rations Co et SF35). L'observation pourrait s'expliquer par différentes hypothèses : la proportion plus faible de C18:0 dans la ration SF70 et la modification du microbiote ruminal et donc la biohydrogénation (comme pour C17:0) et une désaturation plus élevée de C18:0 en C18:1 dans la glande mammaire, comme le montre la valeur élevée de Δ9C18.

La modification du processus de biohydrogénation ruminale pourrait également expliquer la proportion plus élevée de C18:2n-6 et C18:3n-3 dans le lait SF70 (Buccioni *et al*., 2015). Habituellement, environ 90 % de C18:3n-3 et C18:2n-6 sont biohydrogénés dans le rumen (Bernard *et al*., 2009). Ces deux acides gras représentaient 51%, 57%, et 69 % de la proportion d'AGPI observée, respectivement, pour les régimes Co, SF35 et SF70. Un niveau plus élevé du Sulla flexuosa était donc requis pour observer un effet. Les acides gras C20:3n-6 et le C20:4n-6 résultent des allongements et désaturations de C18:2n-6. Une proportion plus faible—pas SF dose-dépendante – de ces deux AG dans le lait SF35 et SF70, malgré une proportion plus élevée de C18:2n-6 dans le lait SF70, est difficile à expliquer. Le Sulla flexuosa pourrait limiter les activités de certains enzymes. La capacité de conversion de C18:3n-3 en AG n-3 à longue chaîne bénéfiques pour la santé (acide eicosapentaénoïque (EPA, 20:5n-3) et acide docosahexaénoïque (DHA, 22:6n-3)) est limitée dans le métabolisme humain, ce qui renforce l'importance de son apport alimentaire. Comme C18:2n-6, la proportion de lait en C18:3n-3 était plus importante dans le groupe SF70 que dans les deux autres groupes (respectivement, 2,73 contre 1,39 g/100g FA). Bien que la concentration en DHA dans le lait soit également significativement plus élevée dans le groupe SF70, les proportions de lait en EPA ne différaient pas entre les trois groupes. Une absence d'augmentation parallèle des AG n-3 et n-6 entre les groupes a été ainsi observée ; en fait, l'impact des tannins sur la compétition enzymatique entre le métabolisme des n-3 et n-6 est pas comprise.

Les sommes, les ratios et les indices des acides gras calculés pour les aliments sont généralement considérés comme des indicateurs au consommateur pour choisir un aliment sain. Par conséquent, les acides gras n-3 et n-6 à longue chaîne sont considérés comme des biorégulateurs d'importants processus cellulaires et sont associés avec la fonctionnalité et le développement du système immunitaire. Les AGMI améliorent la santé du système cardiovasculaire en diminuant l'inflammation et en réduisant les lipoprotéines de basse densité (LDL). Les AGS entraînent des conséquences négatives sur la santé humaine en augmentant le danger de troubles cardiovasculaires et le niveau de cholestérol plasmatique sanguin (Chen & Liu, 2020). Dans le présent essai, en moyenne, les proportions des AGPI et des AGMI observées dans les trois groupes étaient plus élevées et, par conséquent, la proportion d'AGS était inférieure aux valeurs rapportées pour le lait de chèvre, avec respectivement 3,7, 24,5 et 68,8% pour les AGPI, AGMI et AGS (Markiewicz-Kęszycka *et al*., 2013). La proportion croissante du foin de Sulla flexuosa dans les régimes a augmenté les proportions d'AGMI et a diminué les proportions des AGS dans le lait. Le niveau des AGMI significativement plus élevé avec le SF70 était dû aux niveaux plus élevés de C18:2n-6 et C18:3n-3. La différence dans les proportions des AGMI dans le lait selon le groupe était principalement causée par les différences observées dans les proportions de C18:1n-9 ; ce dernier a des effets bénéfiques sur la santé humaine (Chen & Liu, 2020). Semblable à l'étude actuelle, une plus grande proportion d'AGMI et une plus faible proportion d'AGS dans le lait provenant de chèvres et de brebis complétées par des régimes riches en tannins ont été signalés et expliqués par une modification de l'activité de la population microbienne ruminale se manifestant par une diminution de la bio-hydrogénation des AG (De Lucena *et al*., 2018).

Dans le lait de chèvre, le rapport AGMI/AGS varie de 0,04 à 0,18, le rapport n-6/n-3 de 1,49 à 6,60, le rapport AI de 1,89 à 2,77, le rapport TI de 2,04 à 3,20 et le rapport LA/ALA de 1,15 à 10,67 (Chen & Liu, 2020 ; Bodnár *et al*., 2021 ; El Otmani *et al*., 2021a; Kasapidou *et al*., 2022). Les ratios

AGPI/AGS et LA/ALA détectés dans notre étude se situaient dans la marge alors que les autres indicateurs étaient inférieurs. L'incorporation de 70% de Sulla flexuosa a significativement amélioré le rapport AGPI/AGS et les indices AI et TI.

De Lucena *et al*., (2018) ont rapporté une diminution des indices d'athérogénicité et thrombogénique dans le lait de chèvre Saanen recevant jusqu'à 28 g de tannins par kg de MS de régime ; le régime SF70 contenait 15,2 g/kg de MS. L'incorporation du foin de Sulla flexuosa n'affecte pas le rapport n-6/n-3. Le rapport recommandé varie autour de 1-2 :1 (Simopoulos, 2016), ce que nous avons observé dans les trois groupes. Certains auteurs rapportent des ratios arrivant jusqu'à 6,6 dans le lait de chèvre (voir ci-dessus). Un rapport n-6/n-3 optimal pourrait avoir des effets anti-inflammatoires et anticancéreux. En revanche, le C18:2n-6 et les AG n-6 pourraient protéger contre les maladies cardiovasculaires (Liput *et al*., 2021). Certains auteurs (Chen & Liu, 2020) s'interrogent ainsi sur l'utilité de certains de ces indicateurs, arguant qu'ils ne reflètent pas l'effet réel de chaque AG présent dans un groupe d'AG. Par exemple, tous les AG n-3 n'impactent pas le métabolisme humain/animal de la même manière. Dans le présent essai, les AG les plus intéressants, tels que C18:1n-9, C18:2n-6, C18:3n-3 et DHA, ont été observés à des concentrations plus élevées dans le lait SF70.

Le lait SF70 a présenté la teneur la plus élevée en polyphénols, ce qui s'explique par la teneur en polyphénols la plus élevée de ce régime et son transfert au lait via la circulation sanguine (Di Trana *et al*., 2015). Les phénols totaux, jouant un rôle antioxydant, et le FRAP et le DPPH, qui indiquent l'activité antioxydante (Delgadillo-Puga *et al*., 2019), ont montré les valeurs les plus élevées dans le lait SF70.

Di Trana *et al*., (2015) ont également rapporté une corrélation positive entre l'activité antioxydante du plasma de chèvre et l'ingestion des tannins condensés de Sulla coronaria. Les valeurs de DPPH dans la présente étude étaient inférieures aux valeurs de 71% rapportées par Lakram *et al*., (2019) pour les effets du tourteau pressé d'*Argania spinosa* détoxifié sur les propriétés antioxydantes dans le lait de chèvre alpine. Alyaqoubi *et al*., (2014) ont également rapporté des valeurs plus élevées mais probablement en raison de leur méthode d'extraction avec HCl au lieu de l'analyse directe. Autres auteurs (Feng *et al*., 2019 ; Tian *et al*., 2019) ont également rapporté des valeurs proches de celles observées dans le présent essai (18–36 %), avec soit la pulpe de jujube ou le maïs violet (*Zea mays* L.) riche en anthocyanes comme antioxydants.

Pour l'activité FRAP, les valeurs observées (0,760–1,103 mmol $FeSO_4$/L) étaient supérieures à la valeur 0,19 mmol $FeSO_4$/L considérée comme la valeur critique pour limiter les dommages oxydatifs au lait (Amamcharla & Metzger, 2014). L'activité antioxydante dans le lait SF35 était différente pour ces deux paramètres, pas significativement différente de SF70 pour DPPH, et non significativement différente du Co pour FRAP ; ce résultat était probablement dû aux méthodes utilisées. Il est donc important d'analyser les paramètres DPPH et FRAP afin de bien qualifier l'activité antioxydante.

Les proportions plus élevées d'AGPI, AGMI, n-3, n-6 et n-9 dans le lait SF70 pourraient donc également être dues à la protection des AGS contre la biohydrogénation et/ou l'oxydation par les teneurs plus élevées en phénols et en tannins condensés du lait et de la ration.

Les données de cette étude ont montré que l'incorporation du foin de Sulla flexuosa dans la ration des chèvres allaitantes n'avaient pas affecté la production et la composition du lait. Le profile en acides gras a été amélioré par les deux taux d'incorporation (35 et 70% MS). Les sommes, ratios et indices calculés montrent qu'elle pourra avoir un effet bénéfique sur la santé des consommateurs. En revanche, l'incorporation de 70% de MS est nécessaire pour garantir une bonne activité antioxydante.

Ces résultats sont encourageants pour les éleveurs caprins, surtout que le Sulla flexuosa est une plante endémique à la région du Nord du Maroc. Son adaptation aux sols de la région du Nord ainsi que la possibilité de sa conduite sans irrigation rendent son coût de production faible, ce qui nous pousse à la suggérer comme une source de protéines à faible coût. Les rendements qu'elle a donné dans **la première étude** étaient intéressants par rapport à d'autres espèces du genre *Hedysarum* dans la région Méditerranéenne. Les teneurs faibles en tannins n'avaient pas d'effet négatif sur la production laitière.

La culture du Sulla flexuosa peut être un bon précédent cultural pour les cultures de printemps, notamment le sorgho, par sa capacité à fixer l'azote atmosphérique et à diminuer les besoins en fertilisants. La préparation du foin n'a pas nécessité une grande technicité ou bien des heures de travail supplémentaires. Par conséquent, le Sulla flexuosa devrait être suggérée comme une ressource alternative disponible pour le fourrage et les protéines dans l'alimentation des chèvres en lactation.

Dans la région du Nord du Maroc, la production de viande caprine est aussi une activité qui génère des revenus pour les éleveurs. La **deuxième et la troisième étude** ont montré la présence des écotypes d'orobe et de sorgho avec des rendements en grains élevés par rapport aux écotypes Méditerranéens des mêmes espèces ainsi qu'une valeur nutritive intéressante des grains. La paille avait une valeur nutritive faible, c'est pourquoi on a choisi d'incorporer les grains d'orobe et de sorgho dans la ration des chevreaux afin d'évaluer leurs effets sur les performances de croissance et, la qualité de la carcasse et de la viande caprine.

Performance de croissance, caractéristiques de la carcasse et qualité de la viande de chevreaux mâles supplémentés par les grains d'orobe et de sorgho. Cette étude a été menée pour évaluer l'effet de l'incorporation des grains d'orobe et de sorgho dans la ration des chevreaux de race Beni Arouss sur les performances de croissance, les caractéristiques de la carcasse et la qualité de la viande. Les animaux ont été répartis en 3 groupes. Le groupe témoin a reçu une alimentation conventionnelle à base de foin d'avoine, d'orge et de fève. Dans le premier groupe testé, la fève a été remplacée par la vesce amère, et dans le deuxième, l'orge par le sorgho. À la fin de l'essai, les animaux ont été abattus, et les caractéristiques de la carcasse et la qualité de la viande des muscles *longissimus dorsi* (LD) et *semimembranosus* (SM) ont été déterminées.

Plusieurs facteurs influencent la composition de la carcasse des chevreaux, tels que le niveau etle type de concentré, le type de fourrage et le niveau et la source de protéines (Pophiwa *et al*., 2020). Les résultats ont montré que la quantité de matière sèche ingérée a été plus faible pour le groupe qui a reçu les grains d'orobe comme source de protéines. Cette faible ingestion des grains d'orobe pourrait être due à la présence de plusieurs facteurs antinutritionnels, notamment les inhibiteurs de protéases et la canavanine, qui leur confèrent un goût amer ou des effets métaboliques négatifs (Sadeghi *et al*., 2009). La canavanine est un acide aminé non protéique analogue à l'arginine, conduisant à la production de protéines non fonctionnelles (Sadeghi et al., 2009).

La teneur en protéines de la viande dans ce groupe était inférieure à celle des autres groupes. Cependant, l'apport en nutriments n'a pas été influencé dans une autre étude où le tourteau de soja a été remplacé par des grains d'orobe dans l'alimentation des agneaux (Haddad, 2006). Les caractéristiques des écotypes de l'orobe ou bien le niveau d'incorporation des grains (15% pour l'alimentation des agneaux contre environ 30% pour la présente étude) pourraient expliquer une telle différence.

Un pourcentage plus faible d'orobe dans la ration alimentaire pourrait améliorer l'ingestion de la matière sèche. Il est intéressant de noter que l'ingestion de la matière sèche était similaire dans les groupes SRG et Co, tandis que le gain moyen quotidien était plus faible. Le gain moyen quotidien observé dans les groupes testés était donc dû à une ingestion plus faible de la matière sèche et/ou à une efficacité alimentaire plus faible. Par conséquent, le taux de conversion alimentaire était plus élevé pour les deux régimes testés. Ce paramètre était plus élevé que les valeurs rapportées dans la région Méditerranéenne (Titi *et al*., 2008), mais était dans la plage des valeurs calculées pour les chevreaux de la même race recevant le tourteau d'olives et des cladodes de cactus comme ressources alimentaires alternatives (El Otmani *et al*., 2021b). Cependant, les gains moyens quotidiens observés étaient supérieurs aux 35 g/jour rapportés par les mêmes auteurs (El Otmani *et al*., 2021b). Le poids corporel final, le poids de la carcasse chaude et froide et le rendement de la carcasse étaient similaires entre les groupes et dans la marge des valeurs rapportées par El Otmani *et al*., (2021b).

L'amidon dans les grains d'orge est plus fermentescible que dans les grains de sorgho ; il en est de même pour l'amidon dans les grains de fève par rapport aux grains d'orobe (Crépon *et al*., 2010 ; Taghavi *et al*., 2023). Cela pourrait expliquer le pH ruminal inférieur pour le groupe Co, intermédiaire pour le groupe BV (orobe ou bitter vetch) et supérieur pour le groupe SRG (sorgho). Cependant, les trois valeurs étaient dans la même marge de pH normale (5,5-7,5), mais avec des valeurs légèrement inférieures au pH ruminal optimal (6,5).

Les régimes alimentaires ont eu très peu d'effets sur les composantes non-carcasse. Étonnamment, la proportion du tube digestif vide était plus élevée dans le groupe SRG. Cela pourrait être associé à un gain moyen quotidien plus faible associé à une consommation alimentaire inchangée, mais la raison est peu claire. La proportion de peau des groupes BV et SRG était inférieure à celle du groupe témoin. La méthionine et la cystéine sont le premier facteur limitant dans les grains d'orobe (Sadeghi *et al*., 2009). De plus, les grains de sorgho contiennent moins de méthionine que l'orge (Al-Marzooqi, 2020). La cystéine et la méthionine sont généralement les acides aminés limitants pour la synthèse de la kératine et pour la production de viande (Cao *et al*., 2021). De plus, ces acides aminés limitants sont priorisés pour la croissance musculaire plutôt que pour la croissance des téguments. Les différences présumées dans la teneur en ces acides aminés dans les régimes alimentaires pourraient expliquer les différences observées pour la proportion de peau (plus faible dans les groupes SRG et BV) et la teneur en protéines des muscles (plus faible dans les deux muscles BV). L'indice de compacité de la carcasse est un indicateur économique important car le marché de la viande préfère les carcasses plus compactes (Nascimento *et al*., 2018). Les rations distribuées n'ont pas affecté ce paramètre. Cependant, les index musculaires et de conformité étaient inférieurs pour les chevreaux ayant reçu les grains d'orobe ou de sorgho, ce qui pourrait avoir un effet négatif sur leur incorporation et, par conséquent, sur leur utilisation. Cependant, les indices musculaires et de conformité actuels étaient plus élevés par rapport à ceux rapportés par Lahkim Bennani *et al*., (2022) et El Otmani *et al*., (2021b) avec des chevreaux de la race locale Beni Arouss.

Immédiatement après l'abattage, le muscle *semimembranosus* du groupe SRG présentait une valeur de pH plus élevée, et celui de BV présentait une valeur plus faible que le groupe Co. Après la glycolyse, le pH est resté inchangé. De plus, la capacité de rétention de l'eau n'a pas changé en fonction de la ration distribuée. La couleur de la viande est le premier critère utilisé par les consommateurs pour juger la qualité de la viande. La couleur rouge est influencée par le niveau et l'état de la myoglobine (Zhu *et al*., 2022). Vioque *et al*., (2020) ont rapporté que les polyphénols présentent des propriétés antioxydantes qui prolongent la stabilité de la couleur rouge de la viande indirectement en retardant l'oxydation en métmyoglobine (Zhu *et al*., 2022). L'absence de différence de couleur de la viande pourrait être due à la faible concentration en polyphénols. En effet, malgré la plus forte teneur en polyphénols totaux observée dans la ration SRG, les teneurs en polyphénols totaux dans les trois rations étaient inférieures à 20-30 g/kg de MS, rapportées être le seuil pour affecter la stabilité antioxydante de la viande (García *et al*., 2019). La tendreté est le trait organoleptique le plus important contribuant à l'acceptation des consommateurs et à la satisfaction alimentaire de la viande (Garmyn, 2020). Le test de force de cisaillement est généralement utilisé pour mesurer la tendreté de la viande et il est positivement affecté par l'augmentation de la teneur en collagène (Florek *et al*., 2022). L'absence de différences entre les rations pour les valeurs de force de cisaillement dans les deux muscles était attendue en raison des faibles différences de pH final entre les groupes. Le LD cuit actuel était plutôt tendre (61,8 N) par rapport aux valeurs rapportées dans la littérature (47-94 N) (Cao *et al*., 2021 ; Hwang & Joo, 2017 ; Mwangi *et al*., 2017).

Les régimes alimentaires testés ont été complétés avec de l'urée pour être isoprotéiques. Cependant, la viande de BV a montré une teneur en protéines inférieure, probablement due à la teneur plus faible en acides aminés essentiels et limitants (méthionine et cystéine) de l'orobe (Sadeghi *et al*., 2009), comme expliqué ci-dessus, et pourrait également être due à la présence de la canavanine. De plus, les résultats de la teneur en protéines de la viande étaient proches de 23% de MS rapportés par Mazhangara *et al*., (2019) pour la viande caprine. Cette hypothèse sur les acides aminés pourrait être confirmée par la teneur en urée plus élevée dans la ration contenant de l'orobe. Donc malgré l'équilibre de l'azote via l'urée ajoutée dans la ration, la différence en acides aminés des rations pourrait affecter la qualité de la viande.

Par ailleurs, selon Nichols *et al*. (2022), l'apport journalier en urée ne devrait pas dépasser 10g/kg MS ou bien 1% MS. Quant à Chenost et Kayouli (1997), ils énonçaient des normes d'utilisation de l'ordre de 15 g d'urée par jour pour des ovins et des caprins adultes, nourris à base de fourrages pauvres. Dans le lot recevant l'orobe, les animaux ont ingéré 1 346 g/j de ration dont la teneur en MS était de 90,7% ; ils ont donc ingéré 1220,8 g/j MS et donc 14,3 g/j d'urée, la concentration étant de 11,7 g d'urée /kg MS. Selon Nichols *et al*. (2022), cette valeur dépasse la limite quotidienne et pourrait induire une toxicité qui se manifesterait, entre autres, par un pH élevé, aux alentours de 8 ; ce qui ne fut pas le cas pour notre étude (pH = 6,08). Dans leur article de synthèse, Nichols et al. (2022) mentionnaient que la réponse des animaux à l'ingestion de l'urée varie en fonction de la composition de la ration et de la méthode de distribution. Cette atténuation de l'effet de l'urée dans notre étude pourrait être due à la distribution de la ration en deux fois (matin et soir) et à l'adaptation des animaux à l'urée.

L'incorporation des grains d'orobe et de sorgho dans la ration des chevreaux n'altère pas les caractéristiques d'abattage. Cependant, ils avaient montré quelques effets négatifs sur le gain quotidien moyen et l'ingestion de la matière sèche pour le niveau d'incorporation (30%) utilisé dans cet essai.

Le profil d'acides gras a un impact crucial sur la qualité de la viande (Mazhangara *et al*., 2019).Dans la présente étude, les profils en AG de la viande ne sont pas faciles à interpréter en raison de tendances peu claires. Cela se traduit par l'absence de différences dans les sommes de familles d'AG (AGS, AGMI, AGPI). Cependant, dans l'ensemble, le SM a montré des quantités plus élevées d'acides gras saturés à courte chaîne et des quantités plus faibles d'acides gras saturés à longue chaîne. Cela pourrait être dû à la différence dans le métabolisme des AG entre les deux muscles. Les effets des rations alimentaires étaient fortement variables et difficiles à interpréter, comme indiqué ci-dessus. Cependant, il est intéressant de considérer la famille des AGPI fréquemment représentée dans l'alimentation d'origine végétale, c'est-à-dire C18:2 n-6 et C18:3 n-3, car ils sont importants pour améliorer la qualité de la viande chez les ruminants. Il y avait moins de C18:2 n-6 dans le muscle SM. Cela pourrait s'expliquer par le fait que ce muscle contenait plus de graisse, qui diluait le C18:2n-6, principalement d'origine cellulaire membranaire. En revanche, l'absence d'effet musculaire significatif sur le C18:3n-3 est probablement due au faible taux de graisse dans les différentes rations alimentaires. Cependant, le niveau inférieur de cet AG dans les muscles du groupe SRG - et par conséquent le rapport AGPI/AGS inférieur - est conforme à sa quantité inférieure mesurée dans la ration alimentaire correspondante. Les rations alimentaires n'ont pas semblé affecter la séquence de saturation-élongation des AGPI à plus longue chaîne. Compte tenu des faibles niveaux de graisse dans les régimes alimentaires, il n'y avait aucune raison de s'attendre à un tel effet. L'incorporation de grains d'orobe et de sorgho n'a pas affecté le rapport n-6/n-3. Cependant, le type de muscle a impacté cet indice avec 3,83 et 2,17 pour les muscles LD et SM, respectivement. La plage souhaitable pour cet indice est de 14:1 pour aider à maintenir une vie équilibrée et saine (Ponnampalam *et al*., 2021). L'incorporation des grains de sorgho a augmenté l'indice de promotion de la santé dans le muscle SM. Cela a probablement peu d'importance. L'indice HPI était dans la plage des valeurs de 0,16 à 0,68 rapportées pour les produits laitiers (Chen & Liu, 2020). Le régime SRG a augmenté l'indice thrombogène et a diminué l'indice de la valeur nutritive. Des recommandations pour consommer des aliments à faible TI et à NVI élevé ont également été suggérées pour améliorer la santé humaine (Chen & Liu, 2020). Cependant, le NVI de 3,51 était encore plus élevé que les valeurs rapportées pour les chèvres, tandis que le TI de 1,14 était intermédiaire (Taboada *et al*., 2022 ; Yang *et al*., 2022).

L'incorporation des grains d'orobe et de sorgho dans l'alimentation des chevreaux a affecté de manière variable les caractéristiques de la carcasse, la qualité de la viande et le profil en acides gras. Néanmoins, même si le régime a influencé certains paramètres de la qualité de la viande et du profil en acides gras, la plupart de ces paramètres ont rarement été influencés de la même manière pour chaque muscle étudié. Des investigations supplémentaires sont recommandées pour évaluer les effets de différents taux d'incorporations des grains d'orobe et de sorgho sur la production et la qualité de la viande.

Conclusions - Perspectives

Chapitre 9. Conclusions et Perspectives

Ce travail visait à caractériser l'écologie, l'agro-morphologie et la bromatologie des écotypes locaux de trois plantes sous utilisées dans la région du Nord du Maroc, mais qui pourraient constituer des ressources alimentaires alternatives intéressantes dans la région. Il avait concrètement pour objectif d'étudier leurs effets sur la production et la qualité des produits caprins (lait et viande), afin de les introduire comme ressources alternatives.

Le Sulla flexuosa a donné des rendements en matière sèche et matière verte intéressants. Cependant, la variation de cette valeur nutritive au cours des stades nécessite sa préservation sous forme de foin. Pour obtenir la meilleure teneur en protéines et en matière sèche, la coupe au début de la floraison pourrait être un compromis entre le rendement en matière sèche, la teneur en protéines et la digestibilité, pour son exploitation dans l'alimentation animale. Cette étude a montré que l'incorporation du foin de Sulla flexuosa dans l'alimentation des chèvres laitières n'avait aucun effet négatif sur la production laitière et la composition chimique du lait cru. En revanche, le foin de Sulla flexuosa devrait être incorporé à hauteur d'au moins 50% de l'apport en matière sèche pour obtenir une amélioration du profil en acides gras et de l'activité antioxydante. Des études supplémentaires sont recommandées pour tester son utilisation comme fourrage frais ou ensilé et pour explorer son effet sur le microbiote ruminale (bactéries, protozoaires, acides gras volatils, …), et sur les autres produits laitiers de chèvre (yaourt, fromage, …). D'autres essais sont encouragés pour évaluer l'effet du Sulla flexuosa sur la production et la qualité de la viande. De futurs études sont nécessaires afin de poursuivre la domestication et la sélection de Sulla flexuosa, de diffuser cette plante aux sélectionneurs et de conserver les gènes locaux dans une banque de graines. En outre, le changement climatique et les sécheresses récurrentes doivent être pris en compte dans le schéma de sélection.

Les écotypes marocains de sorgho et d'orobe ont également été évalués et ils présentaient une variabilité intéressante quant aux paramètres agro-morphologiques et bromatologiques. Le sorgho a montré des rendements en grains intéressants par rapport à d'autres écotypes de la région Méditerranéenne, cependant l'orobe a donné des rendements en grains modestes. La valeur nutritive des grains était intéressante par rapport à celle de la paille pour les deux plantes. La distance phénotypique des écotypes n'a pas été corrélée à leur distance géographique. Ainsi, la sélection des meilleurs écotypes devrait être basée au niveau des écotypes plutôt qu'au niveau de la zone géographique, en considérant les écotypes à haut rendement avec une valeur nutritive élevée pour les grains mais aussi pour la paille.

Des essais futurs sont nécessaires pour sélectionner des variétés, diffuser les meilleurs écotypes et conserver les gènes locaux dans une banque de gènes. En effet, le changement climatique et l'augmentation des sécheresses récurrentes nécessiteront de maintenir une large biodiversité d'écotypes. En plus de la caractérisation agro-morphologique et bromatologique, une future analyse moléculaire de cette collection d'écotypes pourrait aider à discerner les similitudes et donc à éviter la duplication d'écotypes génétiquement similaires pendant le programme de sélection. Des essais multi-locaux et multi-environnementaux devraient être réalisés pour évaluer et donner des réponses locatives précises pour une meilleure amélioration des cultures pour les traits dépendant de l'environnement.

Les grains d'orobe et de sorgho ont été évalués comme deux ressources alternatives de protéines et d'énergie dans les rations des chevreaux de race Beni Arouss. Les résultats ont montré qu'ils peuvent être incorporés dans l'alimentation des chevreaux sans impacts négatifs sur les caractéristiques d'abattage, malgré certains effets négatifs sur le gain quotidien moyen et l'ingestion de la matière sècheliés au niveau d'incorporation utilisé dans cet essai. Les caractéristiques de la carcasse, la qualité de la viande et le profil en acides gras ont été affectés de manière variable selon les régimes distribués. Des investigations supplémentaires sont recommandées pour évaluer les effets des grains d'orobe et de sorgho sur le microbiote ruminal (bactéries, protozoaires, acides gras volatils, …), et la qualité des dérivés du lait de chèvre (yaourt, fromage, …). Différents niveaux d'incorporation ou traitements des grains devraient également être évalués. Le groupe qui a reçu le sorgho a maintenu la teneur en protéines de la viande alors que le groupe ayant reçu l'orobe a maintenu la teneur en matière minérale par rapport au groupe témoin, ce qui suggère de les tester ensemble dans le même groupe pour évaluer la possibilité de leur complémentation. Sur le niveau agronomique, la possibilité de faire des rotations entre les cultures de Sulla flexuosa et d'orobe avec le sorgho cultivé au printemps doit également être évaluée.

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