Tropical Animal Health and Production (2025) 57:280
https://doi.org/10.1007/s11250-025-04476-7

REGULAR ARTICLES

Incorporation of *Azadirachta indica* kernel in the diet of guinea pigs: effects on digestibility and caecal health

Gina France Djoumessi Tobou[1,2] · Laurette Blandine Mezajouck Kenfack[2] · Emile Miégoué[3] · David Wauffo Fokom[3,4] · Hervé Mubé Kuitche[3] · Zambou Dongmo Delmas Kesnel[3] · Fernand Tendonkeng[3] · Tchiegang Clerge[2] · Jean-Luc Hornick[1]

Received: 7 July 2024 / Accepted: 9 May 2025 / Published online: 21 June 2025

Abstract

The objective of the present study was to assess the effects of neem (*Azadirachta indica*) kernel powder supplementation on the digestibility of feed chemical components and the composition of caecal microbiota in guinea pig. One hundred and thirty guinea pigs were randomly assigned to four groups. For 27 days, the animals were fed once daily with either a standard control diet (complete concentrate) or a concentrated mixture with 2.5, 5 or 7.5% (w/w) neem kernel powder. The results showed a significant increase in feed intake and digestibility with the rate of kernel incorporation. In the caeca content, the log count of lactic acid bacteria and *Clostridium butyricum* increased quadratically ($P < 0.001$) with the supplementation level whereas *Escherichia coli* count decreased. The results suggest that neem kernel powder could be used as a phytogenic supplement for guinea pigs, enhancing both nutrient digestibility and gut microbiota quality.

Keywords *Azadirachta indica* · Caecal microbiota · Digestibility · Feed intake · Guinea pigs

Introduction

Worldwide, and particularly in developing countries, meat consumption continues to rise. Small-scale farming, such as guinea pig farming, can help meet the growing demand for animal protein. In Cameroon, guinea pig production is expanding, especially among low-income families, providing both source of food and additional income (Imoru and Badadipe 2019). However, feeding guinea pigs can be challenging due to their susceptibility to digestive disorders. The use of antimicrobials, for therapeutic, prophylactic, or metaphylactic purposes, is not recommended to address this issue, as guinea pigs are known to have a sensitivity to antibiotics affecting their caecal flora (Somer et al. 1955; Burgevin 2021). Additionnaly concerns about antimicrobial resistance further discourage their use. Recent research has been conducted in Cameroon to study the effects of medicinal plants as effective and natural alternatives to antibiotics in guinea pig farming (Djoumessi et al. 2021).

Medicinal plants contain various secondary metabolites or bioactive compounds that can promote animal health and improve overall performance (Kuralkar and Kuralkar 2021). These compounds can act directly as antimicrobials on pathogenic bacteria (Abd El-Ghany and Smail 2014) or prevent their adherence to the intestinal mucosa by blocking certain membrane receptors (Kuralkar and Kuralkar 2021). They can also act as prebiotics, providing specific substrates and stimulating the growth of beneficial bacteria, or serve as growth promoters (Peng et al. 2020; Abd-Elaziz et al. 2023). Interestingly, plants can modulate the microbiota-intestinal-immune system axis through their extensive antioxidant and anti-inflammatory properties (Gheisar and Kim 2018). In many species, plants increase the activity of digestive enzymes, thereby improving feed conversion and production parameters (Gheisar and Kim 2018; Soltan et al. 2023). Improvements in digestive function also have been linked

Implications Maintaining livestock intestinal health in livestock is a major challenge for breeders. Phytobiotics have shown to be promising substances for improving animal microbiota quality and thus stabilizing physiological digestion. Neem is a well-known plant species used in pharmacopeia. The results of this research revealed that incorporating neem kernels into guinea pigs diet led to significant improvements in nutrient digestibility, as well as marked improvement gut microbiota quality. These findings suggest that neem kernel in diet could be beneficial for guinea pig, promoting better nutrient absorption and improving gut health.

Extended author information available on the last page of the article

to the growth of beneficial bacteria – especially lactic acid bacteria such as lactobacilli and bifidobacterial—in the caeca of broiler chickens supplemented with phytobiotics, (Attia et al. 2017; Sowmiya et al. 2023). These bacterial groups enhance host health by interacting with and training the immune system, enabling the host to allocate resources to production traits (Al-Yassiry et al. 2017; Nath et al. 2023). Garlic, turmeric, and neem (*Azadirachta indica*) are among the plants that showed positive effects on such parameters. Neem kernels contain a wide variety of compounds (flavonoids, terpenoids, lignins, sulfides, polyphenols, carotenoids, coumarins, saponins, and sterols) some of which exhibit antimicrobial activity (Tchinda et al. 2021; Wylie and Merrell 2022). These compounds have shown promising results in improving the health and production parameters of various animals, such as broilers (Mafouo et al. 2019) and rabbits (Mohammed et al. 2021). They enhance energy-related intestinal functions and blood metabolites, contributing to animal health and productivity (Verma et al. 2023; Rehman et al. 2023). They also stimulate bile production and promote its secretion in the intestine, facilitating emulsification, waste elimination, toxin removal, and nutrient absorption (Abd El-Aziz et al. 2025; Odoh and Bratte 2015), thereby contributing to animal health and productivity. In poultry, the addition of neem oil to feed rations had significant modulating effects on growth, intestinal ecosystem, and immune responses (Pliego et al. 2020). To our knowledge, the effects of neem kernel supplementation on guinea pigs have not yet been reported in the literature. This study aims to investigate the impact of neem kernel intake in the diet of guinea pigs on nutrient digestibility and the concentration of certain bacteria in the caecal content.

Materials and method

Geoclimatic characteristics of the study area

The study took place from August to September 2022 at the Application and Research Farm (FAR) of the Faculty of Agronomy and Agricultural Sciences (FASA) of the University of Dschang. Dschang down is located at 05°26 latitude North, 10°26 longitude East, and culminates at an average elevation of 1420 m in the agroecological zone of the highlands of western Cameroon.

Plant material (*Azadirachta indica* kernel) and its origin

Neem seeds were harvested in the locality of Garoua (tropical type climate, mean temperature 25.4 °C; mean rainfall 1005 mm) in the Northern Cameroon region. They were then transported to Dschang, where they were peeled. The kernels obtained were naturally dried at a temperature of 25 to 30 °C to remove residual moisture and grounded using a local mill with a 1 mm diameter mesh. One kilogram of kernel was preserved in hermetically sealed plastic bags to prevent possible contamination and oxidation and later transported to the Laboratory of Foodstuffs and Animal Nutrition of the University of Liege for chemical analyses. The chemical composition of neem kernels was as follows as % DM: crude protein 27.3, ether extract 47.7, crude fiber 18.0, ash 4.7.

Animal and their management

One hundred and thirty (130) English-bred guinea pigs, 450 ± 50 g average initial weight, 3 to 4-months-old, were purchased from breeders in the locality of Dschang. All animals were fed feedstuffs (*Trypsacum laxum* hay) for 7 days to standardize the digestible functions. After that, 40 animals were used for the evaluation of nutrient digestibility and 90 for the caecal microbiota characterization. Vitamin C was fed daily in drinking water at a rate of 240 mg per liter during the entire trial period.

Feed formulation

The ingredients of the total mixed rations were purchased from an approved supplier of livestock ingredients. Four rations were formulated with increasing levels of neem kernel powder (0, 2.5, 5 and 7.5% of the diet). After formulated, the feed rations were granulated and a 100 g sample of each one was taken for further analysis. The proportions of the different ingredients used, and the nutritional value of the diets are reported in Table 1. The ration composition took into account the nutritional needs for guinea pigs (NRC 2006).

Evaluation of *in vivo* ingestion and digestibility

The forty animals were divided into 4 treatment-groups of comparable weights and housed in individual cages according to a completely randomized bloc design with 10 replicates per treatment. Then they were adapted to the experimental rations for 10 days, during which, the quantities of feed served were gradually adjusted to the animal consumption. The experimental period of digestibility measurement lasted 10 days; 40 g of each of the rations was served each morning (8:00 am), after that refusals and faeces had been collected and weighed. Feed intake was calculated as follows:

$$\textit{Feed intake}\ (g) = \textit{Amount of feed served} - \textit{Amount not consumed}$$

The apparent digestibility coefficients of Dry Matter (aDC DM), Organic Matter (aDC OM), Crude Fiber (aDC

Table 1 Ingredients and chemical composition of the experimental rations

Items (%)	R_0	$R_{2.5}$	R_5	$R_{7.5}$
Corn	32.50	31.30	30.20	29.00
Wheat bran	8.00	6.20	4.30	2.50
Soybean meal	10.00	10.50	13.00	14.50
Peanut meal	10.00	11.50	11.00	11.50
Cotton meal	7.00	6.00	5.00	4.00
Shell	1.50	1.70	1.30	1.00
Palm oil	2.00	2.00	2.50	3.00
Azadirachta indica kernel	0.00	2.50	5.00	7.50
Trypsacum laxum	29.00	28.30	27.70	27.00
Total	**100.00**	**100.00**	**100.00**	**100.00**
Nutrient composition (% DM)				
Dry matter	98.30	98.30	98.00	98.10
Organic matter	75.18	77.03	77.37	77.61
Crude fiber	7.60	8.40	7.20	6.60
Crude protein	17.10	19.30	20.50	20.90
Ether extract	2.84	3.03	3.06	3.13
ADF	16.01	17.35	17.69	17.88
NDF	35.01	41.51	41.69	41.94
Ca	3.70	5.00	5.10	3.50
P	5.00	4.90	4.40	4.70
K	12.80	1240	10.70	12.50
Na	0.10	0.20	0.10	0.10
Mg	2.70	2.70	2.40	2.50
Composition of neem kernels (%DM)				
Crude protein	27.30			
Ether extract	47.70			
Crude fiber	18.00			
Ash	4.70			
Ca	0.24			
P	0.48			
K	1.48			
Na	0.01			
Mg	0.23			
Phytochemical analysis of neem kernels				
Total phenols (mg EAG/g)	1.74 ± 0.03			
Total flavonols (mg EQ/g)	0.008 ± 0.002			
Total Flavan-3-ols (mg EC/g)	1.82 ± 0.17			
Total anthocyanins (mg Ku/g)	0.06 ± 0.06			
Total tannins (mg EEC/g)	nd *			
Condensed tannins (mg ECy/g)	1.56 ± 0.03			

ADF Acid Detergent Fiber, *NDF* Neutral Detergent Fiber, *Ca* Calcium, *P* Phosphorus, *K* Potassium, *Na* Sodium, *Mg* Magnesium, (mg EAG/g) Milligram of gallic acid equivalent per gram of extract, (mg EC/g) Milligram of quercetin equivalent per gram, (mg EEC/g) Milligrams of catechin equivalent per gram, (mg EQ/g) Milligrams of quercetin equivalent per gram, (mg ECy/g) (mg Ku/g) Kuromanin milligrams per gram, nd* None determined

CF), Crude Protein (aDC CP), Extract Ether (aDC EE), Acid Detergent Fibre (aDC ADF) and Neutral Detergent Fiber (aDC NDF) were calculated using the formulas proposed by Roberge and Toutain (1999):

$$\text{aDC fraction (\%)} = \frac{\text{Ingested fraction} - \text{Faecal fraction}}{\text{Ingested fraction}} \times 100$$

Chemical analysis

The feed and fecal samples were pre-dried by drying in oven at 60 °C to constant weight and then milled separately through a 1 mm sieve before analysis. Analytical Dry matter was determined according to Method 934.01 from AOAC. Diets and feces were analyzed for crude protein content (CP, Method 954.01 AOAC 1990), Ether extract (EE) with petroleum ether as solvent (Method 920.39, AOAC 1990), Ash (Method 942.05, AOAC 1990), Crude fiber (method962.09), ADF and NDF with Ankom Technology F57 fiber filter bags (method 973.18, AOAC 1990). Phosphorus (P; Method 965.17, AOAC 1990) was obtained using a UV–vis spectrophotometer. Calcium (Ca) was determined by titration with a standardized solution of ethylenediaminetetraacetic (EDTA) as described previously (Hunt 1963). Non-nitrogen extract was calculated by difference.

Characterization of selective caecal microbiota

Following the adaptation of guinea pig to hay (day 0), out of the 90 animals in the second group 10 were randomly selected and sacrificed to determine the initial composition of the caecal microbiota. After a 10 days adaptation period similar to that of the digestibility group, forty animals (10 per group) were sacrificed, and the remaining forty on day 20 of the experiment.

The animals were slaughtered by cervical dislocation and then eviscerated. After each sacrifice, the caecum of the animal was sectioned, and homogeneous samples contents were taken with swabs. The swabs were aseptically stored in sterile boxes in the refrigerator from the FASA Physiology and Animal Health laboratory at −4 °C for bacteria quantification of *Escherichia coli,* Lactic acid bacteria, and *Clostridium butyricum spp.* These bacteria were identified on the following selective and specific cultures: MRS agar, Mac Conkey agar and Reinforced Clostridial Medium (RCA) agar following the method described by Benson (2002).

At the laboratory level, 0.01 g of fecal sample from each animal was collected and placed in Eppendorf tubes filled with 990 µL of phosphate 1 buffered saline solution and

8 dilutions were prepared. The samples were placed on agar plates. After incubation, the bacterial colonies were counted according to Langhout et al. (1999). The counted bacteria were expressed as log CFU g − 1 of caecal digesta. *Escherichia coli* were counted on MacConkey dark pink agar plates after 24 h of aerobic incubation at 37 °C. *Clostridium butyricum* were counted on a Reinforced Clostridial Medium (Merck, Germany), yellowish brown after anaerobic incubation at 37 °C for 24 h (Hirsch and Grinsted 1954). Lactic acid bacteria were counted on a light brown agar of Man, Rogosa, and Sharpe (Merck, Germany) after anaerobic incubation at 37 °C for 48 h (Argyri et al. 2013).

Statistical analysis

The experimental data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System software (version 9.4, SAS Institute, Cary, NC, USA), with a general linear model (GLM). Each animal was considered an experimental unit. Before statistical analysis, fecal microbiota concentrations were log-transformed. The main effects of neem almond powder dose and their interaction with time were tested.

The statistical model was:

$$Yij = \mu + \alpha i + e_{ij}$$

where $\mathbf{Y_{ij}}$ = Performance of animal i having received the treatment j;

μ = Overall mean;
αi = effect of the rate i of incorporation of neem kernels powder;
eij = random residual error N[0, σ].

All dependent variables were tested for normality using the Univariate procedure of SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4). Orthogonal polynomials were performed to determine linear and quadratic effects of increasing level of neem kernels in diets. Data from guinea pigs fed the diets containing kernels were compared with that from guinea pigs fed the control diet using orthogonal contrasts. Lsmeans differences were evaluated using PDIFF option and all results were reported as LSMEANS followed by SEM. When significant, quadratic equations were derived on kernel incorporation level to estimate optima value. Significance levels were defined at $P < 0.05$.

Results

Feed intake

The data presented in Table 2 show that the guinea pigs fed the rations containing neem kernel had higher DM and nutrient feed intake ($P < 0.001$) when compared to the control animals. Nutrient intake both increased linearly and quadratically with the level of kernel incorporation ($p< 0.001$). The CF and ADF intake were particularly the highest in the groups R_5 and $R_{7.5\%.}$ The optimum incorporation of neem kernel powder to maximize dry matter feed intake was found to be 5.84%.

Digestibility

The incorporation of neem kernel significantly ($P < 0.001$) increased the digestibility all chemical components excepted for OM (Table 3). Digestibility parameters values increased linearly and quadratically with the level of neem incorporation, but the three experimental groups did not differ from each other. Considering the significance of the quadratic

Table 2 Apparent feed intake of guinea pigs fed rations containing different levels of neem kernel powders

	[1]Dietary Rations				SEM		P > F	
(%DM)	R_0	$R_{2.5\%}$	$R_{5\%}$	$R_{7.5\%}$		Model	Linear	Quadratic
n	10	10	10	10				
DM	25.44[a]	30.84[b]	31.11[b]	31.31[b]	0.57	0.001	0.001	0.004
OM	19.13[a]	23.20[b]	24.07[b]	24.30[b]	0.45	0.001	0.001	0.001
CF	1.93[a]	2.53[d]	2.24[c]	2.03[b]	0.41	0.001	0.056	0.001
CP	4.35[a]	5.81[b]	6.37[c]	6.54[c]	0.11	0.001	0.001	0.001
EE	0.73[a]	0.92[b]	0.97[bc]	1.00[c]	0.01	0.001	0.001	0.001
ADF	4.14[a]	5.30[b]	5.60[b]	5.70[b]	0.10	0.001	0.001	0.001
NDF	9.06[a]	12.71[b]	13.24[c]	13.38[c]	0.24	0.001	0.001	0.001
NNE	11.39[a]	13.43[b]	13.39[b]	13.89[b]	0.57	0.001	0.013	0.205

[1]dietary treatments: R_0– control diet, $R_{2.5}$, R_5, $R_{7.5}$ – diets mixed with 2.5, 5, and 7.5% of neem kernel, respectively; $n = 10$: number of animals per treatment, SEM – standard error of the mean

[a, b, c] means with different superscripts in the same row are significantly different at $P < 0.05$. *DM* Dry matter, *OM* Organic matter, *CF* Crude fiber, *CP* Crude protein, *EE* Ether extract, *ADF* Acid Detergent Fiber, *NDF* Neutral Detergent Fiber, *NNE* None-nitrogen extract

Table 3 Apparent digestibility of guinea pigs fed rations containing different levels of neem kernel powders

	[1]Dietary treatments				SEM		P-value	
(%)	R_0	$R_{2.5\%}$	$R_{5\%}$	$R_{7.5\%}$		$P > F$	Linear	Quadratic
n	10	10	10	10				
DM	71.84[a]	77.24[b]	77.82[b]	77.76[b]	0.78	0.001	0.001	0.008
OM	76.28	77.21	77.03	77.15	0.74	0.836	0.438	0.678
CF	44.60[a]	61.51[c]	52.53[ba]	47.89[a]	2.71	0.001	0.001	0.001
CP	75.24[a]	84.27[b]	85.54[b]	85.76[b]	0.56	0.001	0.001	0.001
EE	39.39[a]	65.00[b]	73.66[b]	70.77[b]	3.25	0.001	0.001	0.001
ADF	62.26[a]	68.58[b]	68.69[b]	69.58[b]	1.19	0.001	0.002	0.028
NDF	72.38[a]	76.87[b]	76.31[b]	76.25[b]	0.81	0.001	0.004	0.008
NNE	79.27[a]	73.90[b]	73.13[b]	73.13[b]	1.05	0.001	0.000	0.008

[1] dietary treatments: R_0– control diet, $R_{2.5}$, R_5, $R_{7.5}$ – diets mixed with 2.5, 5, and 7.5% of neem kernel, respectively; $n = 10$: number of animals per treatment, *SEM* standard error of the mean

[a, b] means with different superscripts in the same row are significantly different at $P < 0.05$. *DM* Dry matter, *OM* Organic matter, *CF* Crude fiber, *CP* Crude protein, *EE* Ether extract, *ADF* Acid Detergent Fiber, *NDF* Neutral Detergent Fiber, *NNE* None-nitrogen extract

contrast, the optimal incorporation of neem kernel powder to improve dry matter digestibility was found to be 5.43%.

Caecal microbiota

Escherichia coli

The level of *E. coli* was significantly influenced ($P < 0.001$) by both the time and the level of incorporation of neem kernel in the rations, compared to the initial time and control ration (Fig. 1). Guinea pigs fed rations containing 2.5, 5, and 7.5% kernel had lower levels of *E. coli* ($P < 0.001$), compared to the initial time (day 0) and to the control group. Guinea pigs that received the experimental diets had comparable values ($p > 0.05$) across the different treatment groups. On the other hand, the effect of the neem kernel was more pronounced at d20 of the experiment with a significant group x time interaction effect ($P < 0.001$).

Lactic acid bacteria

Both the level of incorporation of *Azadirachta indica* kernel in diet and the time effect significantly influenced ($P < 0.05$) the population of lactic acid bacteria in the caecum of guinea

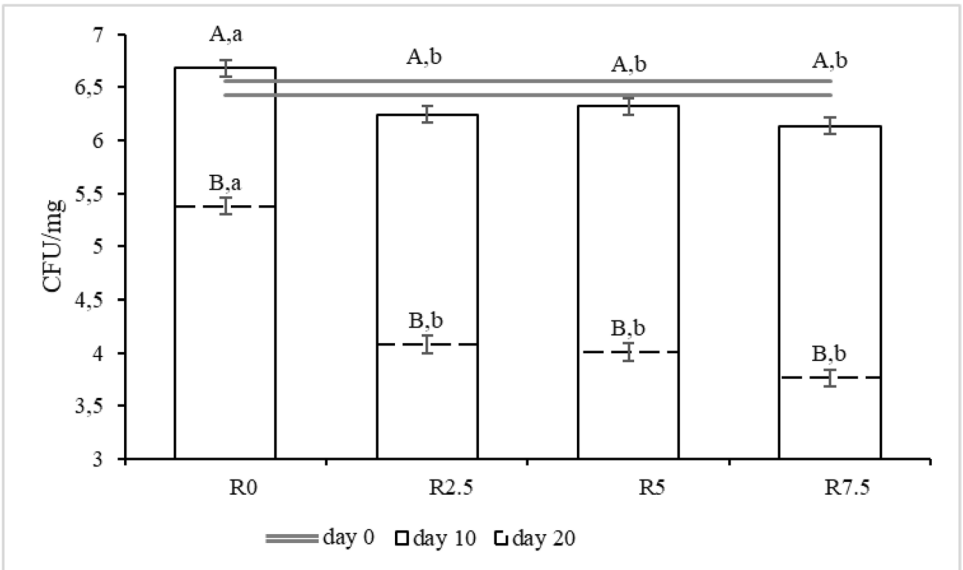

Fig. 1 Concentration of *Escherichia coli* at different time in caeca content of guinea pigs fed rations containing different levels of neem kernel powders. R_0: control ration; $R_{2.5}$, R_5, $R_{7.5}$%—rations containing 2.5, 5 and 7.5% neem respectively; Number of animals per treatment = 10. [a,b] – within time lsmeans are significantly different at $P < 0.05$. [A,B] – within group lsmeans are significantly different at $P < 0.05$. The two parallel lines show the confidence interval of the mean observed at the end of the transition period

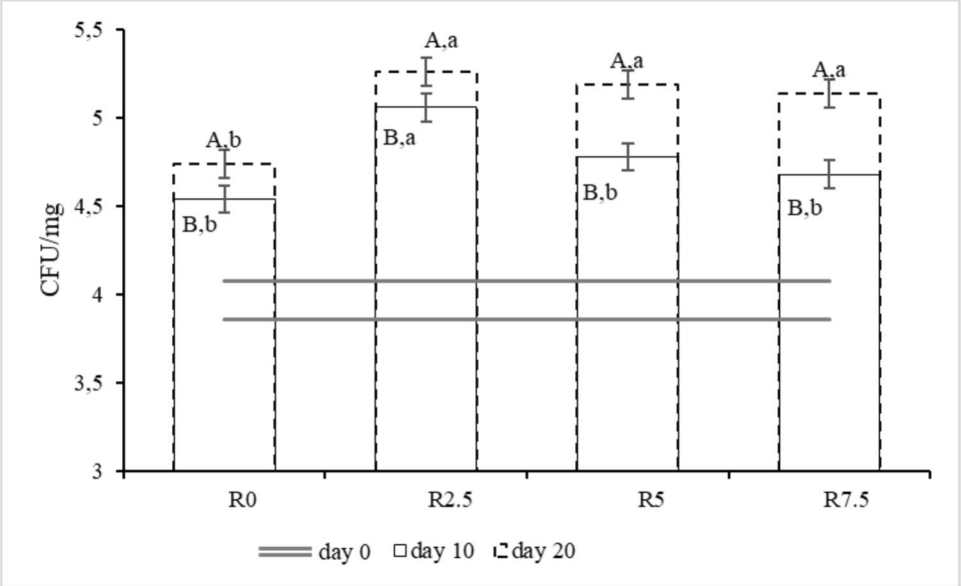

Fig. 2 Concentration of Lactic acid bacteria at different time in caeca content of guinea pigs fed rations containing different levels of neem kernel powders. R_0: control ration; $R_{2.5}$, R_5, $R_{7.5\%}$—rations containing 2.5, 5 and 7.5% neem kernels respectively; Number of animals per treatment = 10. ^{a,b} – within time group-lsmeans are significantly different at $P < 0.05$. ^{A,B} – within group time-lsmeans are significantly different at $P < 0.05$. The two parallel lines show the confidence interval of the mean observed at the end of the transition period

pigs compared to the initial time (day 0) and the control ration (Fig. 2). The level initial before group allocation was significantly lower when compared to d10 and d20. The values increased with time and quadratically with the level of neem incorporation in the rations. A maximum effect was observed at 2.5% incorporation, regardless of the time point considered.

Clostridium butyricum

The level of incorporation of *Azadirachta indica* kernel in the diet as well as the application time significantly influenced ($P < 0.05$) the population of *C. butyricum* in the cecum of guinea pigs compared to the initial time (day 0) and the control ration (Fig. 3). Initially before group allocation, the levels of *C. butyricum* was significantly lower when compared to d10 and d20. The level of the bacterium increased with time and quadratically with the level of incorporation of neem in the rations, a maximum effect being observed at 2.5% incorporation, regardless of the time point considered.

Discussion

The present experiment aimed to evaluate the effect of dietary addition of neem (*Azadirachta indica*) kernel powder on nutrient digestibility and on the composition of selected

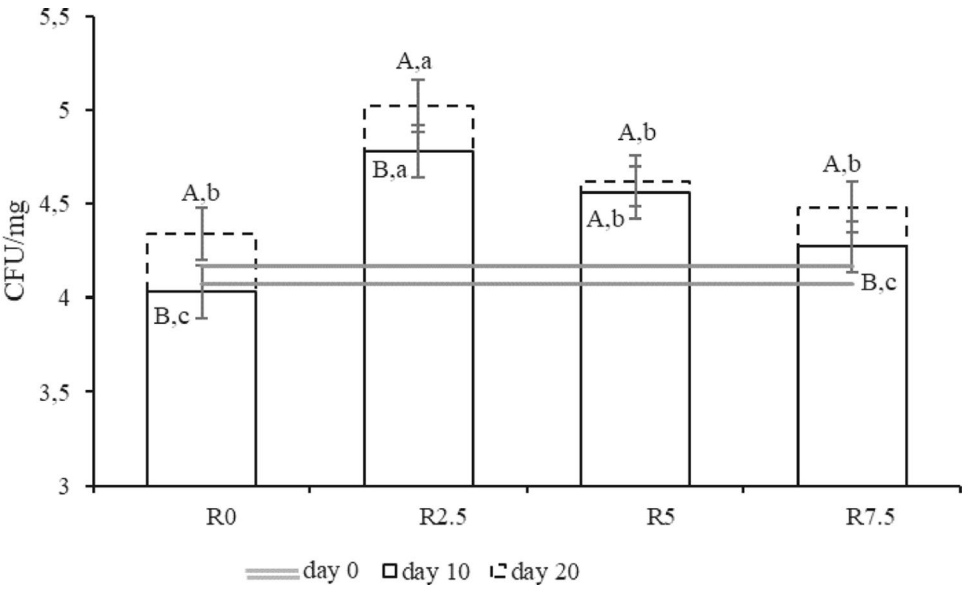

Fig. 3 Concentration of *Clostridium butyricum* at different times in caeca content of guinea pigs fed rations containing different levels of neem kernel powders. R_0: control ration; $R_{2.5}$, R_5, $R_{7.5}$%—rations containing 2.5, 5 and 7.5% neem kernels respectively; Number of animals per treatment = 10. ^{a,b} – within time group-lsmeans are significantly different at $P < 0.05$. ^{A,B} – within group time-lsmeans are significantly different at $P < 0.05$. The two parallel lines show the confidence interval of the mean observed at the end of the transition period

microbial populations of the caecal microbiota of guinea pig. Only one mortality case was reported in the control group and none in the experimental groups. Additionally, guinea pigs fed different rations showed no clinical signs of morbidity. In the context of this experiment, neem kernel can thus be considered as safe for the animals.

Food intake

New approaches are urgently needed to combat the digestive disorders that are inherent to the imbalance of the caeca flora in the guinea pig. The antimicrobial properties of neem as leaves or as dietary supplement have been reported by Tchinda et al. (2021) and Wylie and Merrell (2022), to improve productivity and health of laboratory animals (mice, rats, guinea pigs and rabbits). Consequently, in the present study, neem kernels have been used as a prophylactic method to alleviate this issue. The supplementation of neem kernel had positive effects on feed intake, highlighting the palatability of the product in the rations. Moreover, digestibility and microbial flora were improved.

Feed intake in the experimental groups was higher than in the control one. Contrary to expectations, the bitter taste (Maji and Modak 2021) of the almonds did not have negative effect on feed intake of the animals. Like other plants, neem contains anti-nutritional factors (saponins, azadirachtin), which could induce a decrease in feed consumption if tolerable doses are exceeded (Adjorlolo et al. 2016). Through the findings of this study, it can be established that the incorporation of 2.5 to 7.5% of neem kernel in feed increases the dietary intake of guinea pigs. These results are not in agreement with the work of El-Bolkiny et al. (2022), who showed that the addition of 50 mg/kg of neem leaf extract reduced the dietary intake in rabbits. But the results confirm those of El-Zaiat et al. (2022), and Jack et al. (2020), who showed that the addition of 35 mg/kg of leaf powder in rams and 5% neem fruit in sheep diets was safe and acceptable, with positive effects on their daily intake. However, the maximum effect level of 5.84% neem kernel on DM food intake calculated in the context of this experiment should be interpreted with cautious and may not reflect the real-world conditions.

Digestibility

Neem kernel significantly improved the digestibility of DM of most feed components excepted OM and NNE, the digestibility of which numerically decreased. The lower digestibility of OM thus may be explained by that of NNE. This suggests that, whatever the level of incorporation, neem kernel induced a negative effect either on digestibility of starch or soluble sugar, or decreased the level of soluble fibers fermentation. In the first case, some endogenous enzymatic inhibition could occurred, and in the second hypothesis, some soluble-fiber fermentative population of microbiota may have been impacted. The mode of action of neem in animal feed is known to alter the microbiome ecosystem (Chachar et al. 2022; Rehman et al. 2023). Based on our data, the potential responses of neem kernel may refer to a different selective mechanism, which may have resulted in differences in nutrient intake, fermentation level, and digestion efficiency when compared to the control group. The antimicrobial mechanism of phytogenic supplements can be seen as some growth inhibition of Gram-negative microflora and promotion of Gram-positive microbial proliferation (Chachar et al. 2022). In this experiment, the addition of neem almonds to feed rations have promoted the number of two Gram-positive species or families bacteria (lactic acid bacteria and *C. butyricum*) and decreased that of one Gram-negative species (*E. coli*). Cobellis et al. (2016) observed results in the same vein in rumen with neem essential oil. Neem kernels indirectly could have contributed to stimulate nutrient digestibility, and especially cellulose digestibility, when compared to the control group.

It could be noticed that the incorporation of neem kernel in the diet of guinea pigs resulted in a significant increase in the digestibility of DM, CF, CP, EE, ADF, and NDF compared to the control group. These results agree with Jack et al. (2020), reported by Jack et al (2020), who showed that the addition of neem fruit in the ram's diet, increased the digestibility of hemicellulose. The improvement in nutrient digestibility could be attributed to the phenolics compounds present in the kernels. These compounds stimulate the morphology and activity of the digestive system and improve nutrients absorption (Malik et al. 2020). In addition, phenolic compounds in neem kernel can alter the cell membrane of pathogenic bacteria, inhibiting their growth and survival, while leaving beneficial bacteria relatively intact. This will improve gut physiology and immune response (Lima, et al. 2019). Anthocyanidin in almonds have been reported to increase the growth of potentially beneficial gut bacteria, the activity of endogenous digestive enzymes in the small intestine, and the digestibility and absorption of nutrients (Ketnawa et al. 2022), likely due to change in the structure of the microflora.

The increase in CP digestibility could be a result of the protein binding ability of the phytochemicals of neem kernel formed with dietary CP (Patra and Yu 2012) but in the case of non-ruminant animals, this hypothesis is hard to support because protein is not degraded in a forestomach. Researchers have shown that the inclusion of various plant extracts improves digestion and absorption of nutrients in the intestine. Some authors argued that the inclusion of plant extracts in the diet of chickens leads to an increase in the secretion of intestinal mucus (Liu et al. 2023), thus

increasing the digestibility of nutrients through the action of digestive enzymes that help breakdown nutrients in the digestive tract, especially carbohydrates and proteins. This facilitates digestion and absorption of nutrients (Oluwafemi et al. 2020).

Finally, the optimum level for DM digestibility (5.43%) is compatible with that of DM intake (5.84%).

Microbiota

The use of colony counting of caecal microbiota in this study was chosen for practical and cost reasons. The method is cheaper than advanced ones such as high-throughput sequencing or quantitative PCR. It can be performed with standard laboratory equipment and requires fewer financial resources. This method allows a direct and quantitative measurement of the number of bacterial colonies present in a sample, which can be particularly useful for quantifying bacterial load or evaluating changes in microbiota composition. Finally, the method is well-established and standardized in many laboratories, making it easier to compare results between different studies and laboratories (Benson 2002). Colony counting can reveal significant changes in the composition of the caecal microbiota in response to specific nutritional interventions or treatments. For example, changes in the number of colonies of certain bacterial species may indicate a microbiota response to a particular diet or bioactive agents. This is useful for identifying changes in bacterial composition in response to dietary or therapeutic interventions.

Analysis of the caecal microbiota allows to explore animal health state (Abd El-Aziz et al. 2025; Odoh and Bratte 2015). The microbiota helps to digest poor-quality food, improving host animal use of nutrients and modulating the development and function of the digestive and immune system (Rehman et al. 2023). According to Reda et al. (2020), establishing a state in favor to beneficial microorganisms and detrimental to harmful ones is a crucial factor for improving animal health. In this experiment, neem almond powder, with its bioactive compounds (azadirachtin, phenols, flavonoids, etc.), could have modulated positively gastrointestinal microbial composition and consequently guinea pigs health. These compounds act by forming complexes with certain proteins of the bacterial membrane of pathogens, thus inactivating their enzymes. These disturbances result in the release of cellular content and possibly the death of the microorganism. (Serrano et al. 2009). However, a more comprehensive understanding of the phenomena would require 16 s-DNA analysis of microbiota.

The current study showed that neem kernel supplementation strongly affected the load of some microbial gut. Up to 7.5% of neem kernel in diet decreased in a linear way the concentration levels of pathogenic *E. coli* in caeca content of guinea pigs while they had the opposite effect on families or species of favorable bacteria (lactic acid bacteria and *C. butyricum*). The effect also increased with time. This could be associated with the improved growth performance of the guinea pigs. Neem antimicrobial activity may be related to the presence of triterpenoids, phenolic compounds, carotenoids, steroids, flavonoids and azidarachtin (Odoh and Bratte 2015). This reduction in bacterial load is in line with the work of Rehman et al. (2023), who found that neem antibacterial properties reduced the rate of pathogenic bacteria in broilers. Additionally, Chachar et al. (2022) noticed that the use of phytobiotics decreased the number of *E. coli* in the gastrointestinal tract of compared to the control group. Odoh and Bratte (2015), observed a reduction in enterobacteria in the feces of laying hens while evaluating the feed inclusion of several levels of neem dry leaves. They also observed that a 10% feed inclusion of such leaves could be used as antimicrobial substance and natural growth promoter in diets. These results corroborate previous studies that also demonstrated the efficacy of neem leaf extracts against *E. coli* (Singh et al. 2023).

Escherichia coli is a pathogenic bacteria capable of causing disease in animals. The lower number of pathogenic bacteria recorded in $R_{2.5}$, $R_{5,}$ and $R_{7.5\%}$ rations could indicate that neem kernel had an antimicrobial effect, possibly due to the presence of phytochemicals (triterpenoids, phenolic), which can prevent dysbiosis and preserve gut flora balance (Chachar et al. 2022). Phenolic and terpene compounds characterize neem kernels and are at the origin of their antimicrobial activity. The latter are generally described as a weakening of the cytoplasmic membrane of microorganisms (Mojca et al. 2019). These lead to an increase in permeability that impairs cellular activities such as energy production, membrane transport or metabolic functions. These disturbances lead to a release of the cellular content and possibly the death of the microorganism.

The number of *Lactobacilli spp.* and *C. butyricum* increased significantly with time and almond concentration. *Lactobacillus spp*. and *C. butyricum* are beneficial bacteria that produce organic acids and can modulate the immune response and improve animal resilience, thereby promoting a healthy gut (Wu et al. 2023). The overwhelming conclusion of the majority of these studies is that the phytochemicals of *A. indica* have antimicrobial activities against several pathogens while promoting the multiplication of beneficial bacteria (Ibrahim and Kebede 2020). A healthy gut is a more efficient digestive organ, able to mount an adequate defense against disease and easily cope with nutritional and environmental alterations.

Limitation of study

The intermediate diets ($R_{2.5\%}$ and $R_{5\%}$) were fully formulated and not resulted from mixing from R_0 and $R_{7.5\%}$. This could have led to possible bias in the results observed. Moreover, the different diets were not fully iso-nitrogenous. To some extent, this may have led to partial collinearity.

Conclusion

Up to 7.5% in the diet, neem kernel showed no harmful for fattening guinea pigs and had positive effects on feed intake and digestibility of several chemical components. Moreover, strong positive effects have been observed on caeca microbiota. This suggests that neem kernel could be a useful ingredient in the diet of guinea pigs.

Acknowledgements The authors thank the University of Liege-Belgium and the Academy of Research and Higher Education (ARES) as well as the University of Dschang for their support during this research.

The authors thank Ulrich Darlin Tsafack Fondjeu for him support during this research and their participation in the overall design process.

Author contributions Djoumessi Tobou France Gina generated the research idea, designed the study, designed and conducted experimental trial and analysis, interpreted the results, and wrote the manuscript. Hornick Jean-Luc participated in the design of the study, analysis, and reading of the manuscript. Tendonkeng Fernand participated in the design of the study and reading of the manuscript. Tchiegang Clerge, Mezajoug Kenfack Blandine Laurette, Miegoué Emile and Mubé Kuietché Hervé participated in the overall design process. Fokom Wauffo David and Zambou Dongmo Delmas Kesnel participated in the design of the survey form and in conducting the data collection.

Funding The results of this research were obtained thanks to the Impulse Grant of the University of Liege financed by PACODEL.

Data availability The data will be available from the reviewers upon request.

Declarations

Ethical approval This study was carried out in strict accordance with the recommendations of the ethical approval guide of the Council for Control of Animal Experimentation. The protocol was approved by the Ethics Committee on Animal Experiments of the University of Dschang, Cameroun (license number: 314/14/182/Uds/FASA/DZOO).

Ethics statement Applicable.

Ethical approval Applicable.

Consent to participate Not applicable.

Consent to publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

Abd El-Aziz AH, Ghanima MMA, Kamal M, El-Hack ME, Alagawany M (2025) Chapter 8 - The use of bile acid supplements in poultry feed. Academic Press, In Organic Feed Additives for Livestock, pp 127–138

Abd El-Ghany WA, Smail M (2014) Tackling experimental colisepticaemia in broiler chickens using phytobiotic essential oils and antibiotic alone or in combination. Iranian J Vet Res 15:110–115

Abd-Elaziz RA, Shukry M, Abdel-Latif HM, Saleh RM (2023) Growth-promoting and immunostimulatory effects of phytobiotics as dietary supplements for *Pangasianodon hypophthalmus* fingerlings. Fish Shellfish Immunol 133:108531

Adjorlolo LK, Timpong-Jones EC, Boadu S, Adogla-Bessa T (2016) Potential contribution of neem leaves (*Azadirachta indica*) to dry season feeding of ruminants in West Africa. Livest Res Rural Dev 28(5):75

Al-Yasiry ARM, Kiczorowska B, Samolinska W, Kowalczuk-Vasilev E, Kowalczyk-Pecka D (2017) The effect of Boswellia serrata resin diet supplementation on production, hematological, biochemical and immunological parameters in broiler chickens. Animal 11:1890–1898

Argyri AA, Zoumpopoulou G, Karatzas KAG, Tsakalidou E, Nychas GJE, Panagou EZ, Tassou CC (2013) Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. Food Microbiol 33:282–291

Association of Official Analytical Chemists (AOAC) (1990) Official methods of analysis

Attia G, El-Eraky W, Hassanein E, El-Gamal M, Farahat M, Hernandez-Santana A (2017) Effect of dietary inclusion of a plant extract blend on broiler growth performance, nutrient digestibility, caecal microflora, and intestinal histomorphology. Int J Poult Sci 16:344–353

Benson HJ (2002) Microbiological applications: laboratory manual in general microbiology, 8th edn. Mc. Graw Hill, pp 478

Burgevin C (2021) Antibiotherapie raisonnée du lapin, du cochon d'inde et du furet de compagnie : élaboration d'un guide pratique [en ligne]. [Consulted on March 1, 2025]. Available: http://www2.vetagrosup.fr/bib/fondoc/th_sout/dl.php?file=2021lyon090.pdfT.0296765

Chachar RA, Kamboh AA, Bakhetgul M (2022) Individual and combined effects of Moringa and Neem leaves on the immune response and intestinal microflora of Japanese quails. Pakistan J Zool 1–9

Cobellis G, Trabalza-Marinucci M, Marcotullio MC, Yu Z (2016) Evaluation of different essential oils in modulation of methane

and ammonia production, rumen fermentation and rumen bacteria in vitro. Anim Feed Sci Technol 215:25–36
Djoumessi TFG, Tendonkeng F, Miégoué E, Emalé C, Fokom WD, Hornick JL (2021) Effects of graded levels of *Curcuma longa* powder on in vivo digestibility in guinea pigs (*Cavia porcellus*). Tropicultura 39:1847
El-Bolkiny Y, Monsour M, Kamel K, Tabl G, Rabie H (2022) Effect of sage and neem aqueous leaf extracts on growth, carcass and hematological parameters of growing apri rabbits under summer and winter conditions. Egyptian J Rabbit Sci 32(1):41–58
El-Zaiat HM, Elshafie EI, Al-Marzooqi W, Dughaishi KA (2022) Effects of Neem leaf powder supplementation (*Azadirachta indica*) on rumen fermentation, dietary intake, apparent digestibility and performance in Omani sheep. Animals 12:3146
Gheisar MM, Kim IH (2018) Phytobiotics in poultry and swine nutrition. Ital J Anim Sci 17:92–99
Hirsch A, Grinsted E (1954) Methods for the growth and enumeration of anaerobic spore formers from cheese, with observations on the effect of nisin. J Dairy Res 21:101–110
Hunt JR (1963) Feedstuffs analysis, rapid determination of calcium in feedstuffs. J Agric Food Chem 11:346–347
Ibrahim N, Kebede A (2020) In vitro antibacterial activities of methanol and aqueous leave extracts of selected medicinal plants against human pathogenic bacteria. Saudi J Biol Sci 27(9):2261–2268
Imoru A, Babadipe SS (2019) Minilivestock - the invaluable underutilised genetic species for enhanced protein availability. Afr J Agricult Food Sci 2:27–32
Jack AA, Adewumi MK, Adegbeye MJ, Ekanem DE, Salem AZ, Faniyi TO (2020) Growth-promoting effect of inclusion of waterwashed neem (*Azadirachta indica* A. Juss) fruits in West African dwarf rams. Trop Anim Health Prod 52:3467–3474
Ketnawa S, Reginio FC, Thuengtung S, Ogawa Y (2022) Changes in bioactive compounds and antioxidant activity of plant-based foods by gastrointestinal digestion: a review. Crit Rev Food Sci Nutr 62:4684–4705
Kuralkar P, Kuralkar SV (2021) Role of herbal products in animal production – an updated review. J Ethnopharmacol 278:114246
Langhout DJ, Schutte JB, Van P, Leeuwen WJ, Tamminga S (1999) Effect of dietary high-and low-methylated citrus pectin on the activity of the ileal micro flora and morphology of the small intestinal wall of broiler chicks. Br Poult Sci 40:340–347
Lima MDC, De Sousa CP, Fernandez-Prada C, Harel J, Dubreuil JD, De Souza EL (2019) A review of current evidence of phenolic compounds in fruit as potential antimicrobials against pathogenic bacteria. Microb Pathog 130:259–270
Liu S, Wang K, Lin S, Zhang Z, Cheng M, Hu S, Xiang J, Chen F, Li G, Si H (2023) Comparison of effects of tannins extracted from different natural plants on growth, antioxidant capacity, immunity and intestinal flora of broilers. Antioxidants 12(2):441
Maji S, Modak S (2021) Neem: Treasure of natural phytochemicals. Chem Sci Rev Lett 10:396–401
Malik JA, Bhadauria M, Lone R, Hajam YA (2020) Exploitation of plant phenolics in animal farming. Plant Phenol Sustain Agricult 1:69–89
Mohammed LS, Sallam EA, Elbasuni SS, Eldiarby AS, Mohamed Mohamed S, Aboelenin SM, Shehata SF (2021) Ameliorative effect of neem leaf and pomegranate peel extracts in coccidial infections in New Zealand and V-line rabbits: performance, intestinal health, oocyst shedding, carcass traits, and effect on economic measure. Animals 11:2441
Mojca Z, Žiga P, Tadej SP, David S, Martin P, Dular M (2019) Effects of cavitation on different microorganisms: the current understanding of the mechanisms taking place behind the phenomenon. a review and proposals for further research. Ultrason Sonochem 57:147–165
Nath S, Mandal GP, Panda N, Dash SK (2023) Effect of neem (Azadirachta indica) leaves powder and cinnamon (*Cinnamomum zeylanicum*) oil on growth performance of broiler chickens. Indian J Anim Res 57:340–344
National Research Council (2006) Nutrient requirements of dogs and cats. National Academies Press
Odoh L, Bratte L (2015) Effect of varying levels of neem leaf flour (*A. indica*) in diets on hematological and serological indices and number of fecal bacteria in layers. J Nat Sci Res 5:2224–3186
Oluwafemi RA, Oluwayinka EO, Alagbe JO (2020) Effect of dietary supplementation of neem oil (*Azadirachta indica*) on the growth performance and nutrient digestibility of weaned rabbits. J Sci Comput Eng Res 1:100–105
Patra AK, Yu Z (2012) Effects of essential oils on methane production and fermentation by, as well as on the abundance and diversity of rumen microbial populations. Appl Environ Microbiol 78:4271–4280
Peng M, Tabashsum Z, Anderson M, Truong A, Houser AK, Padilla J, Ahlam A, Jacob B, Cheikl OR, Biswas D (2020) Efficacy of probiotics, prebiotics and prebiotic-type components in common functional foods. Comprehen Rev Food Sci Safety 19(4):1908–1933
Pliego AB, Tavakoli M, Khusro A, Seidavi A, Elghandour MMMY, Salem AZM, Ofelia MM, Rene Rivas-Caceres R (2020) Beneficial and adverse effects of medicinal plants as feed supplements in poultry nutrition: a review. Anim Biotechnol 33(2):369–391
Reda FM, El-Saadony MT, Elnesr SS, Alagawany M, Tufarelli V (2020) Effect of dietary supplementation of biological curcumin nanoparticles on growth and carcass traits, antioxidant status, immunity and caecal microbiota of Japanese quails. Animals 10:754
Rehman R, Hussain K, Zaman MA, Faurk MAZ, Abbas A, Mero WMS, Abbas RZ, Waqas MU, Rani Z, Khan JA, Raza MA, Muhammad NM (2023) Effect of coneflower, neem, and thyme extracts on growth performance, blood chemistry, immunity and intestinal microbial population of broilers. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 29:407–413
Roberge G, Toutain B (1999) Choix des plantes fourragères. Cultures fourragères tropicales. CIRAD, Montpellier, pp 147–184. https://www.doc-developpement-durable.org/file/Culture/Culture-fourrages/8009_Article%20Roberge%20Choix%20des%20plantes%20fourrageres.pdf
Serrano J, Puupponen-Pimia R, Dauer A, Aura AM, Saura-Calixto F (2009) Tannins: current knowledge of food sources, intake, bioavailability and biological effects. Mol Nutrit Food Res 53:310–329
Singh AA, Naaz ZT, Rakaseta E, Perera M, Singh V, Cheung W, Mani F, Nath S (2023) Antimicrobial activity of selected plant extracts against common food borne pathogenic bacteria. Food Human 1:64–70
Soltan NM, Soaudy MR, Abdella MM, Hassaan MS (2023) Partial dietary fishmeal replacement with mixture of plant protein sources supplemented with exogenous enzymes modify growth performance, digestibility, intestinal morphology, haemato-biochemical and immune responses for Nile tilapia, *Oreochromis niloticus*. Anim Feed Sci Technol 299:115642
Somer P, Van de Voorde H, Eyssen H, Van Duck P (1955) A study on penicillin toxicity in guinea pigs. Antibiot Chemother 5:463–469
Sowmiya S, Prathiviraj R, Selvin J, Jasmine R (2023) Analysis of evolutionary imprints among the gut bacteria in phytobiotic supplemented *Gallus gallus domesticus*. Animal Gene 29:200153
Tchinda SJB, Tchebe FMT, Tchoukoua A, Yona CMA, Fauconnier ML, Ndikontar KM, Richel A (2021) Fatty acid profiles, antioxidant and phenolic contents of oils extracted from acacia polycantha and *Azadirachta indica* (neem) seeds using green solvents. J Food Process Preserv 45:15115
Verma B, Nehra R, Sawal RK, Dhuria RK, Kumar S (2023) Effect of neem (*Azadirachta indica*) leaf incorporation on feed intake,

water consumption, digestibility, rumen fermentation parameters, blood biochemicals and nutrient utilization in camels (*Camelus dromedarious*). Indian J Anim Nutrit 40:47–53

Wu J, Wang J, Lin Z, Liu C, Zhang Y, Zhang S, Zhou M, Zhao J, Liu H, Ma X (2023) *Clostridium butyricum* alleviates weaned stress of piglets by improving intestinal immune function and gut microbiota. Food Chem 405:135014

Wylie M, Merrell DS (2022) The potential antimicrobien of the tree Neem Azadirachta indica. Front Pharmacol 13:891535

Authors and Affiliations

Gina France Djoumessi Tobou[1,2] · Laurette Blandine Mezajouck Kenfack[2] · Emile Miégoué[3] · David Wauffo Fokom[3,4] · Hervé Mubé Kuitche[3] · Zambou Dongmo Delmas Kesnel[3] · Fernand Tendonkeng[3] · Tchiegang Clerge[2] · Jean-Luc Hornick[1]

✉ Gina France Djoumessi Tobou
ginafdjoumessi@gmail.com

✉ Jean-Luc Hornick
jlhornick@uliege.be

Laurette Blandine Mezajouck Kenfack
mezajouglaurette@yahoo.fr

Emile Miégoué
migoumile@yahoo.fr

David Wauffo Fokom
fokomwauffod@yahoo.fr

Hervé Mubé Kuitche
mubehervemkh@yahoo.com

Zambou Dongmo Delmas Kesnel
delmaszambou12@gmail.com

Fernand Tendonkeng
ftendonkeng@gmail.com

Tchiegang Clerge
clerge.tchiegang@caramail.com

[1] Department of Veterinary Management of Animal Resources, FARAH Unit, Faculty of Veterinary Medicine, University of Liege-Belgium, Quartier Vallée 2 Avenue de Cureghem 10, 4000 Liege, Belgium

[2] Department of Food Engineering and Quality Control (GACQ), National School of Agro-Industrial Sciences, University of Ngaoundéré-Cameroon, P.O. Box 454, Ngaoundere, Cameroon

[3] Department of Zootechnics, Faculty of Agronomy and Agricultural Sciences, University of Dschang-Cameroon, P.O. 96, Dschang, Cameroon

[4] Multipurpose Research Station, Institute of Agricultural Research for Development, Bangangté, Cameroon