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Substitution of commercial Pregnant Mare Serum Gonadotrophin (Gonaser®) by *Synedrella nodiflora* (L.) Gaertn leaves in rabbits breeding: impact on reproductive performance

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ABSTRACT

The current study aims to compare the effect of *Synedrella nodiflora* (L.) Gaertn and Gonaser on reproductive traits in rabbit do. Then, 60 nulliparous rabbits do and 12 males were divided into 3 lots. In lot 1, the animals hadn't received any treatment. In lot 2, rabbits do were subjected to hormonal treatment based on subcutaneous injection of Gonaser®. In lot 3, rabbits do were subjected to the sex hormone plant (*Synedrella nodiflora* leaves) used as feed supplement. It appears that the highest litter size was recorded in lot 3 and lot 2 (P<0.001) while the highest live weights at birth, at 35 days and at 56 days old were found in lot 1. The greatest average daily gain (32.1 g/day) was recorded in animals of lot 3 (P<0.01). The fertility rate of lot 2 and lot 3 was respectively 90% and 95%, to 88% in the control lot (P<0.001). Similarly, the highest kidding rates and the lowest stillborn rate and mortality rate from birth to weaning were recorded in the lots 2 and 3 (P<0.001). The parturition interval was of 63 days in lot 1 to 45.72 in lot 2 and 45.74 in lot 3 (p<0.001). As found for Gonaser, the main effect of leaves of *S. nodiflora* is to increase litter size and to reduce kidding interval. *Synedrella nodiflora* leaves can therefore be used as Pregnant Mare Serum Gonadotrophin supplier in family rabbit breeding to improve reproductive parameters in rabbit does.

Keywords: Benin, Gonaser®, rabbit, reproductive parameters, *Synedrella nodiflora*.

INTRODUCTION

In Benin, rabbit breeding is an activity of undeniable economic interest which is currently emerging and practiced in all departments. It is a source of ready cash for investment in crop production and purchase of foodstuffs, medicines, school fees and clothing. The manure is also used for soil fertility management. Furthermore, rabbit meat is very appreciated by consumers for its good nutritional and dietary values (Zotte Dalle, 2005). This lean meat type has a high rate of unsaturated fatty acids, low in cholesterol, proteins of high biological value and rich in macro-elements including potassium, phosphorus and magnesium (INRA, 2004; Djago et al., 2007). The statistics of the Association of Rabbit breeders of Benin for the period from 1998 to 2007 indicate that the number of rabbits does increased from 2251 in 1998 to 17200 in 2007 with a numerical productivity at weaning varying from 20 to 25 rabbits per female per year (Akpo et al., 2008). The improvement of this productivity depends on the control of feeding, health monitoring, rabbit breeding management and reducing mortality of young rabbits (Farougou et al., 2005; Kpodékon et al., 2006; Akpo et al., 2008 ; Koutinhouin et al., 2009a, Kpodékon et al., 2010). In tropical conditions, rabbit produces an average of 6.4 rabbits per litter (Djago and Kpodékon, 2000; Lebas et al., 2004), and the litter size at weaning is on average 4.8 rabbits (Kpodékon et al., 2004, Koutinhouin et al., 2009b). The fertility rate is 81% in nulliparous does, 61% in primiparous and 50% in multiparous (Koutinhouin et al., 2009a). The numerical productivity of

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rabbits is therefore low in hot and wet climate of Africa. The average weight per rabbit weaned varies between 483 g and 650 g at 35 days of age (Akpo, 2004; Akpo et al., 2008, Kpodékon et al., 2009.). The average daily gain is ranged from 18 g to 28 g/d (Kpodekon et al., 2009b). The average live weight of rabbits after fattening during a period of 56 days is ranged from 1.9 kg to 2 kg (Djago and Kpodékon 2007; Kpodékon et al., 2009a,b). These performances are considerably lower than those obtained in the European countries. This difference is not only related to the genotype of rabbit and the rearing conditions but also to the improvement of their reproductive performances by hormonal methods (Perrier *et al.,* 2000; Theau-Clement, 2008). Although other methods have proven to be efficient for the estrus synchronization in does (Theau and Boiti, 1998), PMSG (Pregnant Mare Serum Gonadotrophin) is still widely used in industrial rabbit production because of its follicle-stimulating effect. It improves receptivity of does and increases fertility and litter size, mainly in lactating and primiparous does (Bourdillon et al., 1992; McNitt, 1992; Maertens et al., 1995; Castellini, 1996; Koutinhouin et al., 2009b). This hormonal treatment is to administer to rabbits different types and doses of hormones 2-3 days before the copulation (Koutinhouin et al., 2009b). The relatively high cost of these products, the delicacy of their instructions and packaging requirements do not allow all farmers to use it wisely.

Therefore, the valuation of local plant resources becomes necessary. The study carried out by Koutinhouin et al. (2014) on the impact of *Synedrella nodiflora (L.) Gaertn* leaves used in rabbit feeding as feed supplement on the reproductive performance showed that such leaves improves fertility rate and litter size, and reduces significantly the mortality rate from birth to weaning. Then, it will be very interesting to compare the efficiency of *Synedrella nodiflora (L.) Gaertn* leaves used as feed supplement to the to the effect of Gonaser® (Pregnant Mare Serum Gonadotrophin) on the reproductive parameters in rabbit does in order to better valuate this sex hormone plant in rabbit breeding in Benin. This plant is described in the literature as having several medicinal properties (Hidayat, 2001a; Bhogaonkar et al., 2011; Wijaya et al., 2011; Amoateng et al., 2012). Its leaves are reported as containing estradiol, haageanolide and alkane, sterol and triterpenes (Hidayat, 2001b). Moreover, this plant species is particularly rich in sesquiterpene lactones, polyacetylenes, steroids, terpenoids, alkaloids, saponins and various heterocyclic compounds (Bhogaonkar et al., 2011). Although some authors report that the aqueous extract of the leaves of *Synedrella nodiflora* (L.) Gaertn is toxic (Dutta et al., 2012) or causes cellular degeneration of the seminiferous tubules in Wistar rat at the dose of 100mg/rat (Olukunle and Abatan, 2008), it is interesting to assess the effect of the leaves used by oral route through animal feeding since *Synedrella nodiflora* (L.) Gaertn is available freely in Benin on open waste places, along roadsides and consume by rabbit, sheep and goat ad libitum.

The aim of the present work was to compare the efficacy of commercial gonadotrophins (PMSG) to the effect of *Synedrella nodiflora* (L.) Gaertn leaves used as feed supplement on reproductive performances of rabbits does of Local breed reared in Benin.

MATERIALS AND METHODS

Area of study

The study was conducted from March 15 to October 15, 2013 with three rabbit farms in the municipality of Abomey-Calavi of Benin (Figure 1). Situated at latitude of 6° 27' north and at a longitude of 2° 21' east, the Commune of Abomey-Calavi covers an area of 650 km2 with a population of 307745 inhabitants (INSAE 2010). This area exhibits climatic conditions of sub-

equatorial type, characterized by two rainy seasons with an uneven spatial and temporal distribution of rainfall: major (from April to July) and minor (from September to November). These two seasons are separated by a dry season. Average rainfall is close to 1200 mm per year. The monthly average temperatures vary between 27 and 31°C and the relative air humidity fluctuates between 65%, from January to March, and 97%, from June to July.

Vegetal material

The vegetal material used herein is *Synedrella nodiflora (L.) Gaertn.* As reported by Koutinhouin et al. 2014, the genus *Synedrella* is a monotypic genus, belonging to the tribe *Heliantheae,* and taxonomically close to *Wedelia. Synedrella nodiflora (L.) Gaertn* is a highly variable, weedy species. *Synedrella nodiflora (L.) Gaertn* is of Asteraceae family, annual plant and an erect branched ephemeral herb usually 30-80 cm tall (Hidayat, 2001a). This plant is originated from America and now distributed pan-tropically and occurring throughout the West African Region.

Animal management

The current study was carried out on 60 nulliparous rabbits does and 12 males divided into 3 lots of 24 including 20 nulliparous females and 4 males. Does were housed in individual flat-deck wire-mess cages and fed with a balanced pellet diet *ad libitum* (2677 DE/kg, 18.8% crude protein). Water was also supplied *ad libitum*. Lighting program was constant, 16 hours-light: 8 hours-dark, and ventilation was natural.

In lot 1, the animals hadn't received any hormonal treatement based on Gonaser® (Gonaser® (Pregnant Mare Serum Gonadotrophin) injection or feed supplement made of fresh *Synedrella nodiflora (L.) Gaertn* leaves. In lot 2, rabbits do were subjected to hormonal treatment based on injection of Gonaser®. 5000 IU of Gonaser® were diluted in 200 ml of a saline solution (Glucosalina Grifols ®) and 1 ml of this dilution, i.e. 25 IU/ doe was injected subcutaneously in the scruff of the neck 48 hours prior mating.

In lot 3, rabbits do were subjected to hormonal treatment based on feed supplement made of 100g of fresh *Synedrella nodiflora (L.) Gaertn* leaves. This leaves of *S. nodiflora (L.) Gaertn* were used in the lot 3 from the begining of the experiment to the first kidding.

Males were allowed to mate twice with the same doe on day 11 post-parturition. Receptivity was assessed visually during mating. If the doe accepted mating, then it was considered to be receptive. Does treated with different treatments (Gonaser®, *Synedrella nodiflora (L.)* Gaertn leaves) were equally receptive. The mating was carried out by natural insemination by using the reproductive male. The pregnancy was determined by palpation. After kidding, does and their bunnies were housed together up to weaning.

Health monitoring was based on the use of aseptic foot baths at the entrance of each farm buildings; vitamin and antibiotics drenching was used to prevent disease. Preventive treatment against coccidiosis was also done. A standard prophylactic endoparasitic and ectoparasitic control schedule was applied. Occasional diseases were treated specifically according to the clinical signs detected.

Data collection

Data collection was done from the individual data record form of each reproductive animal and young rabbit by lot. In total, the study of reproductive parameters was recorded on 60 rabbit does and the weight growth was evaluated from their young rabbits. The number of females in estrus, the number of mated females and the number of pregnant females found after palpation, the number of kidding females, the number of stillborn, the live weight of kits at birth and the live weight of kits at weaning (35th day post-birth) were recorded.

Statistical analysis

The variables included in the data analysis were: litter size at birth and at weaning, the fertility rate, kidding rate, the stillbirth rate, the mortality rate from the birth to the weaning and the live weight of kits at birth and at 35 days post-birth. These data were analyzed using the SAS (Statistical Analysis System, 2006) software. *Proc GLM* procedure was used for variance analysis. The test of Fisher was used to evaluate the effect of the treatment on the different growth and reproductive traits considered. Means comparisons were made by Student t-test.

RESULTS AND DISCUSSION

Effect of Gonaser® and *Synedrella nodiflora* (L.) Gaertn on the average litter size at birth and at weaning and ADG

The effect of *Synedrella nodiflora* (L.) Gaertn leaves was remarkable (P<0.001) on litter size at birth and at weaning; the live weight of young rabbits at birth, at 35 days and at 56 days post-birth (Table 1). Indeed, the highest litter size were recorded in the lot 3 (7 young rabbits) and lot 2 (6.95 young rabbits), while the lowest values (P<0.001) were recorded in the control group (5.57 young rabbits). Therefore, as Gonaser®, *Synedrella nodiflora* (L.) Gaertn leaves improve significantly the litter size in rabbit does, and then can improve profitability of rabbit breeding in sub-Saharan Africa. According to Apori et al. (2014), litter size in rabbits is regarded as one of the most important economic traits in any breed development and improvement programs for intensive meat production. According to Moce and Santacreu (2010) most maternal lines are selected based on litter size at weaning, since this trait reflects both the prolificacy and mothering ability of the doe.

In the current study, the live weights at birth, at 35 days and at 56 days old of young rabbits from the control group were higher

Table I: Effect of Gonaser and *Synedrella nodiflora* (L.) Gaertn on the average litter size at birth and at weaning and ADG

Variables	Lot 1 (Control lot)		Lot 2 (GONASER)		Lot 3 (*S. nodiflora (L.) Gaertn)*		Test of significance
	Mean	SE	Mean	SE	Mean	SE	
Born alive	5.57a	0.53	6.95b	0.45	7b	0.42	*
Total born	5.8a	0.56	7.2b	0.47	7.3b	0.47	*
Litter size at weaning	5a	0.53	6.15b	0.38	6.15b	0.35	*
Kidding interval	63a	1.37	45.72b	0.22	45.74b	0.23	***
W 35 (g)	740.5a	20.3	608.4b	31.9	560.1c	34.6	**
W 56 (g)	1330.3a	23.2	1135.5b	36	1236.8c	39.7	**
ADG 35-56	28.1a	0.63	25.1b	1.13	32.1c	1.12	**

*SE: Standard Error, *: P<0.05; ***: P<0.001. The means between the classes of the same line followed by different letters differ significantly with the threshold of 5%.*

Table II: Effect of Gonaser and *Synedrella nodiflora (L.) Gaertn* on the average fertility rate, birth rate, stillbirth rate and birth-weaning mortality rate

Variables	Lot1		Lot2		Lot3		Test of significance
	Mean	SE	Mean	SE	Mean	SE	
Fertility rate (%)	88.8c	1.08	90b	0.95	95a	0	***
Kidding rate (%)	88.88b	1.03	100a	0	100a	0	***
Stillbirth rate (%)	7.83a	0.14	5.04b	0.19	4.5c	0.24	***
Birth-weaning mortality rate (%)	10.66a	0.24	11.46a	0.32	12.05a	0.2	NS

*SE: Standard Error, *: P<0.05; ***: P<0.001. The means between the classes of the same line followed by different letters differ significantly with the threshold of 5%.*

(P<0,05) than those recorded at the experimental groups (lots 2 and 3). The average daily gain (ADG) recorded in animals of lot 3 (32.1 g/day) fed with Synedrella nodiflora (L.) Gaertn leaves as feed supplement was the highest followed by the animals of the control lot (28.1 g/day). The average daily gain (ADG) recorded in animals of lot 2 suggested to hormonal treatment based on Gonaser injection was intermediary.

This impact of *Synedrella nodiflora* (L.) Gaertn leaves used as feed supplement for rabbits on the litter size and young rabbit growth comparable to the effect of Gonaser® could be related to the chemical composition of the leaves of *S. nodiflora* (L.) Gaertn, and more specifically to its hormonal content. According to Hidayat (2001b), *S. nodiflora* (L.) Gaertn contains estradiol and haageanolide. Furthermore, leaf contains alkane, sterol and triterpenes. The study of Hidayat (2001b) on the properties of *S. nodiflora* (L.) Gaertn reveals also that upon steam distillation of the leaves, *Synedrella nodiflora* (L.) Gaertn yields a yellow colored essential oil (0.02%), with the terpenes 'beta'-caryophyllene, 'beta'-farnesene, germacrene-D and 'beta'-cubebene as major components. From the ethanol extract of the whole plant, the triterpenoid saponin nodifloside A (oleanolic acid 3-O-'beta'-D-xylopyranosyl-'beta'-D-glucopyranuronosyl methylate) was isolated, together with the triterpenoid oleanic acid-3-O-'beta'-D-glucopyranuronosyl methylate, and the steroids 'beta'-sitosterol, stigmasterol, stigmasterol-3-O-'beta'-D-glycoside and rosasterol. *Synedrella nodiflora* (L.) Gaertn also contains a high content of estradiol. Moreover, since 1978, Mannan and Ahmad (1978) have cited *Synedrella nodiflora (L.) Gaertn* in the 4 main sex hormones plants (*Synedrella Nodiflora* (L.) Gaertn*, Heliotropicum indicum, Belva chal and Phyllanthus neruri*) of Bangladeshi after his preliminary study on sex hormones of medical importance in Bangladeshi plants.

The average total born per litter in the different lots are comparable to those reported by Kpodekon et al. (2004), Akpo et al. (2008), Koutinhouin et al. (2009b), which recorded an average of 5.7 to 6.6 total born ; and total weaned young rabbit ranged from 4.8 to 5.7 per birth in Local rabbits in Benin. However, those performances found herein are lower than those reported by Mahmoud (2008) which recorded an average of 8 young rabbits for litter size at birth and 6 young rabbit for litter size at weaning in rabbit of Néo-Zelandais Blanc, Californien and Géant Blanc breeds reared in a healthier farming system in Canada. This difference could be due not only to the genotype, but can also be linked to the best breeding conditions and climate factors that significantly affect the ability and frequency of females ovulate as was reported Hulot et al. (1981) and (Ouyed, 2006). The variation found in the live weight of the young rabbit at birth, at 35 days old and at 56 days old according the lot in the current study could be due to the difference in the litter size with the low litter size results in high live weight at birth, at 35 days old and at 56 days old. This finding is consistent with the reports of Ouyed et al. (2007) who indicate a negative relationship between litter size and growth performance in rabbit. The live weight increases when the litter size decreases. The gradual increase in body weight of young rabbits with age found herein is consistent with the results of Ouyed et al. (2010) in rabbit in rabbit of Neo-Zelandais Blanc, Californien and Geant Blanc breeds reared in a healthier farming system in Canada.

Effect of Gonaser and *Synedrella nodiflora* (L.) Gaertn on the fertility rate, birth rate, stillbirth rate and birth-weaning mortality rate

Fertility rates, birth rate, stillbirth rate and birth-weaning mortality rate varied significantly according to the lot (P<0.001; Table 2). The fertility rate of animals of lot 2 and lot 3, where rabbit does were

respectively treat with Gonaser® or fed with *Synedrella nodiflora* (L.) Gaertn leaves was of 90% and 95%, to 88% for the control lot (P<0.001). The kidding rate of the experimental lots was 100% while the lowest (88%) kidding rate was recorded in the control lot (P<0.001). Similarly, the highest stillborn rate (7.83%) was recorded in control lot while the lowest stillbirth rate (4.5 -5.3%) were obtained in animals of experimental lots (P<0.001). The highest mortality rate recorded from the birth to the weaning (14.4%) was observed in animals of Lot 1 (P<0.001). This variation of the fertility rate found herein could be related to the sex hormones content of the plant notably the estradiol content (Mannan and Ahmad, 1978; Hidayat, 2001b). According to Mukasa-Mugerwa (1989), prepubertal ovaries also respond when transplanted to mature animals and injecting oestradiol results in LH release in calves as young as 3 months old. The possible causes of sexual maturation at puberty appear to be an increase in pituitary hormones output culminating in increased size and activity of the ovaries and maturation of the hypothalamo-pituitary axis, resulting in secretion of gonadotrophins. Therefore, the highest fertility rate and litter size found in the current study in the rabbit suggested to *Synedrella nodiflora (L.) Gaertn* leaves used as feed supplement in the diet may be related to the hormonal profile of the plant.

Ouyed (2006) found fertility rates of palpation of 92.1% and 93.6% respectively in white New Zealand and Californian rabbits reared in a healthier farming system in Canada. The average stillborn recorded in the current study are significantly lower than those reported for the same rabbit breed in the literature in Benin (Akpo, 2004; Kpodékon et al., 2004; Lebas, 2004; Akpo et al., 2008; Koutinhouin et al., 2009a) in White rabbit of Algeria (Zerrouki et al., 2007).

This difference may be related to the kidding rank because fertility rate at palpation increase with the kidding rank of rabbit does (Ouyed et al., 2007). In the current study, we have used primiparous females whose maternal instinct might be more developed compared to nulliparous does used by Akpo (2004); Kpodékon et al. (2004); Lebas (2004); Akpo et al. (2008); and Koutinhouin et al. (2009b). This mortality rate from birth to weaning obtained herein is comparable to that recorded by Fellous et al. (2012) in rabbits of the Algerian high experimental station population, but remains lower than 21.5% and 36.61% respectively recorded by Akpo (2004) in the same rabbit breed reared in the same agro-ecological area of Benin. Number range may cause discrepancies between the different results. Mortality rates birth-weaning recorded in the four experimental groups in this study are consistent with the standard (10-15%) indicated by Lebas (2004). Bolet et al. (2004) had reported mortality rate from birth to weaning ranging fron 9.5 to 38.5% in rabbits of Argenté de Champagne, INRA 9077, Thuringer, Vienna White, Fauve de Bourgogne, Belgian Hare, Chinchilla, English and Himalayan breeds fed with a commercial diet *ad libitum* in France.

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Conflict of Interest

The authors declare that they have no conflict of interest

CONCLUSION

As found for Gonaser, the main effect of leaves of *S. nodiflora (L.) Gaertn* is to increase litter size and to reduce the interval between kidding. *Synedrella nodiflora (L.) Gaertn* leaves can therefore be used as Pregnant Mare Serum Gonadotrophin supplier in family rabbit breeding to improve reproductive parameters in rabbit does. Since *Synedrella*

nodiflora (L.) Gaertn is an available plant known as advent or weed in Benin, the current results will be useful for local rabbit breeders in the improvement of the profitability of rabbit breeding in sub-Saharan Africa.

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