Comparative effect of lemon seeds and albendazole a veterinary anthelmintics on gastrointestinal parasites of farmed grasscutter: case of breeding grasscutter in southern Benin

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Abstract:
The financial damage caused by parasitic diseases in "farmed grasscutter breeding" is important and seriously affects the profitability of this activity. Apart from toltrazuril (7mg/kg) and Ganidan (sulfonamides) used in intestinal infections at a rate of 2 tablets/day for 3 days per os), grasscutter breeders frequently use lemon seeds extracted from the fruit twice a day to treat their animals. The objective of this study was to compare the efficacy of lemon seeds and albendazole, a veterinary anthelmintic, on gastrointestinal parasites of grasscutter. The medothology adopted is to assess the effects of lemon seeds compared to albendazole on gastrointestinal parasites of grasscutter. Using the quantitative method of Mac Master, a coprological analysis of 118 samples of grasscutter feces was carried out in station. The efficacy of lemon seeds was compared to albendazole on the basis of OPG (eggs per gram). Coprological examinations showed a considerable decrease in OPG from 7000 to 200 with the use of albendazole at a dose of 7.5 mg/kg body weight and lemon seeds extracted from the fruit at a dose of 0.25 mg/kg to 30 mg/kg body weight. This study showed that lemon seed extract is as effective as albendazole for the treatment of gastrointestinal parasites in grasscutter farming. It opens perspectives for further research on lemon seeds in order to develop a bioactives molecules with anthelmintic properties.

Keywords: Grasscutter, Lemon seeds, Albendazole, Anthelmintic properties, Benin.

Effet comparé des pépins de citron et de l'albendazole un anthelminthique vétérinaire sur certains parasites gastro-intestinaux des aulacodes d'élevage : cas des aulacodicultures au Sud du Benin

Résumé :
Les dégâts financiers causés par des maladies parasitaires en aulacodiculture sont importants et portent des préjudices graves à la rentabilité de cette activité. A part le toltrazuril (7mg/kg) et du Ganidan utilisés dans les infections intestinales à raison 2 comprimés/jour pendant 3 jours per os), les aulacodiculteurs font usage de manière fréquente des pépins de citron secs (2 fois par jour) pour soigner les animaux. L'objectif visé par la présente étude est de comparer l'efficacité des pépins de citron et de l'albendazole sur certains parasites gastro intestinaux des aulacodes. La méthodologie est basée sur l'exploration des effets de pépins de citron comparée à l'albendazole sur certains parasites gastro intestinaux des aulacodes. A l'aide de la méthode quantitative de Mac Master, une analyse coprologique de 118 échantillons de fèces d'aulacodes a été effectuée en station. L'efficacité des pépins de citron a été comparée à celle d'un anthelminthique vétérinaire (albendazole) sur la base des OPG (œufs par gramme). Les examens coprologiques montrent une baisse considérable des OPG de 7000 à 200 avec l'usage de l'albendazole à la dose de 7,5 mg/kg de Poids Vif et des pépins de citron à la dose de 0,25 mg/kg à 30mg/kg de Poids Vif. Cette étude a montré que l'extrait de pépins de citron est aussi efficace que l'albendazole pour le traitement des parasites gastro-intestinaux dans l'élevage des aulacodes. Elle ouvre des perspectives pour de nouvelles recherches sur les pépins extraits de citron afin de développer une molécule bioactive aux propriétés anthelminthiques.

Mots-clés: Aulacodes, Pépins de citron, Albendazole, Propriétés anthelminthiques, Bénin.

Introduction

Parasitic diseases are responsible for significant morbidity and mortality of animals worldwide, as well as considerable losses to food production (Kumarasingha et al., 2016). In Africa, these health problems encountered in aulacod breeding by farmers have a considerable socio-economic impact. Indeed, they are the cause of reduced yields. The management of these pathologies creates a significant economic burden due to the cost of their care (Adjahoutonon et al., 2007). In Benin, parasitic diseases are among the most frequent animal pathologies in grasscutter farms (Sacramento et al., 2022). A study by Adjahoutonon et al. (2007) reported a frequency of infestation with digestive helminths and coccidiosis. In Ivory Coast, work conducted by Bi et al. (2013) reported infestation of grasscutter by several types of parasites namely *Oesophagostomum venulosum, O. radiatum, O. columbianum, Strongyloides papillosus, Bunostomun trigonocephalum, B. phlebotomum, Trichuris skrjabini, T. ovis, T. globulosa, Cooperia punctata,*

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Trichostrongylus spp, Nematodirus spp and Taenia spp (Bi et al., 2013). Among these, gastrointestinal parasitosis is important because it is the cause of low livestock productivity. For example, in the Atlantic Department in southern Benin, the prevalence of gastrointestinal helminth infestations in wild grasscutter was 95% compared to 2.5% for farmed woodcocks in 2009. (Assogba et al., 2009).

However, the treatment of parasitic diseases is generally done using conventional anthelmintic. Their effectiveness has been demonstrated by several reports in the scientific literature (Voigt et al., 2022). However, in certain specific conditions their ineffectiveness is somewhat observed (Areskog et al., 2013). In developing countries such as Benin, where poverty is rife, breeders sometimes find it difficult to effectively provide the conventional treatment recommended for animal breeding in general and for grasscutter in particular (Sacramento et al., 2022). One of the alternative solutions used by farmers is the use of medicinal plants. Medicinal plants are a main source of bioactive molecules with antihelmintic properties. (Kumarasingha et al., 2016; Liu et al., 2020). In Benin, endogenous ethnoveterinary knowledge reports the use of several plants, including lemon seeds, in the treatment of parasitic diseases of grasscutter (Sacramento et al., 2022). Unfortunately, very few scientific studies attest to the effectiveness of lemon seeds. This study is part of the valorization of endogenous knowledge related to lemon seeds in order to maximize the yields of grasscutter farming. The objective was to explore the comparative effect of lemon seeds and albendazole on gastrointestinal parasites of the grasscutter.

Materials and Methods

Material

Animal material used in this study is a 7-month-old grasscutter (Thryonomys swinderianus) Temminck, 1827). Their average body weight is 3 to 3.5 kg. Albendazole was used as classic veterinary antihelmintic. Lemon seeds were used as plant material.

Study area

This study was carried out on farms in the Plateau and Ouémé departments in southern Benin (Figure 1) where samples of droppings were collected. The experimentation (coprological analysis OPG) was carried out in the experimental grasscutter breeding of the School of Management and Exploitation of Livestock Systems (EGESE) at the Laboratory of Animal and Halieutic Sciences (LaSAH) of the National University of Agriculture (UNA) located in Kétou.

Methodology

The study was conducted in two steps:

- a first phase of investigation e devoted to identify the grasscutter breeding of Oueme and Plateau in southern Benin in those who use lemon seeds and why breeders use it was conducted. The question of why some grasscutter breeders of the same department of Benin didn't used lemon seeds has been investigated.
- a second phase consisted in carrying out the coprological analysis (OPG) of the droppings collected in the breedings surveyed at the Laboratory of Animal and Fisheries Sciences (LaSAH) of the National University of Agriculture.

Ethnopharmacological survey (Sacramento et al., 2022)

Ethnopharmacological survey was conducted in the following steps:

- In the first phase, we conducted a literature review
- identified grasscutter breeders in the Plateau department with a considerable herd
- defined the study parameters (health status, treatment of diseases, use of endogenous treatments livestock population management);
- identified the main grasscutter's gastrointestinal diseases and their endogenous treatments;
- develop the field survey questionnaire

To avoid possible difficulties in recovering as much information as possible from the breeders concerned, we conducted a pre-survey of a dozen grasscutter breeders to finalize our questionnaire. The formal survey phase consisted of collecting information from 118 grasscutter breeders

The survey questionnaire has five main sections and were conducted in the form of interview:

- Geographic location of the grasscutter breeding;
- Identification of the grasscutter breeder (Social, professional and economic characteristics;
- Sanitary state of grasscutter breeding;
- Information on the health status of grasscutter (animal care, deworming);
- Zootechnics informations (reproduction, adaptation to specific grasscutter's needs).

The criteria used for the selection of grasscutter breeding to be used for the station tests are:

- Presence of livestock,
- Presence of breeding follow-up sheet,
- No bankruptcy of the farm,
- Size of the breeding.

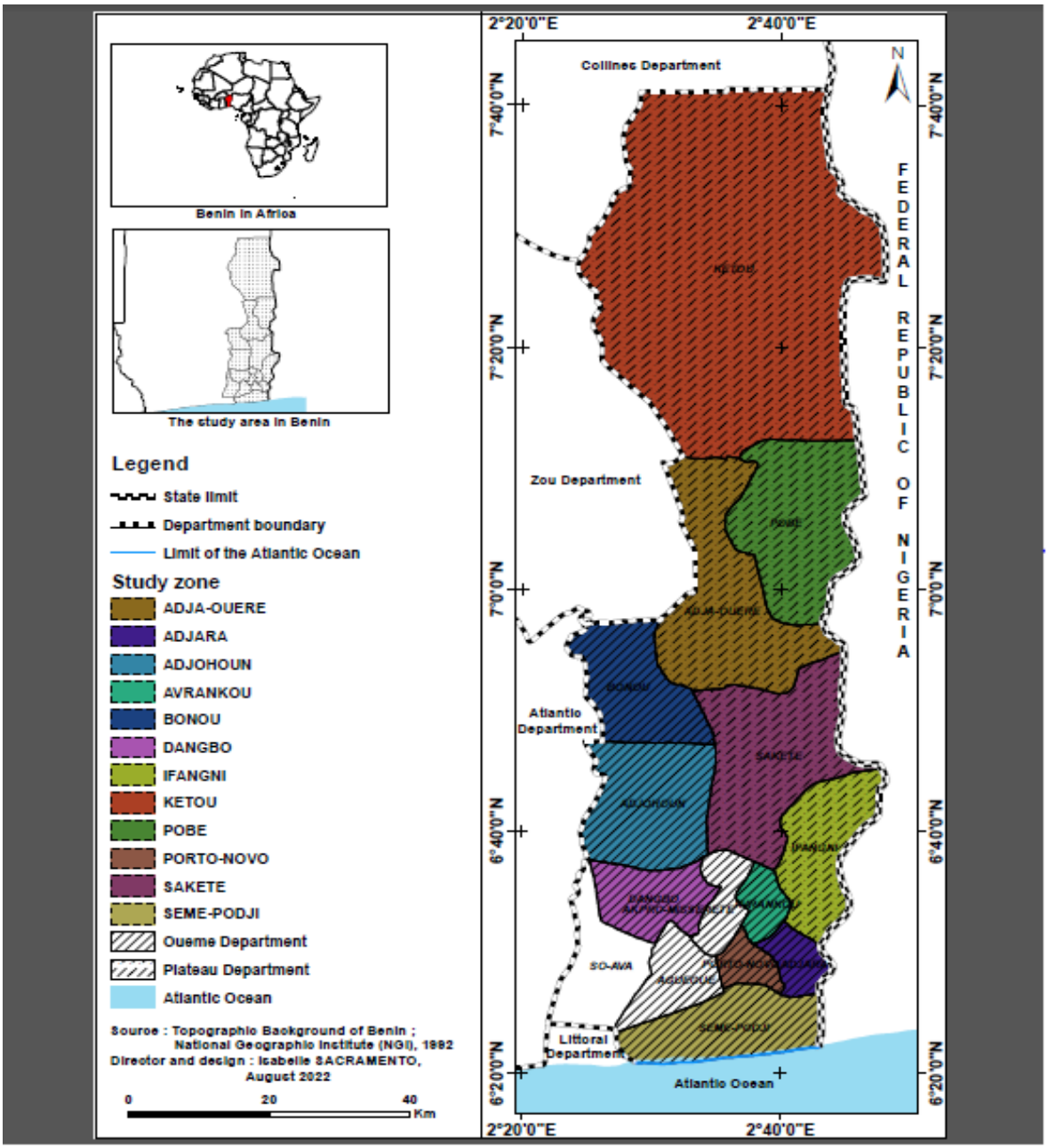

Figure 1 : Map showed the study zone in southern Benin

The Experimental device (Sacramento et al., 2022)

- ***In the field***

A total of 180 breedings were surveyed and only 118 farms were selected considering the size of the workforce, reproduction management, sanitary hygiene. The experimental animals were selected from 16 targeted farms with 30 grasscutters per farm, for a total of 480 animals. Eight breedings were selected as the control group (breedings not using lemon seeds) and the other eight breedings were the treated group (breedings using directly lemon seeds as an antihelmintic mixed with food supplements). The field experimental design was a simple randomized block with two treatments (lemon seed use and non-use). Thirty (30) grasscutters samples from each breeding site were considered. The grasscutter were feed ad libitum with conventional forages and supplementary feed. The conventional forages were composed of *Panicum maximum* (25 %), *Pennisetum purpureum*

(12.5 %), *Paspalum vaginatum* (12.5 %) while the supplementary feed was composed of corn bran, corn, roasted soybeans, leucaena or moringa leaflets, cassava roots, sweet potato tubers, cooking salt and oyster shell powder.

- ***In station***

A total of 35 (30 experimental and 5 reserve) grasscutter with a body weight of 3 kg and 7 months of age were used on station. Six (6) batches of 5 animals were constituted: one untreated control batch, 3 test batches and 2 reference control batches. The 3 test batches were treated with three doses of lemon seed (7.5, 15 and 30 mg/kg) for twenty-five (25) days.

The two reference control batches were treated with albendazole (7.5 and 8 mg/kg) three times at seven (7) day intervals. The animals were fed ad libitum with the same conventional forages and supplementary feeds.

- ***Coprology methods***

Regarding the coprological analysis method, we used the Mac Master quantitative method according to "Diagnosis of verminosis by coprological examination" (Sacramento et al., 2022). Briefly, 3 g of droppings were taken and triturated in a mortar with 42 mL of the saturated NaCl solution. The resulting triturate was then sieved to remove coarse elements. Using a pasteur pipette, the 2 cells of the Mac Master slide were filled while avoiding the formation of air bubbles. After 5 minutes of waiting to allow the eggs to stick to the cover slip, we proceed to the observation with a photonic microscope and a magnifying glass and then to the counting of the eggs.

The OPG (eggs per gram) was determined by the following formula:

OPG = (n1 + n2) × 50

With n1 = number of eggs counted in cell 1 and n2 = number of eggs counted in cell 2.

- ***Statistical analysis of data***

The data obtained were analyzed using SPSS 13.1 and Graph Pad Prism 7 software. Quantitative variables were presented as mean. The student test was used to compare the values of the different coprological examinations OPG, presence or absence of intestinal parasites of the treated animals with those of the control lot. The significance threshold was set at 5 %.

Results

1. *Social and Economic Characteristics of grasscutter 's breeders*

Botanical The social characteristics of grasscutter farmers in the departments of Ouemé and Plateau (Tables I and II) showed that they were 2.25 ± 12.10 years old and had an average experience of 5 ± 3 years in grasscutter farming.

The oldest was 79 years old and the youngest 24 years old. More than half were agro-breeders (64.50 %) and had been trained by another breeder (22.3 %). However, one third (23.80 %) of them were also trained by the Project for the Promotion of grasscutter breeding in sub-Saharan Africa (PPAS). More men (76.27%) are involved in this activity than women (23.73%).

Grasscutter breeders were from the following socio-cultural groups:

Goun (28.34 %), Fon (25.2 %), Hollidje (16.2 %), Nago (29.36 %), Mahi (0.5 %), Adja (0.2 %) and Yoruba (0.2 %). Breeding grasscutter need much greater use of family labor combined with wage labor.

The average annual income of an grasscutter breeders were more than 250,000 FCFA (387,30 USD).

2. *Practice of deworming in grasscutter breeding*

Most of the grasscutter breeders (60.16 %) preferred to use lemon seeds (69.49 %) because of their availability and low cost. Only 13.55 % of them used veterinary anthelmintics. Other plants such as *Crateva religiosa, Ocimum basilicum, Eucalyptus globulus, Newbouldia laevis* and *Vernonia amygdalina*, are also used in the treatment of other grasscutter pathologies (11.87%) (Table III).

3. *Using of lemon seeds*

Grasscutter breeders (69.49 %) used lemon seeds in grasscutter's feed for health reasons (Table III). They considered lemon seeds as pest control while 13.55% used veterinary pest control despite the high cost of the latter. Nearly 86.45 % of breeders used an approximate dose between 0.25 mg/kg to 30mg/kg per kilogram of Body weight and used small used beer cap as measuring spoons with precision in the food supplement for grasscutters. Some breeders (16.94%) considered lemon seeds as vitamins and only 13.55% were unaware of its using. Some preferred using seeds of yellow lemon (23.72 %) and others those seeds of green lemon (76.28 %) in grasscutter feeding.

Table I: Socio-professional characteristics of grasscutter breeders

Parameters	Characteristics of grasscutter breeders	Percentage (%)
Sex	Male	76.27
	Female	23.73
Socio-cultural group	Nago	29.36
	Goun	28.34
	Fon	25.2
	Hollidjè	16.2
	Mahi	0.5
	Yoruba	0.2
	Adja	0.2
Main activity	Agro-breeders	64.50
	Breeders	22.3
	Traders	9.6
	Official	3.6
	Small business	3.40
Breeder training	By a breeder	52.54
	PPAS/SEAG/GTZ	33.9
	Songhaï Center	6.78
	PEEANC	6.78
	Not trained	0
Use of family labor	Yes	100
	No	0
Breeding objectives	Economic	90.67
	Social	5.93
	Leisure	3.40

Table II: Age and years of experience of the visited breeders

Characteristics	Age of breeders (years)	Years of experience of breeders (years)
Average	42.25 ±12.12	5± 3
Minimum value	24	2
Maximum value	79	15

4. Doses of use of lemon seeds

The dose of lemon seed used by breeders varies from 0.25 mg/kg to 30 mg/kg of Body weight for a group of animals (5 grasscutters). It should be noted that 60.16% of grasscutter' breeders were literate and have a clear idea of treatment dosage. There were 13.55% of breeders who had no knowledge of its using as an endogenous anthelmintic and prefer the using of veterinary anthelmintics. The measure of the dosage of lemon seeds in breeding grasscutters is a beer cap brim if it is seeds of dried lemon seeds of and others having already acquired over time experience preferred just to cut four lemons and remove the seeds and mix them in the food supplement for grasscutters.

5. Antiparasitic effect of lemon seeds

- Coprology examination

Figure 2 showed that whipworms and Strongyloides were mostly found in grasscutter breedings in Ouémé and Plateau. The oocysts of Coccidia and Strongyles were not very representative in the breedings of these 2 departments of study but also cause health damage in the grasscutters.

Thus, breedings are mostly infested with whipworms with OPGs approaching up to (7000 OPGs). Then come strongyloides (5800 OPGs) and coccidia (2800 OPGs) and Strongyles with maximum OPGs of 2000.

Table III: Practice of deworming in aulacode breeding

Use of anthelmintics		Number	Frequency (%)
Veterinary Anthelmintic	Yes	16	**13.55**
	No	102	**86.45**
Application of doses of veterinary anthelmintics	Yes	16	**13.55**
	No	102	**86.45**
Using Lemon seeds	Unknown	16	**13.55**
	Vitamins	20	**16.94**
	Deworming	82	**69.49**
Reason for using Lemon seeds	Economic /Availability	102	**86.45**
	Restrictive	16	**13.55**
Application of doses of lemon seeds	0.25 mg/kg to 30mg/kg of Body Weight	102	**86.45**
	Unknown	16	**13.55**
Literacy rate of grasscutter breeding	Literate	71	**60.16**
	No literate	47	**39.84**
Antiparasitic medicinal plants	Lemon seed	82	**69.49**
	Papaya seed (*Carica papaya*)	22	**18.64**
	Crateva religiosa, Ocimum basilicum, Newbouldia laevis, Vernonia amygdalina	14	**11.87**
Type of lemon seeds	Yellow	28	**23.72**
	Green	**90**	**76.28**

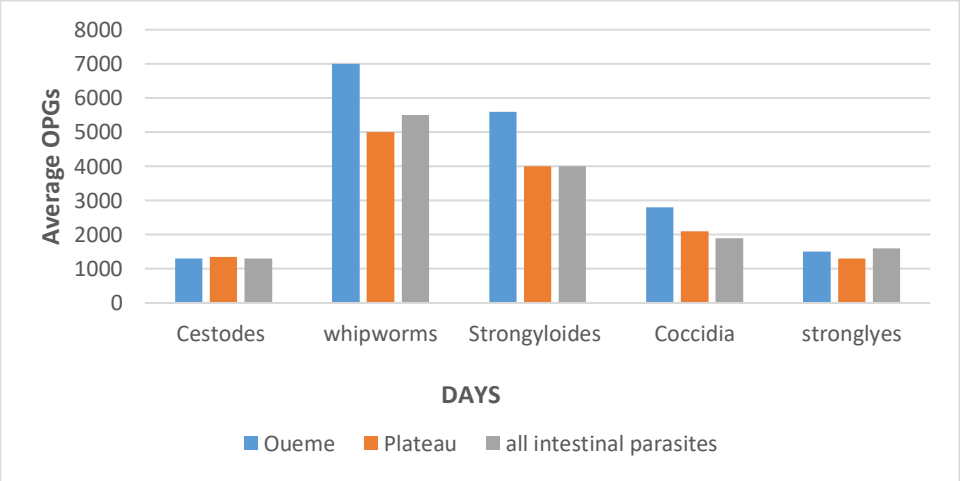

Figure 2: Level of infestation in the studies departments

Figure 3 showed the batches of grasscutter from breeds that didn't use lemon seeds or albendazole. The OPGs showed a very high level of infestation in the Oueme and Plateau grasscutter breedings. Figure 4 showed the grasscutter breedings that used lemon seeds as anthelmintics in their breeding. The observation made is that there is a considerable drop in OPG compared to grasscutter breeding which didn't using lemon seeds or albendazole.

Figure 5 showed that the farms in which albendazole is used the OPG were at least 200.

Figure 6 shows the OPG results at the station with the use of lemon seed at a rate of 30mg/Kg/PV. It is noted from this figure that the OPGs were at least 200 as shown by the OPGs in Figure 5.

All these figures clearly showed that there was a considerable reduction of OPG in grasshopper farms using lemon seeds as anthelmintic. This reduction in OPG is very remarkable at a dose of 30 mg / kg / PV (considerable decrease in OPG from 7000 to 200).

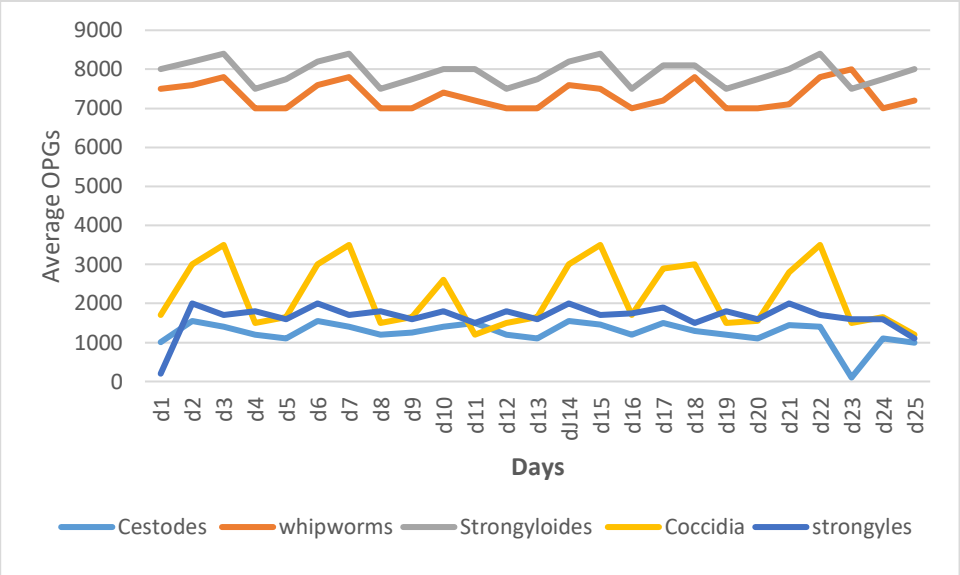

Figure 3: **Level of infestation to grasscutter breedings without deworming**

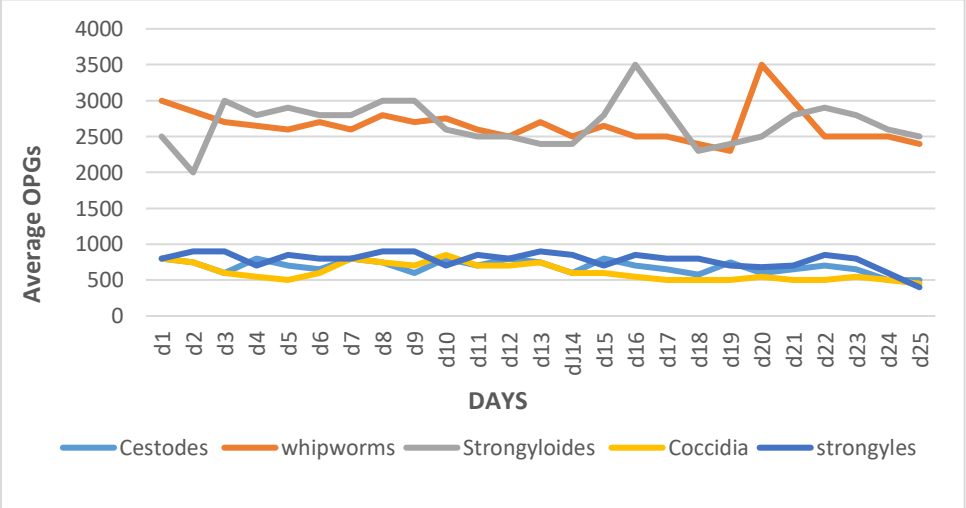

Figure 4: Level of infestation of grasscutter breeding's using lemon seeds

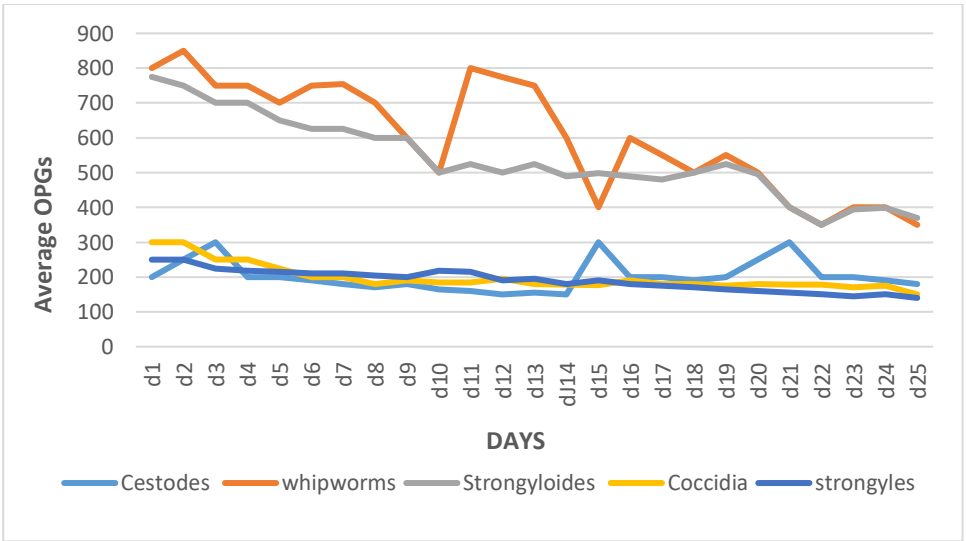

Figure 5: Level of infestation of grasscutter breeding's using Albendazole

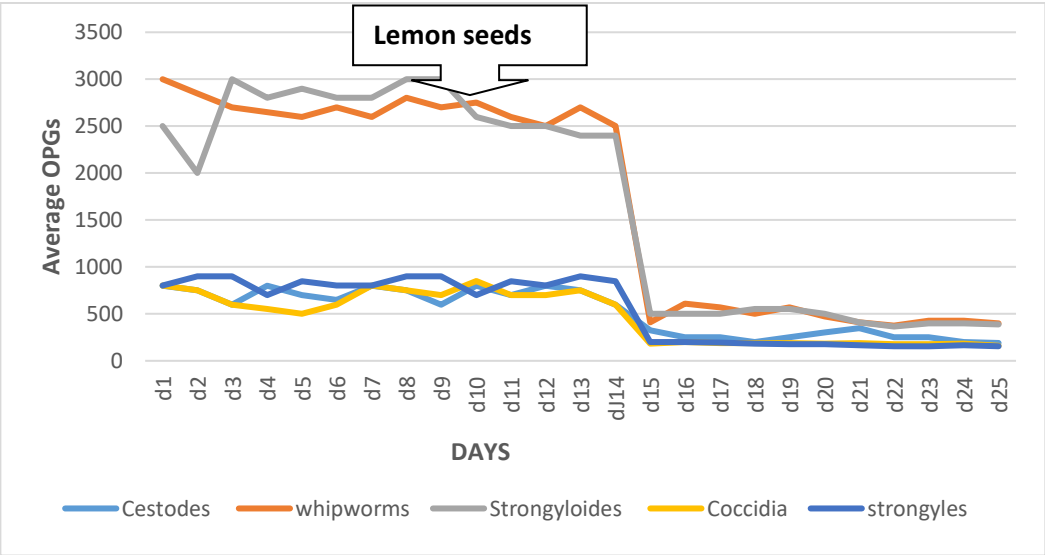

Figure 6: Level of infestation of grasscutters treated with lemon seeds in the laboratory

Discussion

- ***Social and economic Characteristics of grasscutter breeders***

The grasscutter breeders surveyed in the two departments visited are 24 years old on average with at least 5 years of experience. This trend confirms the observations made by Mensah et al. (2001) and Adjahoutonon (2005) in the departments of Ouémé and Plateau. grasscutter breedings operations are typically family-owned and operated with family labor, as confirmed by the work of Soulé (2000). This approach increases the profit margin and allows for good animal monitoring. Soulé (2000), confirmed our work by showing that the grasscutter breedings are mainly family breedings, which increases the profit margin by four times more than grasscutter breeding enterprises using salaried labor (Mensah et al., 2007). The average number of years of experience (5 years) of all the breeders surveyed in our study is higher than that (2.5 years) reported by Mensah et al. (2007). Many breeders start with a trial breeding before later establishing a full-fledged grasscutter breedings with larger numbers. In relation to the gender of the grasscutter breeders in the study departments (76.27% of the breeders are men and only 23.73% are women), their socio-cultural groups (Fon, Aïzo, Hollidje, Mahi, Yoruba, Nago, and Goun in the majority) and their main activity (2/3 farmers or breeders) are also consistent with those reported in the same area by Mensah et al. (2007). The same is true of our observations on their training locations, with training by other association of grasscutter breeders and grasscutter breeders followed by training at the grasscutter breeding station of the Project "Promotional of grasscutter breeding in Sub-Saharan Africa (PPAS)". All breeders are trained either by one of their own or by a structure that is skilled and experienced in the field. The neglect of the gender approach during training would be the cause of the reluctance and low participation rate of women in this activity. The presence of commercial poles due to the proximity of the Nigerian borders is another reason for this state of affairs. The observations made by Adjahoutonon (2005) in the departments of Oueme and Plateau in Benin are generally agreeing with our own, since all of the grasscutter breeders surveyed are male and more than two-thirds of them use labor, while nearly half are farmers.

- ***Parasites diseases encountered in grasscutter breeding***

Several parasitic pathologies described in the breedings visited are similar to those mentioned by various authors in Southern Benin (Adjahoutonon, 2005), then in Ivory Coast (Bi et al., 2013). Mensah et al. (2007) found this to be the case in almost a third of grasscutter breeders, with the digestive tract of grasscutter being severely attacked by acute illnesses (70%). Digestive pathologies kill more than a quarter of the animals, and this is in line with the observations made by Assogba et al. (2009) in Benin who already described them as one of the main causes of mortality in grasscutters breeding.

However, compared to the level of infestation of grasscutters by parasites of the digestive tract, we find that the grasscutters breedings visited are infested by Strongyloides and whipworms The critical periods of infestation are during the rainy

season and, according to Mensah and Ekué (2003), the simple drying of green fodder reduces their importance. In addition, the OPGs of whipworms and Strongyloides are very high without apparent symptoms observed in the grasscutter breedings, assuming the climate is favorable to the development of whipworms and Strongyloides. In addition, it should be noted that the presence of Coccidia oocysts which, despite their OPG, devastate grasscutter breedings. Mensah and Ekué (2003) advised treating coccidiosis in grasscutters with amprol (3 g/kg PV) or ganidan (2 tablets/d) for 3 days.

- ***Use of lemon seeds in the diet of grasscutter***

The treatment of parasitic diseases uses lemon seeds (69.49%) in different doses and medicinal plants was mentioned by Mensah et al. (2007) who stated that respectively 86.45% of the breeders trust the pharmacopoeia.

The effectiveness of treatments recorded with lemon seeds is variable but effective because their breeders used the variable dosage. This leads us to continue the research in station to find the optimal dose of the lemon seeds, and the best mode of administration.

The results obtained showing a decrease in OPG after the use of lemon seeds are similar to the effectiveness obtained for Albendazole used as reference molecule. These data of the present study allow to envisage future investigations for in-depth studies that could lead to bioactive molecules with anthelmintic properties.

Conclusion

The purpose of this study was to compare the efficacy of lemon seed and albendazole (veterinary anthelmintics) on gastrointestinal parasites of grasscutter. The results obtained from the ethnopharmacological survey conducted in the departments of Oueme and Plateau confirmed the traditional uses of lemon seeds in the treatment of gastrointestinal parasitosis of grasscutter. Efficacy tests provided scientific validation of the anthelmintic effect of lemon peels with a potential comparable to albendazole used as a reference drug for the treatment of gastrointestinal parasites in grasscutter farms. This study allows to consider further studies on lemon seeds in order to develop a bioactive molecule with anthelmintic properties.

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