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Effects of the Ethanolic Extract of Avocado (*Persea americana* M.) Seed Powder on Growth Characteristics and Reproductive Performance of Female Cavies (*Cavia porcelus* L.)

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ABSTRACT

Faced with the negative side effects (like hepatotoxicity, nephrotoxicity, reprotoxicity and antibiotic resistance) of some synthetic molecules usually used in animal husbandry, the use natural molecules from medicinal plants seems to be part of the solution to this problem. The general objective of the present study is therefore to contribute to improve the productivity of livestock animals, by the use of avocado seeds. More specifically to show in female cavies (*Cavia porcelus* L.), the effects of the cavies ethanolic extract of the avocado (*Persea americana* M.) seed powder on growth characteristics and reproductive performance. To attend this objective, 48 female cavies with regular estrous cycles were use. To attend this objective, 48 female cavies with regular estrous cycles were use. They were selected from a herd of 90 females. They were randomly distributed into 4 groups of 12 females each, comparable in terms of body weight (464.25±60. 13g). The control group received, by gavage 2 ml/kg bw of distilled water for 120 days, while the treated groups received 100, 200, and 400 mg/kg bw of the seed extract for the same duration. The latter groups were designated EE100, EE200, and EE400 groups respectively. After 51 days of gavage, half of the females of each group were sacrificed in estrus and the ovaries and uterus collected for analysis. The other half was bred with proven boars. The extract administration continued until delivery (parturition). The main results are as follows: The weight and the weight gain of the females at delivery generally increased in all groups treated with the extract. However, the highest ($p<0.05$) value was obtained in the females of the EE200 and EE400 groups with reference to the control. The weight of the pups at weaning increased in females treated with extracts, but significant ($p<0.05$) only in the subjects of the EE400 group, compared with the control. In view of these results, the ethanolic extract of the avocado seed powder improved the reproductive performance of female cavies.

Keywords: Avocado Seed; Ethanolic Extract; Cavies; Growth; Reproductive Performance

Introduction

Food insecurity in Africa in general and Cameroon in particular is a serious public health problem [1]. Indeed, there is an exponential growth of African population and Cameroonian in occurrence without a commensurate growth in agricultural (food) production, resulting in a food deficit. In other words, demographic growth thoroughly leads to an increase in demand. Despite measures taken by governments, the offer remains insufficient to cover not only national, but also international needs [2]. Faced with this problem, the promotion of livestock, including non-conventional species, like

cavies (*Cavia porcelus* L.) can be a solution to overcome this deficit. To boost the productivity of livestock, breeders generally use synthetic molecules, in the form of hormones, antioxidants and antibiotics. However, many studies have shown adverse effects of these synthetic molecules on livestock and human population that consume these livestock products [3,4]. For example studies conducted by Lanigan and Yamarik [5]; Nadeau [4] showed that BHT (butyl-hydroxytoluene) and BHA (butyl-hydroxyanisole), reference antioxidants used in animal husbandry to preserve the smell, taste and color of the feed, can be hepatotoxic, nephrotoxic and reprotoxic in livestock animals and in humans. In addition, the chemical synthesis of bioactive molecules by pharmaceutical firms, leads to emission of large amount of waste, polluting the environment, hence the need for a return to more ecologically friendly solutions [6].

The use of natural products rich in bioactive molecules, originating from living matter like plants (whole plants, plants extracts and plant's essential oils), with hormonal, antioxidant and antibiotic activities, can serve as a solution to increase animal productivity. Indeed, in addition to being therapeutically effective, these are generally available, less expensive and better tolerated by the body than many synthetic substances. These synthetic substances are however physiologically very active but their side effects are mostly unpredictable [7,8]. The seed of *Persea americana* M. (pear) is part of this category of natural products. Considered as food waste, pear seed contains many bioactive molecules such as phenols, phytosterols, triterpinetoids, flavonoids, carotenoids, alkaloids, saponins, tannins, proanthocyanidines, proteins, fatty acids, sugars, vitamins (A, C and E) which endows them with many proven pharmacological properties and therefore provide a gateway to many research works. This includes antioxidant, estrogenic, anti-inflammatory, anti-carcinogenic properties, etc. [9,10].

Daramola et al. [11] demonstrated the ability of phenols contained in pear seed to protect the sperm of goat against oxidative stress during cryopreservation. Mvondo et al. [12], have shown the antiproliferative effect of the ethanolic extract of avocado seed powder on the uterine endometrial hyperplasia induced by Tamoxifen in Wistar rats. Similarly, Minko et al. [13] provided proof that the ethanolic extract of avocado seed induces the regression of endometrial implants and restores ovarian dynamics on an endometriosis model in Wistar rats.

The presence of antioxidant in avocado seed extracts, could make them remarkable phyto-additives, capable of improving production performance in cavies. Indeed, in the face of the various endogenous and/or exogenous factors likely to generate stress and therefore oxidative stress, these extracts could prevent radical phenomena, harmful for production. Thus, the energy mobilized by the animal to cope with this oxidative stress could be mobilized for production. Moreover, the flavonoids, alkaloids and tannins contained in the extracts of avocado seed, by their anti-inflammatory, anticarcinogenic, antibiotics and antimicrobial activities, could strengthen the immunity of animals, allowing them to resist diseases and reproduce more efficiently. Although avocado seeds have been the subject of numerous studies, to our knowledge, very few of them deal with their use in improving livestock productive performance. It is with this in mind that this study was designed to examine the improvement of the productivity of livestock animals, through the use of medicinal plants. More specifically, to evaluate, the effects of ethanolic extract of avocado seed powder on growth characteristics and reproductive performances of female cavies.

Materials and Methods

Animal Material and Housing

A total number of 48 adult females' cavies (*Cavia porcelus* L.) of English breed, aged 3 months and average weight of 464.25±60.13g were used for this study. They came from the Training and Research Farm of the University of Dschang (TRF-UDs) and were housed in the caviaculture building, with an ambient temperature between 20 and 30ºC and a lighting rhythm of 12 h day and 12 h night. This building was equipped with concrete cages, made up of wire nets on the front façade. Each of the cages had dimension 100 cm x 80 cm x 60 cm (length x width x height) and equipped with feeders and drinkers respectively. Those cages were split into 6 compartment and each containing a cavy.

Feeding

Throughout the period of the test, the guinea pigs of all the batches received ad libitum drinking water fit for human consumption and a compound food whose ingredients were purchased in a market, with the exception of Pennisetum purpureum which was harvested at the TRF. This Pennisetum purpureum was wilted and incorporated into the feed. The bromatological characteristics of this ration are as follows: digestible energy (kcal/kg DM), 2750; Dry matter (%), 97.82; Organic matter (% DM), 86.06; Gross protein (% DM), 16.79; Gross cellulose (% DM), 15.80; Ash (% DM), 13.94.

Plant Material

The Fruits of *Persea americana* M. (avocado) were harvested at maturity, on the same tree, in a farm in Mbouda (Bamboutos Division, Cameroon Western Region) in September 2018. For authentication, branch samples (comprising leaves and flowers) and bark harvested from the avocado trees were preserved and forwarded to the National Herbarium Center of Yaoundé (Cameroon). This specimen was referenced under number 18604/srf/cam, according to the comparison model with the hardware of Daniel Dong number 80. The fruits were cleaned and the extracted seeds were crushed into fine particles, then directly boiled at 100 °C for 15 minutes (using a constant pressure pot) and dried in the shade in accordance with the method described

by Talabi et al. [9]; to reduce the quantity of ant-nutritional factors (cyanhydric acid). The dried particles were then crushed to obtain a homogeneous powder, used for the preparation of ethanolic extract.

Preparation of the Extract

Ethanolic extract of avocado seed powder was prepared according to the maceration method described by Egbuonu et al. [14] (Figure 1). The extract thus obtained was introduced in opaque vials and kept at 4°C until use.

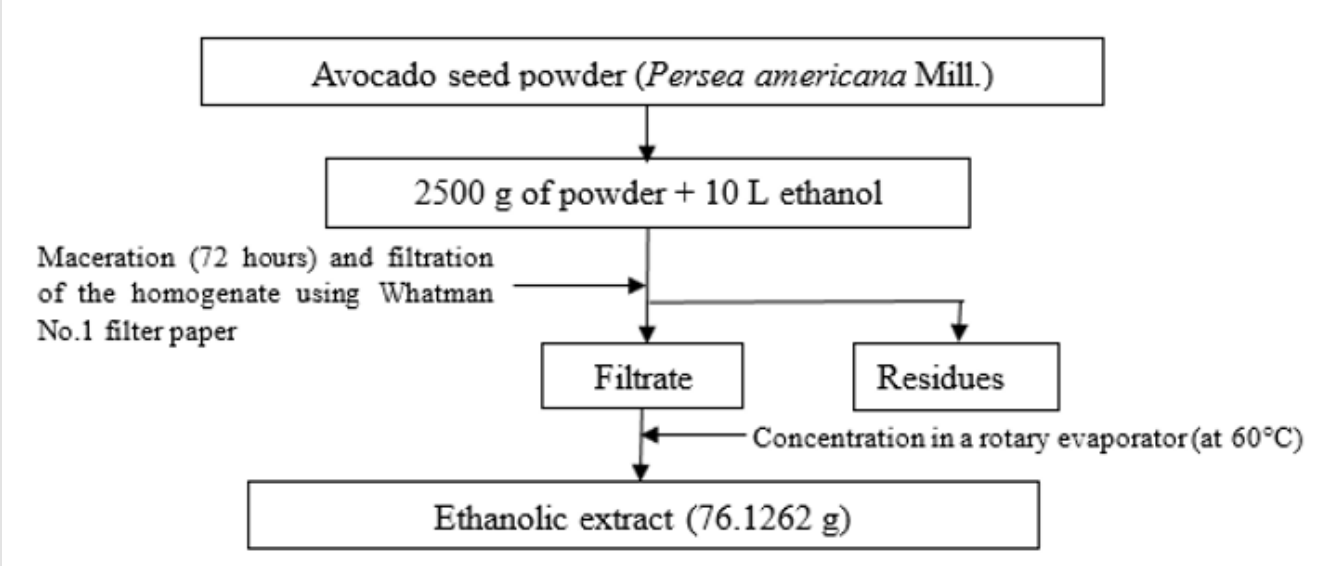

Figure 1: Diagram of the preparation procedure of the ethanolic extract of avocado seed.

Phytochemical Composition of the Extract

A phytochemical study was carried out on the ethanolic extract of avocado seed powder to reveal the different groups of bioactive compounds contained in it. This is how the qualitative method described by Ramde-Tenderbeogo et al. [15] was used to highlight the presence of the bioactive compounds present in the extract. The method described by Padmaja et al. [16] made it possible to determine the quantities of phenols and flavonoids.

Choice of the Different Doses of Extract to Administer

The different doses of ethanolic extract (100, 200 and 400) of avocado were chosen under the basis of the results of the work of Ozolua et al. [17] and Padilla-Camberos et al. [18] who revealed a LD50 of 1200.75 mg/kg bw in rats by administration through oral route the aqueous and phenolic extracts of avocado seed for 28 days. Indeed, after a sub-acute toxicity study, these authors concluded that the LD100 of avocado seed extracts would be above 10 mg/kg bw because at this dose they did not obtain 100% mortality. The same is true for the study made by Pahua-Ramos et al. [19], which made it possible to obtain following the oral administration of the methanolic extract of avocado for 6 days in mice, a LD50 of 1767 mg/kg bw.

Conduct of Trial

Forty-eight (48) female cavies with regular estrous cycles were used in this study. They were selected out of a number of 90 females after studying 4 consecutive estrous cycles (by the vaginal smear method described by Sahar and Abeer, [20]. These females were weighed using a 5 kg capacity balance and 1 g sensitivity, then randomly distributed in 4 groups (Control, EE100, EE200 and EE400) of 12 females each, corresponding to the different treatments. Thesegroups were comparable in terms of body mass (464.25±60.13g). Each of the females was marked with an eartag and considered as an experimental unit. These animals received by gavage throughout the period of the test (120 days), 2ml/kg.bw of distilled water for those of the control; 100, 200 and 400mg/kg.bw of ethanolic extract of the avocado seed powder respectively for the EE100, EE200 and EE400 groups. The gavage solutions were prepared by introducing 2.5, 5 and 10 g of extracts into 50 ml gauged vials, then completing with distilled water to the gauge line, so as to obtain concentration solutions 50, 100 and 200 mg/ml respectively for doses 100, 200 and 400 mg/kg bw. These were kept in opaque vials, then administered to the females' cavies every morning (between 6am and 8am). These animals were weighed individually every 7 days and distilled water volumes and

solutions of avocado seed powder extracts adjusted. After three consecutive estrous cycles (51 days) of treatment, half of the female cavies of each batch (6/group) were anesthetized with petroleum ether and sacrificed in estrus (vaginal smear characterized by the predominance of cornified epithelial cells and anucleate). Subsequently, after laparotomy, the uterus and ovaries of each of the sacrificed females were collected for analysis. The other half (6/group) was then paired with sexually matured, proven males. To do this, the males were introduced into the lodges of the females following a sex ratio of a male for six females and rotated after 5 days, so that in 20 days, each of them made the 4 lodges representing the different treatments. Extract administration as well as weighing continued until littering.

Parameters Studied and Data Collection

Growth Characteristics

Consumption Index: The food was weighed and distributed daily (every morning between 7 and 8 am), and then the remnants of each treatment were weighed at the end of the week using a 5 kg and 1g precision capacity electronic scale. Subsequently, cavy's consumption index (CI) was determined according to the formula described by Kana et al. [21]

$$CI = \frac{Feed\ consumption}{Weight\ Gain}$$

The feed consumption was determined by finding the difference between the amount of feed served and the remnants at a given period.

Weight Gain: The animals were weighed each week with the help of an electronic balance of 5 kg capacity and precision 1 g until the end of the test. The weight gain (WG) of guinea pigs was obtained arithmetically, by finding the difference between the live weight of the animal at the end of the trial (W_n) and that at the beginning (W_{n-1}) [21].

$$WG = W_n - W_{n-1}$$

Histological Study of Uterus and Ovaries: To realize the histological sections of the ovary and the uterus, immediately after laparotomy, the right ovary and right uterine horm of each cavy were collected and fixed in formol at 10%. They were cut off in a transverse plane and arranged in plastic and labeled cassettes. The fixed tissues were cleared of any trace of water by successive immersions in 8 tanks of alcohols, at the rate of two hours in each tank in the following order: 1 ethanol tank at 80% (1 x 2 h), 4 tanks at 95% ethanol (4 x 2 h) and 3 absolute alcohol tanks (3 x 2 h). After dehydration, the tissues were put successively into two baths in xylene (2 x 2 h). The cassettes containing the organ fragments stayed 4 hours (2 h x 2 baths) in warm and liquid paraffin. The tissues were placed in molten paraffin stainless steel molds (60 °C), then put on a cold surface (4 °C) for solidification after appropriate orientation of the tissue in the block. The blocks obtained were cut with a microtome. Cut ribbons of 5 µm thick obtained, were spread in a water-bath containing gelatinous water. By means of clean and labeled objects blades, the cuts were subsequently collected and dried at the oven at 45 °C for 24 hours. The cuts were cleared of immersion paraffin in xylene baths and rehydrated in alcohol baths at decreasing concentrations up to distilled water. Each bath having a duration of 5 minutes.

Blades containing organ cuts were successively passed in the hematoxylin baths of Harris, tap water, alcohols at 70 and 95%, alcoholic eosin and finally tap water, at the rate of 5 minutes per bath. The cuts were dried before being mounted. This operation was made in two stages: the first consisted of dehydration in three absolute liquor baths (3 x 5 minutes) and the second, lightening in three xylene baths (3 x 5 minutes). The microscopic analysis of the cuts was performed in the Laboratory of Animal Health and Physiology (LAPSAN) of the University of Dschang using a Leica (DM500, Leica®) brand optical microscope, connected to a computer. The images were filmed (with magnifications 100 and 400) using the microscope integrated camera and the Leica LAS EZ software version 2.0.0 of the Leica Microsystem.

Determination of the Sizes of the Uterine Epithelium: After transferring the images into a computer, the measurements of the epithelium were made using the software Image J [12].

Quantitative Evaluation of Ovarian Follicles: This was evaluated by counting tertiary follicles, follicles from Graaf and yellow body found on the ovarian cuts. For the same ovary, three different sections were arranged on the same blade (the tenth, the fifteenth and twentieth), then the different follicular types were counted by direct observation at the optical microscope at 400X magnification. The final result was obtained in average observations made on the three sections of the same ovary [13,22].

Reproductive Performance

Gestation Length

The gestation length was obtained by counting the duration between the day of mating and the day of littering. Successful mating was verified by the presence of spermatozoa in the vaginal smear of the female. The presence of spermatozoa in the vaginal smear marked the first day of gestation [23-25].

Fertility Rate

The fertility rate was obtained from the following formula described by Lienou et al. [26]:

$$Fertility\ rate\% = \frac{number\ of\ female\ given\ birth}{number\ of\ female\ mated} \times 100$$

Litter Size

It was obtained by counting the number of pups by each female cavy [27].

Weight of Pups at Birth

It was obtained by taking the individual weight of the pups at birth directly, using an electronic weighing balance, with a capacity of 5000 g and a precision of 1 g [27].

Weight of Pups at Weaning

It was obtained by taking the individual weights on the pups at 21 days postpartum. Only the pups from groper births were considered [27].

Weight Gain of Pups at Weaning

This is the difference between weight at birth (Wb) and weight at weaning (Ww) [21].

$$WG = Ww - Wb$$

Viability Rate of Litter

Pups were closely monitored from littering until weaning to detect any mortalities. The rate of viability of the litter was therefore calculated from the following formula [26,27]:

$$Viability\ rate\ of\ litter\ (\%) = \frac{number\ of\ viable\ pups}{Litter\ size} \times 100$$

Statistical Analysis

The data obtained were subjected to one-way analysis of variance (ANOVA) to test the effects of the different doses of ethanolic extract of the avocado seeds powder on the parameters studied. Duncan test was used to separate means when there were significant differences. The results were expressed as a mean ± standard deviation and the mean threshold was 5%. SPSS version 25.0 Statistical Software was used for data analysis.

Results

Phytochemical Composition of Ethanolic Extracts of Avocado Seed Powder

Table 1: Phytochemical composition of ethanolic extracts of avocado seed powder.

Bioactive compound	Ethannolic extract	Test carried out
Flavonoids	+	Shinada test
Alkaloids	_	Dragendorff test
Phenols	++	Ferric Chloride test
Triterpenes	+	
	Lieberman Buchard test	
Steroids	+	Lieberman Buchard test
Saponins	++	Foam test
Tannins	++	Ferric Chloride test

Note: -: Absent; +: Present; ++: Present in a large quantity.

Table 1 shows the phytochemical composition of the ethanolic extract of avocado seed powder (*Persea americana* M.). It revealed the presence of bioactive molecules such as flavonoids, phenols, triterpenes, steroids, saponins and tannins.

Effects of the Ethanolic Extract of the Avocado Seed Powder on Growth Characteristics in Female Cavies Before Breeding and During Gestation

Tables 2 & 3 shows the variations of growth characteristics according to the doses of ethanolic extract of avocado seed powder in female cavies before breeding and during gestation. Before breeding, the consumption index increased significantly ($p<0.05$) in the cavies of the group EE100 compared to the other groups. During gestation, this characteristic was not significantly affected by the extract. On the other hand, the weight of the females at delivery generally increase in the groups treated with the extract. However, the highest ($p<0.05$) value was obtained in the females of the groups treated with 200 and 400mg/kg bw of ethanolic extract with reference to control. Before breeding, the weight gain of the female cavies was high in the EE200 and EE400 treated groups but with significance ($P<0.05$) only in the EE200 group. However, during pregnancy, it increased generally in all the females treated with avocado seed extract. However, this increase was significant ($p<0.05$) in females receiving 200mg/kg bw of ethanolic extract with reference to the control group.

Table 2: Variation of some growth characteristics according to the different doses of ethanolic extract of avocado seed powder in female cavies before breeding.

Growth characteristics	Control (n=12)	Doses of ethanolic extract of avocado seed powder			
		EE100(n=12)	EE200 (n=12)	EE400 (n=12)	p
Initial weight(g)	464.17±53.39	464.25±68.19	464.33±72.37	464.25±52.08	1.00
CI /animal	72.84±35.24a	103.82±47.10b	48.22±20.08a	64.67±27.86a	0,00
Final weight (g)	491.42±45.65	484.75±60.57	502.58±62.51	494.67±41.47	0.87
Final WG (g)	27.25±10.82ab	20.50±9.46a	38.25±10.99c	30.42±13.59bc	0.00

Note: n = number of samples; (a, b, c): on the same line, the affected values of the different letters are significantly different ($p<0.05$); p = probability; Control: 1 ml/kg bw of distilled water; (EE100, EE200, EE400): ethanolic extract of avocado seed powder at the respective doses of 100, 200 and 400 mg/kg bw; CI = Consumption Index and WG = Weight Gain.

Table 3: Variation of some characteristics of growth according to the different doses of ethanolic extracts of avocado seed powder in female cavies during pregnancy.

Growth characteristics	Control (n=6)	Doses of ethanolic extract of avocado seed powder			
		EE100 (n=6)	EE200 (n=6)	EE400 (n=6)	p
Initial weight(g)	479.67±22.89	479.67±16.61	481.67±38.44	490.20±20.50	0.88
CI /animal	18.20±4.28	15.53±4.31	12.07±2.26	16.40±5.72	0,20
Final weight (g)	633.75±21.07^{a}	674.00±56.03^{ab}	712.60±64.62^{b}	699.80±46.90^{b}	0.04
Final WG (g)	154.08±38.3^{a}	194.33±47.83^{ab}	230.93±41.64^{b}	209.60±53.44^{ab}	0.04

Note: n = number of samples, (a, b, c): on the same line, the affected values of the different letters are significantly different ($p<0.05$); p = probability; control: 1 ml/kg bw of distilled water; (EE100, EE200, EE400): ethanolic extract of the respective doses of 100, 200 and 400 mg/kg bw of avocado seed powder; CI = Consumption Index and WG = Weight Gain.

Effects of Ethanolic Extract of the Avocado Seed Powder on the Histomorphometry of Ovary and Uterus in Cavies

Effects on the Number of Tertiary Follicles, De Graaf and Yellow Body

Figure 2 presents the effects of ethanolic extract of avocado seed powder on the histology of the ovary. It appears that this extract did not alter the histological structure of this organ. From figure 3 showing the effects of the avocado seed powder extract on the numbers of tertiary follicles, De Graaf and yellow body in cavies, it appears that the different doses of this extract were comparable ($p>0.05$) to the control group, for the numbers of tertiary follicles, De Graaf follicles and yellow body in the cavies. However, the highest De Graaf follicles was obtained from the females of the EE200 group.

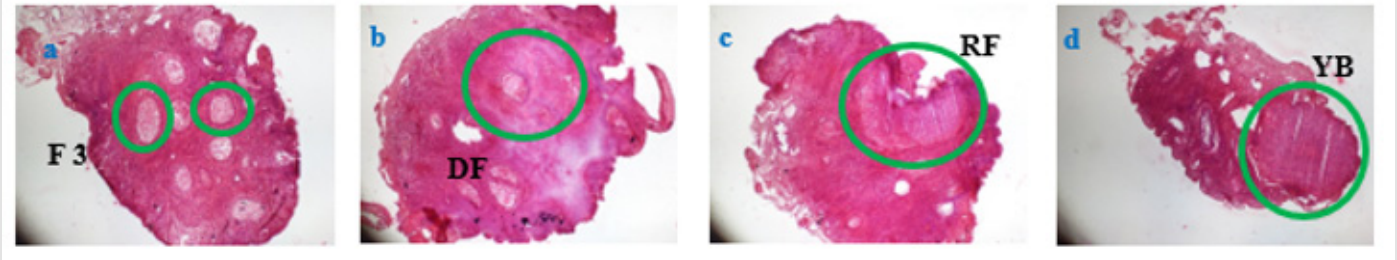

Figure 2: Photomicrograph of ovaries of cavies treated with avocado seed (x 400, H&E).

Note: F3: Tertiary Follicle, DF: De Graaf follicle, RF: Ruptured Follicle, YB: Yellow Body, A: Control group receiving 1 ml/kg bw of distilled water; b, c, d: EE100, EE200 and EE400 groups receiving the ethanolic extract of avocado seed powder at the respective doses of 100, 200 and 400 mg/kg bw.

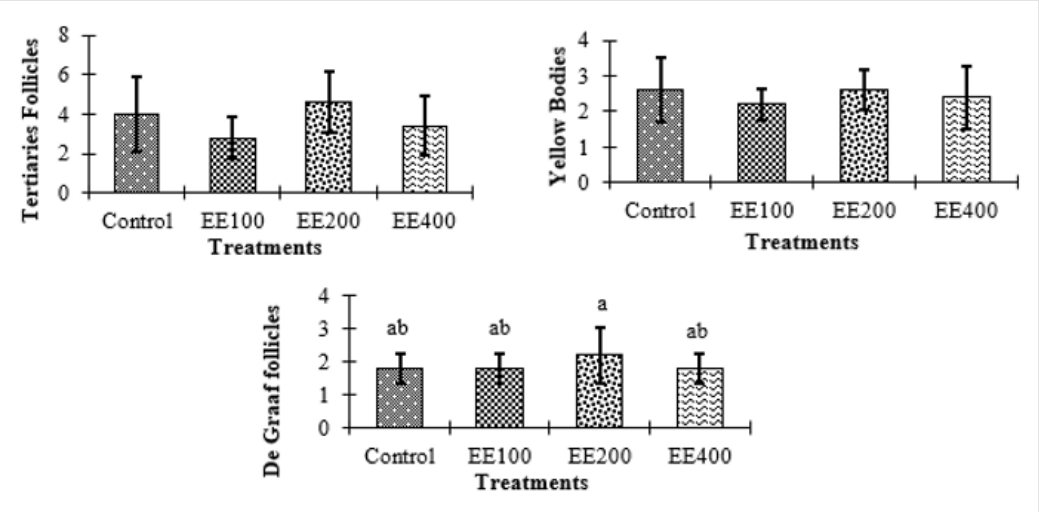

Figure 3: Effects of the ethanolic extract of avocado seed powder on the number of tertiary follicles, De Graaf follicles and yellow body in cavies' ovaries.

Note: (a,b): On the same histogram, the bars having different letters are significantly different ($p<0.05$); Control: 1ml/kg bw of distilled water; (EE100, EE200, EE400): ethanolic extract of the avocado seed powder at the respective doses of 100, 200 and 400 mg/kg bw.

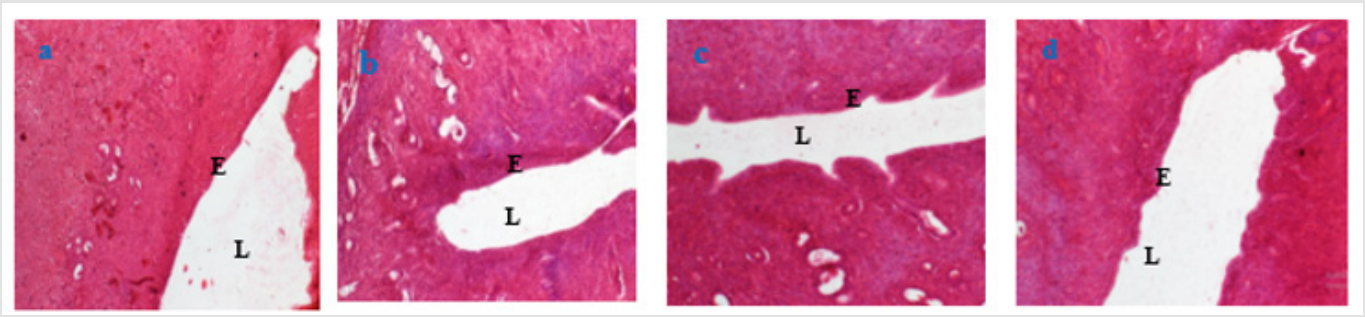

Figure 4: Photomicrograph of the uterus of cavies treated with avocado seed (x 400, H&E).

Note: E: epithelium, L: light. a: Control group receiving 1 ml/kg bw of distilled water; b, c, d: EE100, EE200 and EE400 groups receiving the ethanolic extract of avocado seed powder at the respective doses of 100, 200 and 400 mg/kg bw.

Effects on the Histology of the Uterus and the Size of Uterine Epithelium

Figure 4 shows the effects of the ethanolic extract of the seed powder on the histology of the uterus. It appears that the extract did not alter the histological structure of this organ. From figure 5 showing the effects of the avocado seed extract on the size of the uterine epithelium, it appears that this extract caused an increase in the size of the uterine epithelium. However, this increase was significant ($p<0.05$) only in females of the EE200 group with reference to those of the control group.

Effects of Ethanolic Extract of Avocado Seed Powder on Reproductive Performances in Female Cavies

Effects of the Treatment on the Fertility Rate: Figure 6 presents variations in the fertility rate in the different groups of female cavies receiving different doses of ethanolic extract of avocado seed. The extract at different doses led to an increase in the fertility rate of treated females, compared to that of the females of the control group; however, the highest rates were obtained in the females of the EE100 and EE200 groups.

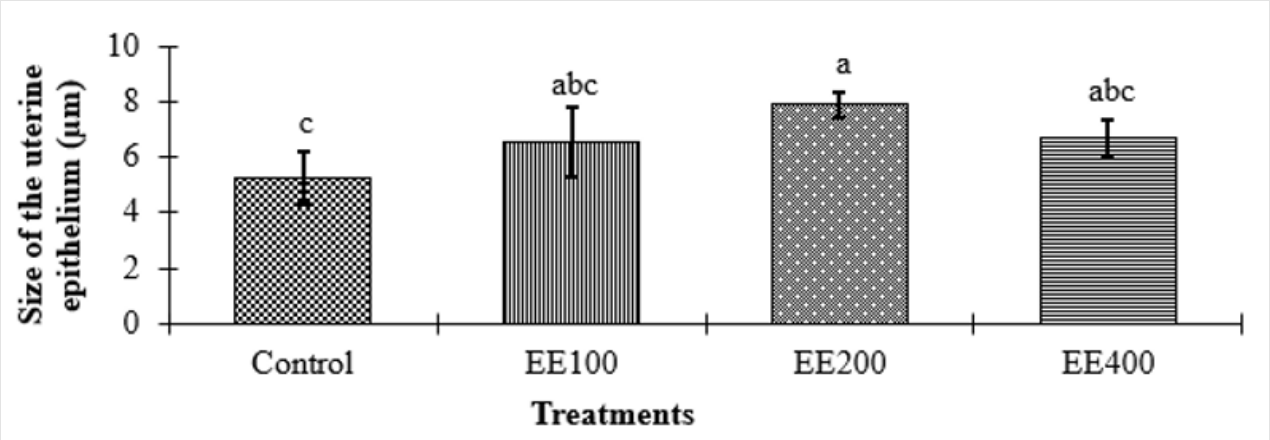

Figure 5: Effects of the ethanolic extract of the avocado seed powder on the size of the uterine epithelium.

Note: (a,b,c): on the same histogram, the bars having different letters are significantly different ($p<0.05$); Control: 1 ml/kg bw of distilled water; (EE100, EE200, EE400): ethanolic extract of the avocado seed powder at the respective doses of 100, 200 and 400 mg/kg bw.

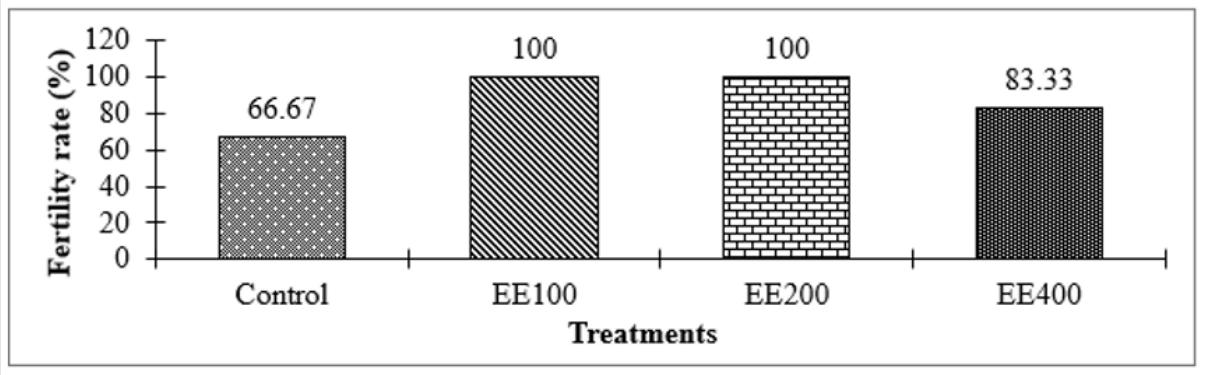

Figure 6: Variation of the fertility rate in different groups of female cavies receiving different doses of ethanolic extract of the avocado seed powder (*Persea americana* M.)

Note: Control: 1 ml/kg bw of distilled water; (EE100, EE200, EE400): ethanolic extract of avocado seed powder at the respective doses of 100, 200 and 400 mg/kg bw.

Effects of the Treatment on the Gestation and the Postpartum: Table 4 shows the effects of the different doses of ethanolic extract of avocado seed powder on the gestation length, litter size, weight of pups at birth and at weaning, weight gain to 21 days and daily weight gain of the pups. It appears that in general, this treatment induced no significant effect ($p>0.05$) on the gestation length, the litter size of female cavies, the weight of pups at birth and their weight gain to 21 days postpartum (weaning). However, there was an increase in features such as litter size and the weight of pups at weaning in subjects treated with avocado seed extracts. The weight of pups at weaning generally increased in females receiving extracts of avocado seed powder with significant increase ($p<0.05$) in subject receiving 400 mg/kg bw of the extract.

Table 4: Effects of ethanolic extract of avocado seed powder on the gestation length, litter size, weight of pups at birth and at weaning and weight gain of pups at weaning.

Reproductive characteristics	Control (n=6)	Doses of ethanolic extract of avocado seed powder			
		EE100 (n=6)	EE200 (n=6)	EE400(n=6)	P
Gestation length (days)	68.00±1.82	69.00±1.22	69.00±1.58	67.80±1.92	0.55
Litter size	1.25±0.50	1.40±0.55	1.80±0.45	1.20±0.45	0.25
Weight of pups at birth (g)	80.00±1.41	82.75±8.46	86.17±4.24	98.00±4.24	0.30
Weight of pups at weaning (g)	136.00±18.38b	135.00±15.05b	151.83±11.16ab	169.00±2.83a	0.04
Weight gain of pups at weaning (g)	56.00±16.97	52.25±22.62	65.67±15.63	71.00±1.41	0.55

Note: N = number of samples, (a,b): on the same line, the values having different letters are significantly different ($p<0.05$); p = probability; Control: 1 ml/kg bw of distilled water; (EE100, EE200, EE400): ethanolic extract of avocado seed powder at respective doses of 100, 200 and 400 mg/kg bw.

Effects of the Treatment on the Viability Rate of Pups: Figure 7 shows the variation of viability rate of pups in different groups of female cavies receiving different doses of ethanolic extract from the avocado seed powder. It appears that the viability rate of the pups from littering until weaning was 100% in all groups.

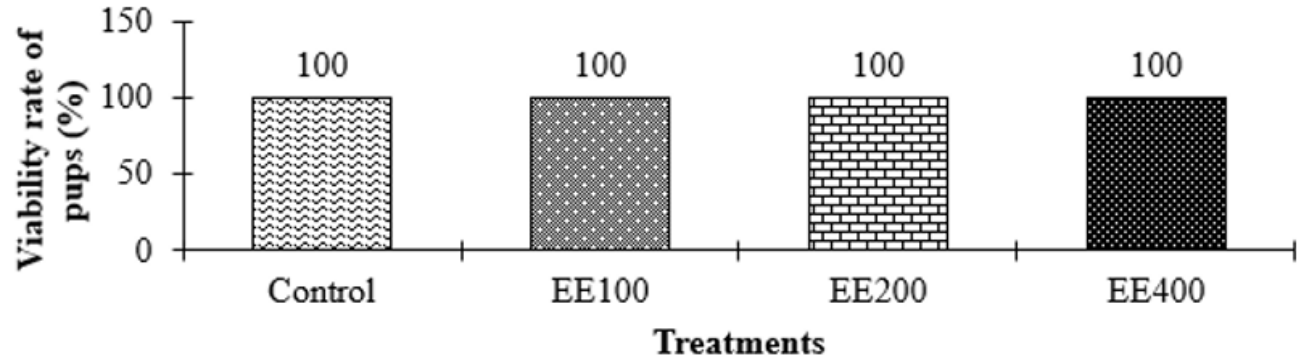

Figure 7: Variation of the rate of viability in different groups of female cavies receiving different doses of ethanolic extract of avocado seed powder in female cavies.

Note: Control: 1 ml/kg bw of distilled water; (EE100, EE200, EE400): ethanolic extract of the powder of avocado seed at the respective doses of 100, 200 and 400 mg / kg bw.

Discussion

The phytochemical analysis of the ethanolic extract of the avocado seed powder (*Persea americana* M.) realized in this study revealed the presence of bioactive molecules such as flavonoids, phenols, triterpenes, steroids and tannins. This result is similar to those obtained by Tremocoldi et al. [28]; Bahru et al. [29]. The presence of the above mentioned molecules confers to avocado seeds, many pharmacological properties (estrogenic, antioxidants, anti-inflammatory etc.) [11,30,31]. Due to antioxidant properties of the avocado seed powder, it can be used to fight oxidative stress, thus preventing free radical phenomena, harmful to reproduction and therefore improve production performance in cavies. Also, the phyto-estrogens contained in avocado seed, by positive feedback exerted on the hypothalamic-pituitary axis, could stimulate hyper-ovulation, thus increasing reproductive performances of females. On the basis of the above, this study was initiated in order to contribute to the improvement of the productivity of farm animals by the use of the avocado seed. It is recommended to put females into reproduction when they have achieved at least 80% of their adult weights at puberty. Thus, they can achieve the optimum of their reproductive potential [32]. Age at puberty in female cavies is between 2 and 3 months. At this time the animal usually weighs between 350 and 450 g [33].

During gestation, the live weight of the female increases due to the increase in the weight of the uterus, embryonic appendices as well as the conceptus (embryo or fetus). The results of this study show that before breeding and during gestation, the different doses of ethanolic extract of the avocado seed, caused an increase in the live weight and the weight gain of the female cavies. This result is similar to that obtained by Pahua-Ramos et al. [19] in mice treated with 125, 250 and 500 mg/kg bw of ethanolic extract of avocado seed powder for 5 days. Indeed, the flavonoids, phenols and terpenoids contained in the avocado seed powder could favor the absorption and assimilation of

nutrients contained in feed thereby increasing anabolism to the detriment of catabolism, resulting in an increase in body weight. Phytoestrogens (flavonoids, triterpenes and sterols) contained in some plants such as the avocado seed have molecular structures similar to estrogen, which give them the ability to attach to the α and β receptors of estrogens present at the target tissue and induce agonist or antagonistic effects of estrogen depending on dose and target tissue [34,35]. In the case of this study, the intake of phytoestrogens by the extract would have increased the serum concentration of estrogen, thus stimulating by positive feedback the synthesis of LH and the FSH.

Estrous, ovarian and uterine cycles are processes that occur simultaneously in animals and cannot be dissociated [36]. It is the set of anatomical, physiological and behavioral changes that take place repeatedly in the ovaries and the uterus during the sex cycle of the animal. It is mainly folliculogenesis (ovary) and the cyclic evolution of the endometrium (uterus). The results of this study show that, the different doses of ethanolic extract of avocado generally led to an increase in De Graaf number of follicles in treated females. This result is similar to that obtained by Zougrou et al. [22] following 50 and 100 mg/kg bw of *Cnesetis ferruginea* extract for 15 and 30 days in Wistar rats. It is the same for Adaay and Mosa [37] who obtained a similar result following the oral administration of 100 and 200 mg/kg bw of aqueous extract of *Tribulus terrestris* for 2 and 4 weeks in Swiss white mice. This result could be explained by the fact that, the phytoestrogens from this extract have stimulated by positive feedback the synthesis of the FSH (follicle-stimulating hormone) by the anterior pituitary gland, inducing a recruitment of the primordial follicles and their growth to De Graaf or mature follicle from which the increase in their number in the cavies treated with ethanolic extract of avocado seed powder. Similarly, the administration by gavage of ethanolic extract of avocado seed powder in the present study generally caused an increase in the size of the uterine epithelium. This result corroborates that obtained by Zougrou et al. [22], following the oral administration of 50 and 100 mg/kg bw of *Cnesetis ferruginea* aqueous extract for 15 and 30 days in Wistar rats. However, this is in contrast to the report of Solomon et al. [38] following the administration by gavage of methanolic extract of *Rumex Steudelii* at doses 2.2, 2.5, 2.8 and 3 g/kg bw for 30 days in albino rats. The increase in the size of the uterine epithelium in the present study, which was more pronounced in the EE200 group, could be attributed to the anabolic effect of phytoestrogens contained in the avocado seed extract.

Fertilization in mammals by the fusion of a male gamete and a female gamete to produce a zygote, that is typical of a species [39]. According to Kenmogne [40], fertilization can be evaluated through features such as fertility and fertility rates and can be influenced by many factors. The results of this study revealed that the ethanolic extract of the avocado seed powder caused an increase in the fertility rate in female cavies. These observations are consistent with those obtained by Zougrou et al. [22] with the oral administration of 200 mg/kg bw of aqueous extract of the leaves of *Cnesetis ferruginea* for 30 days in the rats (*Rattus norvegicus*). It is the same for Awoufack et al. [25] who obtained similar results following the administration by gavage of 20, 110 and 200 mg/kg bw of aqueous and methanolic extracts of the leaves of *Myrianthus arboreus* for 30 days in the Wistar rats. On the other hand, contrary effects were observed by Salah and Wagner [41], after oral administration of 50 and 100 mg/kg bw of extract from the mixture of N-hexane, chloroform, ethyl acetate and methanol of the leaves of *Ruellia preacetermissa* in rats. The increase in fertility rate reported in the present study could well be due to increase in the combined effects of reproductive hormones (LH, FSH and estradiol) on oogenesis and folliculogenesis, stimulated by biochemical molecules contained in the avocado seed extract [42].

Gestation is controlled by endocrine hormones [43]. According to Boyd [44], there is a negative correlation between litter size and the gestation period (the greater the litter, the shorter the gestation period) and a positive correlation between litter size and the weight of the pups at birth (the weight of pups increases with the gestation length). These features can be influenced by some medicinal plants. The administration of the different doses of ethanolic extract of avocado seed powder has induced generally, an increase in litter size and weight of pups at birth in female cavies. These results corroborate those of Ainehchi and Zahedi [45] resulting from the intraperitoneal administration of *Artemisia lanata* hydroethanolic extract at dose 200 and 400 mg/kg bw of the 2nd at 8th day of gestation in Wistar rats. Similar, Abd- El Ghany et al. [46] recorded similar results after 5 and 7.5 g/kg bw on aqueous and ethanolic extract of *Agnus-castus vitex* leaves for 4 weeks before breeding in New Zealand rabbits. These results would be due to the fact that, some molecules such as polyphenols, proteins, fatty acids and vitamins contained in the avocado seeds would have allowed females to cover both their maintenance and reproductive needs. Indeed, according to Meyer [47], under nutrition during gestation can reduce the survival of calves and their weights at birth. During pregnancy, a constant and considerable carbohydrate contribution (especially glucose), amino acids, minerals and vitamins by the placenta is necessary for the realization of the new individual [48].

Maternal weaning is the gradual transition period of a diet exclusively based on breast milk to a solid food. According to Roux [33], weaning in cavies takes place between 2 and 3 weeks of age, small ones between 150 and 200 g. From this study, it appears that the administration by gavage of the ethanolic extract of avocado seed powder has generally induced an increase in weight of kids at weaning. These results are closer to those obtained by Abd- El Ghany et al. [46] following the administration of 5 and 7.5 g/kg bw of aqueous and ethanolic extracts of the *Vitex angus-castus* leaves for 4 weeks before breeding in New Zealand rabbits. Similarly, Matysiak et al. [49] have obtained similar results after all 100 mg/kg bw of the mixture extract from 5.4% Oregano spp., 3.2% Cinnamomum spp. and 2.2% Capsicum

annum of the 90th day of gestation with weaning (28 days postpartum) in the sow. These results could be explained by the fact that bioactive compounds (flavonoids, phenols and terpenoids) contained in avocado seed could favor the absorption and assimilation of nutrients and led to an increase in the quantity and quality of milk produced by the female cavy and therefore the increase in the weight of the pups. These results could also be due to the transfer of dam's immunity to the neonate during the first days of lactation. Wolfensohn and Loyd [50], had observed that after parturition in cavies, although pups start early to eat solid food and drink water, about 50% of the neonates do not survive if they do consume breast milk the first days of their life. Lactation provides for newborns, not only nutrients, but also immune protection and proponent compounds of growth [51].

Conclusion

At the end of this study, which focused on the effects of ethanolic extract of the avocado seed powder (*Persea americana* M.) on the characteristics of growth and reproductive performance in female cavies (*Cavia porcelus* L.), it can be concluded that the ethanolic extract of avocado seed powder induced an increase in the consumption index and the live weight of females cavies. It also induced an increase in the number of De Graaf follicles and the size of the uterine epithelium, thus increasing the chances of potential fertilization and maintenance of gestation. Finally, avocado seed induces an increase in the fertility rate, litter size, pups' weights at parturition and weaning. In the case of its utilization, the dose 200 mg/kg bw is recommended.

Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics Statement

The experimental protocols used in this study were approved by the Department Animal Sciences of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang, Cameroon and in strict compliance with guidelines for care and manipulation of laboratory animals, accepted internationally. The latter were also in accordance with the European Union's 86/609/EEC guidelines adopted by the Ethics Committee of the Ministry of Scientific Research and Innovation of Cameroon.

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Competing Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' Contributions

MA, AD and LF designed the project, conducted the experiment, collected, analyzed the data and wrote the first draft of the manuscript. NM assisted in carrying out the experiment and in the laboratory analysis. BM and CN assisted in the laboratory analysis and checked the draft of the manuscript. FN supervised, designed the project, corrected the draft of the manuscript, and finally approved it for submission. All the authors approved the final draft of the manuscript to be submitted.

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