EFFECTS OF AQUEOUS EXTRACT OF GUAVA (*PSIDIUM GUAJAVA*) LEAVES ON REPRODUCTIVE PERFORMANCES OF ROSS 308 BREEDERS

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Abstract

The present study was conducted to assess the effects of aqueous extract of guava (Psidium guajava) leaves on the reproductive performances of Ross 308 breeders. For this purpose, 48 Ross 308 breeders of 80 weeks old were used for a period of 4 weeks; they were divided into 4 groups of 12 breeders each. Each group consisted of 2 males and 10 females with an average weight of 4050± g and 3750±3g respectively. They received by gavage distilled water for the control (T0) and the aqueous extract of guava leaves at doses of 75 (T1); 100 (T2) and 150 (T3) mg / kg of body weight. From the first to the last day of the test, eggs were collected daily, sorted, weighted, and labeled in order to assess the laying parameters (weekly egg production, egg productivity, weight and egg surface). Collected eggs were incubated and candled on the 18th day to determine the number of fertile eggs. Then handed over to the hatcher and monitored until hatching to assess the percentage of incubated eggs, the embryonic mortality rate, hatching rate, viability, numeric productivity, the average weight and the rate of marketable chicks. The results of this study showed that aqueous extracts of Psidium guajava (75, 100, 150 mg / kg of body weight) leaves improved laying parameters, incubation and day old chick of Ross 308 breeders although not significant (p> 0.05). Generally, the lowest dose (75mg / kg) of the aqueous extract of Psidium guajava induced the best results for all parameters evaluated except the laying and fertility rates. Aqueous extract of guava leaves at 75mg/kg of body weight could be used as an alternative to improve reproductive performances, especially in female parents.

Key words: Aqueous extract, *Psidium guajava*, egg characteristics, incubation, laying hen

INTRODUCTION

Improvement of reproduction seems to be an alternative to optimize the availability of animal products (meat, eggs and others) and thus to remedy the animal protein shortage. It is in this perspective that breeders resort to the use of huge and diversified quantities of chemical products (enzymes, antibiotics, and growth factors) in order to boost reproduction. However, because of microbial resistance and the presence of many side effects associated with the use of synthetic products [18], many countries have been led to ban the use of antibiotics and synthetic hormones [8]. Following this ban and given the disastrous consequences, many researchers have invested in finding alternatives to these molecules, which have now been eliminated from the list of drugs and feed additives for farm animals. Among these alternatives, there are natural plant-based products such as essential oils and plant extracts. Plant extracts are concentrated substances that contain essential nutrients and are obtained from dry matter using a solvent. They have wide range of activities: phytoestrogenic, anti-infectious, antifungal, antiparasitic, antibacterial, anti-inflammatory, antimutagenic, antimicrobial, antioxidant, antitoxin [9]. These properties are exploited in animal production [12], [17], [24], [26], [31] to increase productivity.

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Aqueous extract of *Psidium guajava* (Myrtaceae family) is among the products already used to improve zootechnical performances [6], [10], [11].

All parts (roots, fruits, leaves, etc...) are used in traditional medicine for the treatment of diseases such as: gastrointestinal and respiratory disorders, diabetes, cancer, etc. [23], [25], [30]. Studies conducted on *Psidium guajava* leaves extracts and their essential oils showed that they possess immunological, phytoestrogenic, anti-inflammatory, antibiotic, analgesic, hepato-protective and antioxidant activities [2], [15], [27], [28]. More recently, Aruna *et al*. [4] reported the positive effects of aqueous and methanoic extracts of *Psidium guajava* leaves on testicular and epididymal weights, sperm motility and epididymal concentration, hematocrit and white blood cell count in rats.

In animal productions, a limited number of studies on the effects of *Psidium guajava* leaves extracts on growth and reproductive performances in male guinea pigs have been carried out [21]. Although these studies show the positive effects of these guava leaf extracts on reproductive performance, very little data exist regarding its impact on reproductive performance of broiler breeders. The overall objective of this work was therefore to contribute to the improvement of knowledge on the effects of the aqueous extract of *Psidium guajava* leaves on reproductive performances of Ross 308 breeders (broiler parents). Specifically, the effects of the aqueous extract of *Psidium guajava* leaves on egg laying rate, external eggs characteristics, incubation performances and chick viability were evaluated in broiler breeders.

MATERIAL AND METHOD

Study Area

This study was carried out at the farm of NGV-Traco Company of Souza located in the Moungo Department, Littoral Region of Cameroon. It is located between 4°03' North longitude and 9°42' East latitude. Average altitude is 13 m. The area is characterized by a succession of four seasons in the year (two rainy and two dry seasons). Average annual temperature is 27°C and rainfall is 2402.8 mm/year.

Plant material and preparation of the extract

The mature guava leaves (Picture 1) were harvested in the morning before sunrise at the University of Dschang. They were dried in the shade and then ground into very fine particles (Picture 2).

Photo 1: Fresh guava leaves

Photo 2: Dried fresh guava leaves powder

Aqueous extract was obtained following the process described by Yakubu *et al*. [33]. Therefore, 250 g of ground leaves were introduced into a recipient containing 1.5 liter of distilled water and the whole was kept at rest for 72 h. The maceration was then filtered using Wathman paper number 3. Subsequently, the filtrate obtained was dried in an oven at 45°C until a solid extract was obtained, which was kept in a hermetically sealed flask until use.

The extraction yield was determined according to the following formula:

Extraction yield (%) = (Mass after extraction, g) / (ground leaves mass before extraction, g) x 100

Animal equipment, housing and prophylaxis

A flock of 48 breeders (40 females and 8 males) of Ross 308 strains imported from Belgium and aged 80 weeks (62 week of laying) was used. Mean live weights of roosters and hens were 4050±5 g and 3750±3 g respectively.

The birds were housed in wired pens and divided by plywood at a density of 4 subjects/m^2. Each pen was 2m long and 1.5m wide.

All pens were disinfected in advance with a solution of Virunet (10 g in 15 liters of water) sprayed in all compartments of the experimental pens and left over a period of 10 days before animals were introduced. They were given an anti-stress (Aliséryl: 5 g dissolved in 10 liters of water) during the adaptation phase in the different experimental groups.

Feeding

Throughout the test period, birds received water *ad libitum*. In addition, each animal received 150 g of a commercial standard feed (Crude Protein: 15.34 %, metabolisable Energy: 2518.52 kcal/kg of feed) per day,

Experimental design and execution of the test

Birds were divided into 4 similar groups of 12 subjects (2 males and 10 females). In addition to water and feed, birds in groups 2, 3 and 4 received daily throughout the treatment period aqueous extract of *Psidium guayava* leaves by gavage at doses of 75, 100 and 150 mg/kg body weight, respectively, while those in the control group received 5ml of distilled water. Each of these doses was administered randomly to animals of one of the groups in a completely randomized system consisting of 4 treatments (T0, T1, T2 and T3 respectively).

Eggs collected daily were sorted, weighted, marked, incubated and set on day 18 to determine the number of fertile eggs. Eggs were then returned to the hatchery and monitored until hatching. The following parameters were evaluated: number of fertile eggs, embryo mortality and hatching rate, average weight and viability of the chicks at hatching.

Data collection and studied parameters

Throughout the trial period, the following data were collected daily and by treatment on:

- Daily total oviposition
- Number of downgraded eggs (number of broken, malformed eggs or eggs outside of 50-70g mass interval)
- Weight and surface area of the egg;
- Number of fertile eggs;
- Embryonic mortality rate;
- Hatching rate;
- Chick mass;
- Viability of the chicks;
- Number of marketable chicks (having a weight ≥ 40g with adequate conformation and showing no signs of disease).

These data enabled the evaluation of the following parameters:

Laying performance and egg characteristics

Egg-laying rate determined according to the formula described by Sauveur [29]:

Laying rate (%) = Q/ (N*K) ×100 where

Q= total number of eggs produced in the treatment in K days
N= number of females placed in the building
K= number of laying days

Downgraded eggs rate (TD)

DER (%) = (Downgraded eggs number) / (Number of eggs laid) ×100

- Average egg weight, determined using an electronic scale (5 Kg±1g)
- Egg surface area, determined according to the method of Mongin and Bonnet (1968) cited by Sauveur [29] was calculated using the following formula S=K.P2/3 where

S = egg surface area in cm^2,
P = initial weight of the egg in g,
K = constant which takes the values 4.67; 4.68 or 4.69, respectively, depending whether on egg weight is less than 60 g, between 60 g and 70 g or more than 70 g.

Incubation performance and characteristics of day-old chicks

Percentage of incubated eggs (PIE),
PIE (%) = (Number of Incubated Eggs) / (Number of egg laid) ×100

Fertility rate (FR),
FR (%) = (Number of fertile eggs) / (Number of Incubated Eggs) ×100

Embryonic mortality rate (EMR),
EMR (%) = (Number of dead embryos)/ (Number of Incubated Eggs) ×100

Total hatching rate (THR),
THR (%) = (Number of hatched chicks)/ (Number of Incubated Eggs) ×100

Real Hatching rate (RHR),
RHR (%) = (Number of hatched chicks) / (Number of fertile eggs) ×100

Sellable chicks rate (SCR),
SCR (%) = (Number of sellable chicks) / (Number of fertile eggs) ×100

Sellable chicks viability rate (SCVR),
SCVR (%) = (Number of sellable chicks) / (Number of fertile eggs) ×100

Statistical analysis of data

Data obtained were subjected to a one-factor analysis of variance (ANOVA) and Duncan's post hoc test was used to separate the means at 5% when there were significant differences. Data were expressed as mean ± standard deviation. SPSS 21.1 software was used for the analysis.

RESULTS AND DISCUSSIONS

Laying rate

The weekly change in laying rate as illustrated in figure 1 shows a saw teeth variation. The different doses of the aqueous extract of *Psidium guajava* leaves did not significantly affect ($p>0.05$) the laying rate, which however remained higher in the hens of the control group compared to the values recorded in the hens of the other treatments.

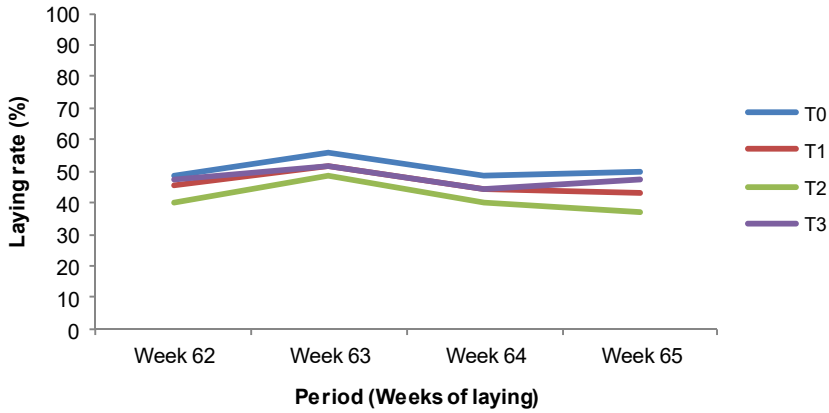

Fig. 1 Weekly change in laying rate as a function of the doses of aqueous extract of *Psidium guajava* leaves

Incubation performance of Ross 308 laying hens' eggs as a function of administration doses of aqueous extracts of *Psidium guajava* leaves.

Numerical productivity and external characteristics of eggs

Table 1 shows the influence of aqueous extract of *Psidium guajava* leaves on physical characteristics of eggs of Ross 308 hens. It appears that egg number, weight, surface area, rate of hatchable eggs and downgraded eggs were not significantly affected ($p>0.05$) by the aqueous extract of *Psidium guajava* at any dose. Nevertheless, for all these parameters, the highest values were recorded with hens given the dose of 75 mg/kg body weight.

Table 1 Egg physical characteristics of Ross 308 breeders incubation eggs as influenced by the doses of aqueous extract of *Psidium guajava* leaves

Treatments	Parameters				
	Egg number	Egg weight (g)	Egg surface (Cm^2)	Hatchable egg Rate (%)	Downgraded Eggs rate (%)
T_0	31.75±2.62	60.43±1.89	72.06±1.50	96.94±4.28	3.05±4.28
T_1	36.00±2.16	62.48±1.18	73.69±0.93	97.16±2.26	2.83±2.26
T_2	31.00±2.44	62.20±2.15	73.46±1.70	95.86±3.31	4.13±3.31
T_3	31.00±3.95	60.98±1.13	72.50±0.90	95.49±7.22	4.50±7.22
Mean	32.43±3.34	61.52±1.71	72.93±1.36	96.36±4.22	3.63±4.22

T0= (control) administration of distilled water (5ml/bird); T1= administration of aqueous extract of *Psidium guayava* leaves at 75 mg/kg body weight; T2= administration of aqueous extract of *Psidium guayava* leaves at 100 mg/kg body weight; T3= administration of aqueous extract of *Psidium guayava* leaves at 150 mg/kg body weight.

Fertility and embryonic mortality rate

Figure 2 shows the variation in fertility and embryonic mortality rate with different treatments. Fertility rate of eggs increased with increasing dose of guava leaf aqueous extract except for treatment T3 which recorded the lowest fertility rate (58.48±10.23) compared to the control. However, no significant difference was found. Embryonic mortality rate of Ross 308 layers was also not significantly ($p>0.05$) affected by *Psidium guajava* leaves aqueous extract, whatever the dose used. However, the embryonic mortality rate increased with the dose of the extracts. The lowest embryonic mortality rate (2.94±5.88) was recorded for hen eggs treated with 75 mg of aqueous extract of *Psidium guayava* leaves/kg body weight.

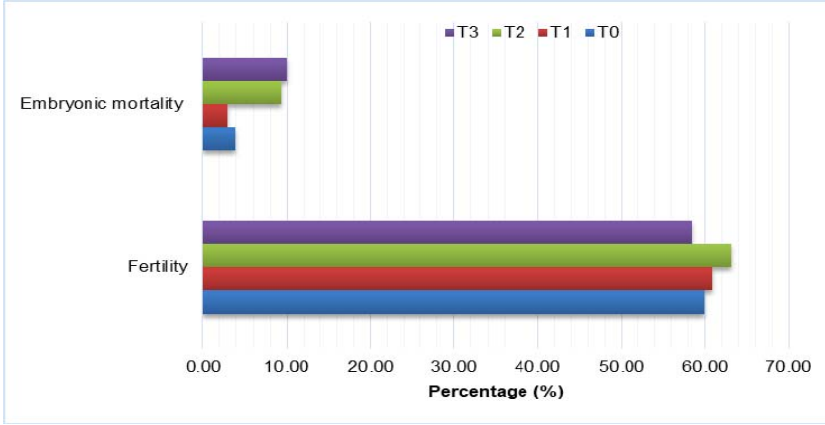

Fig. 2 Embryonic mortality rate as influenced by the doses of aqueous extract of *Psidium guajava* leaves

Hatching performances and viability of day-old broiler chicks

Table 2 shows the influence of *Psidium guajava* leaves aqueous extract on total hatching rate, average weight and viability of Ross 308 day old broiler's chicks. These parameters were not significantly ($p>0.05$) affected by the rates of *Psidium guajava* leaves aqueous extract used. Nevertheless, for these three parameters, the highest values were recorded with the T1 treatment. The different doses of *Psidium guajava* leaves aqueous extract did not significantly ($p>0.05$) affect the real hatching rate of Ross 308 incubation eggs. However, T1 treatment recorded the relatively highest hatching rates (94.59±7.27).

At all doses considered, aqueous extracts of *Psidium guajava* did not significantly ($p>0.05$) influence the sellable chicks rate. However, the T1 treatment had the highest rate of sellable chicks (56.34±7.60).

Table 2 Hatching parameters as a function of the doses of aqueous extract of *Psidium guajava* leaves

Treatments	Parameters				
	Total hatching rate (%)	Real hatching rate (%)	Chicks rate (%)	Chicks viability rate (%)	Sellable chicks rate (%)
T0	50.75±12.6	83.78±11.03	43.68±1.57	95.90±10.8	49.13±13.56
T1	57.08±8.47	94.59±7.27	44.65±1.76	98.80±5.29	56.35±7.69
T2	53.75±8.37	85.24±2.11	43.79±1.28	85.03±2.30	47.48±10.32
T3	48.50±9.98	84.34±8.60	42.97±1.11	87.03±16.74	43.46±10.89
Mean	52.52±9.56	86.99±7.26	43.77±1.44	91.69±8.22	49.10±10.61

T0= (control) administration of distilled water (5ml/bird); T1= administration of aqueous extract of *Psidium guayava* leaves at 75 mg/kg body weight; T2= administration of aqueous extract of *Psidium guayava* leaves at 100 mg/kg body weight; T3= administration of aqueous extract of *Psidium guayava* leaves at 150 mg/kg body weight.

The present study revealed that aqueous extract of *Psidium guajava* leaves did not have a significant effect on egg laying rate, and physical characteristics (numerical egg laying, egg weight and surface area) of the eggs laid by Ross 308 breeders. These results are out of agreement with those obtained by other researches which used botanical extracts, such as Yildinm *et al.* [34], who found that incorporation of Korean ginseng root extract (*Panax ginseng*) in laying hens feed significantly improved egg yield and external quality (eggshell characteristics and egg weight) and Henriéta *et al.* [16] who found that Oregano and pollen essential oils increased the numerical egg productivity of laying hens. This might be due to the fact that plant extracts have wide range of virtues, such as improving feed conversion rate and digestion [32], they also have antimicrobial and antioxidant properties [14], and their effectiveness on laying hens performance such as increasing egg production and improving conversion rate [35].

Administration of aqueous extracts of *Psidium guajava* leaves to Ross 308 breeders had no significant effect on the rate of downgraded eggs and therefore did not significantly increase the rate of eggs appropriate for incubation. Nevertheless, the highest rate of eggs appropriate for incubation and the lowest downgraded eggs rate were recorded with hens given the dose of 75 mg/kg body weight. These results are consistent with those of *Mazuranok et al.* [19], who showed that the inclusion of the combination of carvacol, cinnamic aldehyde and oleoresin in the feed (100 g/t) of laying hens has an improving effect on egg weight and percentage of incubable eggs and those of Asghar *et al.* [5], who reported that the supplementation of plant phytogenic feed additives (garlic, tagetes, fennel seed and thyme) at 8 and 12 g/kg feed improves eggs physical and chemical characteristics and thus the rate of hatchable eggs. This could be due to the fact that *Psiduim guajava* leaves have diverse biological activities, related to their chemical composition, the functional groups of the majority compounds (alcohols, phenols, terpene and ketone compounds) and their synergistic effects [9].

Fertility, hatching and embryonic mortality rates of Ross 308 ready to incubate eggs were not significantly improved following administration of aqueous extracts of *Psidium guajava* leaves at 75, 100, 150 mg/kg body weight. These results are contradictory to those of *Ndubuisi et al*. [20] who reported that the use of other botanical bioactive extracts, such as *Curcurbita pepo, Hibicus esculentus* and guava root powder at respective doses of 50g/kg feed, 50ml/l water and 1kg/5l in the feed of laying turkeys significantly improved eggs number, fertility, embryonic mortality and hatching rate. These results could be explained by the fact that species may have a different response to the administration of plant extracts. They could also be due to the fact that the chemical composition and biological properties of plant extracts vary from one plant species to another and according to the doses and mode of use [3].

Aqueous extract of *Psidium guajava* leaves at 75, 100, 150 mg/kg body weight has a positive effect on day old chicks viability, numerical eggs productivity, weight and rate of marketable chicks of Ross 308 layers, although it is not significant. These results corroborate those of Nzenwa *et al*. [22] who found that antiparasitic plant extracts have positive effects on hatching and chick parameters in birds. This would be due to the fact that plant extracts of guava leaves have a wide range of biological activities: phytoestrogenic [1], anti-infectious, antifungal, antiparasitic, antibacterial, anti-inflammatory, antimutagenic, antimicrobial and antioxidant [13]. *Psidium guajava* also has various therapeutic properties attributed to compounds such as tannins, phenols, flavonoids and quercetins [13]. These therapeutic properties have synergistic effects [9] in addition to the antioxidant properties it possesses, which are able to bind free radicals [7]. These different therapeutic properties are beneficial for the development of embryonic tissues during incubation and in the first days of chick's life.

CONCLUSION

At the end of the study, it emerged that administration of the aqueous extract of *Psidium guajava* leaves at different doses to Ross 308 parent stock resulted in an increased level of laying, incubation and day-old chick performance, although not statistically significant. Best results were observed in breeders given 75mg/kg body weight, specifically on the number, average weight and average surface area of eggs, downgraded eggs, incubation, embryonic mortality and total and real hatching rates, mean weight, viability rate, marketable chick.

Thus these results show that aqueous extract of guava leaves at 75mg/kg body weight could be used as an alternative to improve reproductive performance, especially in depleted parent stock, passing the end of their first reproduction cycle.

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