Evaluation of pawpaw seed powder *(Carica papaya)* as feed additive on blood parameters and libido in male rabbits

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Abstract

Pawpaw seed powder (PSP) and its effect on sexual urge (libido) in adult male rabbit was investigated. Thirty-six pure New Zealand breed rabbits of 5 to 6 months old were divided randomly into three groups of twelve animals each. Group I (control), Group II (50% PSP), Group III (100% PSP) at ratio 0, 0.25 and 0.5 g to 1 kg of feed inclusion respectively. Animals were fed *ad libitum* for 8 weeks. Phytochemical test was carried out on the PSP. At 8th week of feeding trial, blood samples were collected from each animal for hematological parameters, serum testosterone and libido rate were evaluated. Data generated were subjected to one-way analysis of variance using general linear model. The results of the phytochemical test revealed that PSP contain saponins, tannins, glycosides, phenolic, carbohydrates, proteins and no flavonoids. Measured hematological parameters were within values recommended for healthy rabbits of the same breed, sex and age. The libido (secs) and blood testosterone (mg/l) of experimental animals were significantly ($p < 0.05$) affected by the treatment. Rabbits in group III had (low libido) and lowest value for testosterone. In conclusion, PSP as feed additive has a potential to influence libido in male rabbits.

***Key Words**:* Pawpaw seed powder, rabbits, hematological indices, libido, testosterone

Introduction

The application of artificial insemination (AI) has increased mean productivity of rabbit farms (Castellini, 1996) and enhances more homogeneous cycled production (Maertens et al., 1995) with very prolific genetic strains (Bolet et al., 2004; Castellini, 2007). However, several problems related to the welfare of adult and matured bucks may also appear during production cycle, for example, indiscriminate mating, inbreeding, cannibalism, high mortality and hypo-fertility (Facchin et al., 1999). The improvement of reproductive pattern should correspond to welfare as well as an economic point of view, because rearing costs of young bucks are relatively high. The optimization of reproductive performance is one of the main facts that assure high productivity in rabbit farms.

Medicinal plants such as *Moringa oleifera* and Pawpaw (*Carica papaya*) have the potential to be used for the manipulation of gender differentiation in tilapia, due to the fact that they contain bioactive components which have

an antifertility action in animals. Some of these plants, for example pawpaw, have been used successfully to induce sterility in male rats (Prakash and Gupta, 2005) and humans in various countries (Kumar et al., 2012). Studies have identified phytochemicals**,** such as the triterpenic acids of Oleanolic acid (OA) and ursolic acid (UA) and their derivatives, e.g., glycoside (Das, 1980). Oleanolic acid-3β-glucoside, OAG, and saponin (steroidal or triterpenoid glycoside) isolated from plants have abortifacient properties, indicating their potentials to be used as an antifertility treatment in animals (Makkar et al., 2007, Souad et al., 2007).

Pawpaw (*Carica papaya*) seed administered orally to male albino rats, effectively controlled their reproduction (Udo and Kehinde, 1999) and reduced sperm motility (Pathak et al., 2005). The seeds of *Carica papaya* have been emerging as a potential post-testicular antifertility drug (Liu et al., 1995, Furtado et al., 2008, Zhou et al., 2011). Crude extracts from the seeds of *Carica papaya* induce variable responses depending on dose, duration, and route of administration in laboratory animals (Lohiya et al., 1999).

The use of plant or plant-based products to stimulate sexual desire, for fertility and for infertility has been practiced for a very long time. Thus plants and their extracts have been used to reduce or increase libido thus a large number of pharmacological agents are consumed over the years with the purpose of controlling libido. According to Arif in 2000, some phytochemicals such as saponins has been clinically proven to improve sexual desire via the conversion of protodioscine to DHEA (De-Hydro-Epi-Androsterone). Phytosterols in large quantity are also suggested to reduced testosterone levels and sexual behavior by affecting Leydig cell proliferation, and cholesterol trafficking (Qasimi et al., 2017) also androgenic and gonadotropic activities, assigning to the components flavonoids and/or saponin present in plants, have been reported as capable of altering androgen levels thus altering sexual desire (Yakubu and Akanji, 2010).

Studies have shown the efficacy of antifertility properties in *Carica papaya* seeds via oral administration at different doses and extraction methods. However, studies on utilization of the crude seed powder and its reproductive potentials are rare. Therefore, this study aimed to evaluate the effect of crude *Carica papaya* seed powder as feed additive on heamatological, serum testosterone and libido of adult male rabbit.

Materials and methods

Experimental Unit.

The study was carried out at the Rabbitry Unit, Teaching and Research farm, Bowen University – Iwo, Osun State. The laboratory work was conducted at the Animal Science laboratory and Department of Chemistry, Bowen University – Iwo. Rabbits were raised in an intensive system rabbitry hutch. The use and handling of animals were in accordance with the approval granted by the Bowen University ethics committee (BUEC).

Experimental Animals.

Thirty-six (36) sexually matured pure New Zealand breed rabbit were allotted to three treatment diets in a completely randomized design with 6 replicate each. Animals allotted were 5 to 6 months old, weighing 1.5 to 2.0kg. They were housed individually in wire mesh cages and fed with compounded feed mixed with pawpaw seed powder (PSP). Seasonal vegetables i.e; *Tridax procubens*, and water were provided *ad libitum*. The animals were maintained in the departmental animal facility under good supervision.

Phytochemicals of Carica papaya seeds.

Carica papaya seeds of the honey dew variety were used in the present study. The seeds were oven dried at 100 °C until even weight, coarsely powdered, and the seed powder was stored in air tight dark coloured container. Phytochemical screening was carried out on the stored seed powder for steroids, phytosterols, glycosides, carbohydrates, tannins, gum and mucilages, saponins and flavonoids as described by Harborne (1973), Trease and Evans (2009), Sofowora (1993), Ogbuewu (2008).

Feed preparation.

The feed composition as presented in table 1. The pawpaw seed powder was added to the feed at ratio:

0% – No pawpaw seed powder

50% – 0.25 g pawpaw seed powder into 1 kg of feed

100% – 0.5 g pawpaw seed powder into 1 kg of feed

Weight – weight content of active ingredient in PSP as was adapted as described by Lohiya et al. (2006)

Measurement of libido

At 12th week of the experiment, the libido rate was measured. Libido is a sexual desire, that is influenced by biological, psychological and social factors. The libido was measured as a reaction time (secs) of buck with the first attempt to mount the doe. As adapted from (Seleem, 2003). The reaction time of buck to mount the doe was measured using a stop watch.

Blood collection and analysis of hematological indices

Blood samples from the bucks were taken at day 90, in less than 2 min from the marginal ear vein of 12 rabbits each per treatment. Blood samples were collected using a stainless steel needle, into heparinized (hematological parameters) and plain tubes (serum testosterone) which were centrifuged at 3000 rpm for 20 min and kept in deep freezer (-20 °C) until further analysis. Blood serum testosterone concentration was determined using RIA kits (Immunotech, A coulter co., France) according to the manufacturer procedures.

Statistical analysis

Data were subjected to one-way analysis of variance, using the general linear model procedure of SAS (2001). Means with significant differences among the treatments were separated using the Duncan's option of the same software.

Results

Hematological analysis of experimental animals

As reported in Table 3, PSP had no significant ($p > 0.05$) effect on measured blood hematological parameters of fed rabbit. Using pawpaw seed powder as a treatment for libido was checked in buck at different concentrations: 0% PSP; 50%

Table 1. Gross composition of the rabbit diet.

Ingredients	(%)	Group II 0.25gPSP/kg	Group III 0.5gPSP/kg
Maize	49.3	49.3	49.3
Wheat offal	16	16	16
Groundnut cake	12.5	12.5	12.5
Soybean meal	14.5	14.5	14.5
Oyster shell	1	1	1
Bone meal	2	2	2
Vitamin premix	2.5	2.5	2.5
Salt	2	2	2
Methionine	0.1	0.1	0.1
Lysine	0.1	0.1	0.1
PSP	-	0.25	0.5
Total			

[a]*Calculated values; Metabolizable Energy (ME) 2800.5 kcal*kg^{-1}; Crudeprotein (CP)20.1; Crude Fibre (CF)4.98; Ether Extract (EE)5.5.*

[b]*Premix to provide the followings per kg of feed: Vitamin A– 500 iu, Vit. D3 – 1,200 mg, Vit. E – 11 mg, Vit. K3 – 2 mg, Riboflavin – 20 mg, Nicotinic acid – 10 mg, Panthothenic acid – 7 mg, Cobalamin – 0.08 mg, Choline chloride – 900 mg, Folic acid – 1.5 mg, Biotin – 1.5 mg , Iron – 25 mg, Manganese – 80 mg, Copper – 2 mg, Zinc – 50 mg, Cobalt – 1.2 mg and Selenium – 0.1 mg.*

PSP and 100% PSP. The result revealed that PSP as feed additive had a significant ($p < 0.05$) effect on buck libido (table 4). Mean libido at 0% PSP is 17.56 secs, at 50% 27.34 secs and at 100% 74.80 secs. Increasing the quantity of PSP in the diet, resulted in decreasing of buck libido over time.

Investigating the effect of pawpaw seed powder on the secretion of testosterone hormone in buck showed that PSP as feed additive had a significant ($p < 0.05$) effect on serum blood testosterone. The mean blood testosterone at 0% PSP was 20.40 mg/l, 50% pawpaw – 10.71 mg/l and 100% pawpaw seed – 7.03 mg/l. The amount of measure blood testosterone reduces with increase in quantity of PSP in rabbit diet over time (table 4).

Table 2. Phytochemicals of pawpaw seed powder

S/NO	Phytochemical	Result
1	Saponins	+
2	Tannins	+
3	Carbohydrates	+
4	Flavonoid	-
5	Phytosterols	+
6	Glycosides	+
7	Gum and Mucilages	+
8	Proteins	+
9	Phenolic compounds	+

KEY: + Present , - Absent

Discussion

Since phytochemicals affect hormones, the seeds of pawpaw which is rich in phytochemicals can control or affect sexual behaviors by controlling the number of several hormone and serum testosterone produced, thus various herbs have been used by people of different cultures as contraceptives, to treat conditions of male infertility, for treatment of reproductive disorders etc. They have also been advocated for affecting sexual desire as well as sexual performance / behavior and erectile dysfunction, through several mechanisms such as vasodilatation, increased testosterone level, brain monoamines, effect on

Table 3: Effect of *Carica papaya* seed powder as a feed additive on hematological parameters of male rabbits (bucks)

Parameters	Control (No pawpaw seed)	50% pawpaw seed	100% pawpaw seed
WBC (10^9L^{-1})	45.67 ± 14.74^a	41 ± 43.02^a	40.00 ± 16.42^a
RBC ($10^{12}L^{-1}$)	7.63 ± 0.30^{ab}	7.11 ± 0.51^{ab}	7.77 ± 2.46^a
Platelet (10^9L^{-1})	$6.7x\ 10^4 \pm 30.17^b$	$7 \times 10^4 \pm 10.00^{ab}$	$5.7x10^4 \pm 23.86^a$
PCV (%)	46.00 ± 2.65^a	43.33 ± 2.52^a	42.67 ± 14.36^a
Lymphocytes(%)	67.00 ± 2.66^a	68.67 ± 2.52^a	65.00 ± 14.36^a
Monocytes(%)	2.00 ± 0.00^a	2.33 ± 0.58^a	2.33 ± 1.53^a
Hemoglobin (%)	15.00 ± 0.87^a	14.13 ± 0.90^a	13.87 ± 4.86^a

^{ab} Means along the same row with different superscripts are significantly ($P < 0.05$) different using Duncan's test as post hoc analysis. Where; WBC – White blood cell, RBC – Red blood cell, PCV – Packed cell volume

Table 4. Effect of *Carica papaya* seed powder as feed additive on measured reproductive parameters of male rabbits (bucks)

Parameters	0% (Control)	50% pawpaw seed	100% pawpaw seed
Testosterone mg/l	20.40 ± 3.27^b	10.71 ± 1.67^a	7.03 ± 0.2^a
Libido (secs)	17.56 ± 4.20^a	27.34 ± 5.77^a	74.80 ± 17.87^b

^{ab} Means along the same row with different superscripts are significantly ($P < 0.05$) different using Duncan's test as post hoc analysis

pituitary-gonadal axis and so on (Chauhan et al., 2010; da Cruz. et al., 2017).

The study on phytochemical analysis of pawpaw seed powder revealed the antioxidative, antimicrobial and antifertility potential of papaya seed as shown in table 3. Hence, the use of this seed could be of beneficial in the management and treatment of some oxidative stress in human and animal ailments. Table 2 shows the presence of saponins, tannins, carbohydrate, phytosterols, gum & mucilages, protein, glycosides, phenolic compounds and absence of flavonoid. Saponin has relationship with sex hormones like oxytocin. Saponin, acts to form nitrous oxide and may lead to the relaxation of the smooth muscle by means of Larginine/nitrous oxide (Yakubu and Akanji, 2010). In addition, the absence of flavonoids in plant extracts will definitely affect the level of androgen.

The extraction of tannins and saponin from pawpaw seed using ethanol have also been reported as compounds capable of affecting the reproductive potential in male animals (Vincken et al., 2007).

As reported in table 3, all measured hematological blood parameters are not significantly different ($p > 0.05$) between the treatments and are within the range recommended for healthy animal. This stability of measured blood indices such as RBC, Hbc, and PCV in rabbits across the various dietary treatments in this study is an indication that PSP supports or does not interfere with normal haemopoiesis processes (Oloruntola et al., 2016, Oloruntola et al., 2018). The red blood cell carries oxygenated blood, values reported in this study are within the range for a normal male rabbit which is between 4.9–7.8 x 10^6ul*L (Edgar, 2011). White blood cell helps in fighting infections, they circulate in the blood so that they can be transported to an area where an infection has developed (Marshall, 2008). Increase in WBC, is a sign of an infection or strange agent in the blood. Platelet helps blood to clot by forming platelet plug, they are formed in the bone marrow from large cells called megakaryocytes, which break up into fragments. Hemoglobin is contained in the red blood cell, it is responsible for distributing oxygen, and to a lesser extent, carbon dioxide throughout the circulatory system (Amory et al., 2007). Main function of monocytes in the immune system are the production of cytokines, phagocytosis and antigen presentation (Page et al., 2006). Lymphocytes are involved in the production of cytokines and immunoglobins and helps to kill infected cells and tumor cells. Packed cell volume helps in checking the volume of active living cell in the blood, when PCV level is less than 30%, it can lead to anaemia and when higher than 50% it can be harmful to the animal (Marshall, 2008). At the end of this experiment it was observed that the PSP administered in the feed does not have significant effect ($p < 0.05$) on experimental animal blood profile. The report agreed with Lohiya et al. (1999) who stated that choloroform extracted *Carica papaya* seed did not have any toxicological effect in male rabbit.

In this study, it was observed that the libido and testosterone of experimental animals were significantly ($p < 0.05$) affected by treatment diets. The libido rate increased along the treatment with Treatment 1 (0% PSP) had the shortest reaction time i.e high libido. Treatment III (100% PSP inclusion) had the lowest value for blood testosterone which was significantly ($p < 0.05$) different from other treatment. Testosterone level ranged from 0.5 to 10 mg/ml and it is a hormone responsible for male reproduction (Mahabadi et al., 2009).

The contraceptives efficacy of seeds of *Carica papaya* has earlier been demonstrated in rats and mice. Das (1980), reported that the aqueous suspension of ripe papaya seeds at 20 mg/animal/d, given orally for 8 weeks induced 40% sterility in male rats without affecting the body weight of the genital organs, spermatogenesis, and the mortality of the spermatozoa (Chinoy et al., 1995). Vyas and Jacob (1984) also reported a similar effect in rabbits treated with an aqueous suspension of seeds orally at 100 and 200 mg/animal/d.

Lohiya et al. (1999) reported that the contraceptive efficacy of *Carica papaya* seeds is a post-testicular, associated with alternation of the epididymal mileu, which adversely affected sperm motility in rats. However, the result of this study disagree with Chinoy et al. (1996) who re-

ported that treatment with *Carica papaya* seeds does not alter gonadotropin and testosterone levels, further study shall be carried out to validate this observation.

Conclusion

This present study comes to introduce *Carica papaya* seed powder, which are cheap, easy to obtain and non-commercial as a natural agent for reversible male contraceptive to ensure good management, reduce economic loss and promote animal welfare. The buck treated with PSP at 100% has very low libido and least testosterone value in treated buck as compared to 50% and 0% inclusion rate. This is a preliminary result on utilization of PSP as feed additive in rabbit. However, the study shows that *Carica papaya* seed powder as feed additive in buck diet has potential contraceptive ability in male rabbit as evidence through reduction in blood testosterone and libido rate. However, further studies should be carried out to validate the potential of this feed additive as a male contraceptive.

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