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The Impact of Graded Levels of *Talinum fruticosum* Leaf Meal on Reproductive Indices and Organs of Rabbit Bucks

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ABSTRACT

The aim of this study was to investigate the influence of Talinum fruticosum leaf meal (TFLM) supplement in the diet of 24 New Zealand rabbit bucks (crosses) on their semen parameters, reproductive hormones production and selected internal organs. TFLM was incorporated into their diets at 0, 5, 10, and 15%, representing treatments (T) 1 (Control), 2, 3, and 4 respectively. After 8 weeks of exposure to the diets, data were collected, analysed and the results showed that TFLM produced no effect on semen volume, concentration, normal morphology, mortality, livability and colour, but resulted in significant rise ($p<0.05$) in the percentage of abnormal sperm cells (19.00, 20.00, and 15.50% in T2, T3, and T4 respectively) compared to the 11.50% found in the control group (T1). Furthermore, diets with varying levels of TFLM significantly ($p<0.05$) enhanced the production of Testosterone, Thyroid Stimulating Hormone (TSH), and Follicle Stimulating Hormone (FSH) in the rabbit bucks. The relative weights of the internal organs and the testicular morphometric parameters of the rabbit bucks were the same with those on the control diet, except for the kidney and left testis, which were significantly ($p<0.05$) heavier in rabbit bucks that received different inclusion levels of TFLM. This study concludes that Talinum fruticosum leaf meal should not be included in the diets of rabbit bucks beyond 5% to prevent impairment of semen quality.

INTRODUCTION

Proteins are important components of human diets as a result of their numerous functions. Unfortunately, from a survey carried out in 2022 by Obayelu *et al.*, it was reported that up till 2019, the average daily protein consumption among Nigerians was 45.4g, a value less than the recommended 53.8g by the Food and Agriculture Organization (FAO). The implication of this is that Nigeria has been bedeviled by a shortage in protein intake (Akerele *et al.*, 2017; Protein Challenge, 2020). There has therefore been a call for investment in domestic animals with relatively short period of gestation and low production cost like rabbits as a way of mitigating protein deficiency.

Also, using antibiotics in livestock production especially during the breeding season has been reported to have a positive impact on reproduction as well as utilization of dietary nutrients (Petersson-Wolfe *et al.*, 2007), but the dangers linked to the administration of antibiotics have necessitated the quest for alternatives to antibiotics and other feed additives of plant sources. In spite of the availability of Talinum fruticosum and its abundance in nutrients carotene, and Omega-3 fatty acids, limited information is available about its effects on the male reproductive system (Manikandan & Gayathri, 2022).

Preparation of Talinum fruticosum Leaf Meal (TFLM)

Talinum fruticosum leaves were gathered around the site. The leaves were dried in the shade to an appropriate moisture level that would allow efficient milling of the leaves into powdery form.

Experimental Diets

The diets were formulated to meet the nutrient needs of the bucks, as shown in Table 1, with diets 1, 2, 3, and 4 incorporated with 0, 5, 10, and 15 g/100g of Talinum fruticosum leaf meal, respectively.

Table 1: Composition of the Experimental Diets g/100g

Ingredients	Diet 1(0% TFLM)	Diet 2(5% TFLM)	Diet 3(10% TFLM)	Diet 4(15% TFLM)
Maize	21.00	21.00	21.00	21.00
Palm Kernel Cake	10.50	10.50	10.50	10.50
Rice Husk	20.00	20.00	20.00	20.00
Wheat Offal	25.00	25.00	25.00	25.00
Soyabean Meal	20.00	20.00	20.00	20.00
DCP	2.00	2.00	2.00	2.00
Limestone	1.00	1.00	1.00	1.00

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Vitamin/Mineral Premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Calculated Nutrients				
Crude Protein (%)	17.51	17.51	17.60	17.64
Crude Fibre (%)	13.99	14.07	14.14	14.22
Metabolizable Energy (Kcal/kg)	1,802.22	1,739.42	1,676.62	1,613.82

DCP = Dicalcium phosphate, TFLM = Talinum fruticosum Leaf Meal.

Experimental Animals Design and Management

Twenty-four New Zealand White rabbit bucks, with average age of 15 weeks were utilized for the research. These bucks were obtained from a trusted farm located in Ekiti State, Nigeria. Upon arrival, the rabbits were housed in individual hutches and were given a two-week acclimatization period before the experiment started. Throughout the experiment, good management practices and hygiene were observed. The rabbits were randomly allotted to four different treatment groups (Table 1), each containing varying levels of TFLM. All the treatments had six rabbits each in a completely randomized design.

Data Collection and Sample Analysis

Semen was harvested with the aid of an artificial vagina in the presence of a female rabbit before 9 a.m. with a 72 h interval for two weeks, while semen analysis was achieved following the procedure outlined by Ogunlade *et al.* (2019). Blood samples were collected according to the method reported by Olatundun & Ogunlade (2020).

Determination of Hormones

In each of the serum samples collected, the levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone and thyroid stimulating hormone were evaluated with chemiluminescence immunoassay techniques utilizing kits, based on the standard procedures of the kit manufacturer, AUTOBIO DIAGNOSTICS CO., LTD., located in Zhengzhou, China (Zade *et al.*, 2013).

Organ and Testicular Characteristics

At the end of 8 weeks, 2 bucks were selected at random from each treatment. They were weighed and sacrificed followed by careful removal of organs in such a way as to prevent rupture. The organs' weights were measured with a sensitive scale, while the circumference of the testis was measured using thread and a measuring ruler. The volumes of the organs were calculated by measuring the volume of water displaced in a measuring cylinder (Archimedes' principle).

Statistical Analysis of Data

All collected data were analyzed using analysis of variance (ANOVA) with statistical analysis software (SAS, 1999), and means were differentiated using Duncan's procedure within the same software.

RESULTS AND DISCUSSIONS

Table 2 shows the influence of diets with TFLM on the semen parameters of bucks. The findings revealed that TFLM did not produce any significant ($p>0.05$) effect on quality indices of spermatozoa, except the number of abnormal morphology which was elevated as the level of TFLM increased.

Table 2: Semen characteristics

Parameters	T1(0% TFLM)	T2(5% TFLM)	T3(10% TFLM)	T4(15% TFLM)	SEM
Volume (ml)	0.45	0.45	0.40	0.45	0.05
Concentration (x106/ml)	94.20	93.00	93.10	93.00	3.04
Normal	88.50	81.00	80.00	84.50	2.48
morphology (%)	11.50^{b}	19.00^{ab}	20.00^{a}	15.50^{ab}	2.02
Abnormal morphology (%)	75.00	72.50	72.50	65.00	3.54
Motility (%)	86.00	82.50	85.00	77.50	3.39
Livability (%)	86.00	82.50	85.00	77.50	3.39
Colour	Creamy white	Creamy white	Creamy white	Creamy white	

Table 3 below also shows the impact of dietary TFLM on the synthesis of reproductive hormones in the experimental rabbits. The levels of testosterone, thyroid stimulating hormone, and follicle stimulating hormone were significantly influenced ($p<0.05$), while luteinising hormone was not significantly affected by the dietary TFLM levels.

Table 3: Reproductive hormones production

Hormone Level(I.U/L)	T1(0% TFLM)	T2(5% TFLM)	T3(10% TFLM)	T4(15% TFLM)	SEM
Testosterone	3.30^{ab}	4.00^{a}	3.20^{ab}	2.5^{b}	0.3
TSH	1.20^{b}	2.25^{a}	1.85^{a}	1.90^{a}	0.13
LH	12.50	16.50	12.00	11.00	1.32
FSH	9.50^{ab}	12.00^{a}	9.00^{b}	10.50^{ab}	0.65

The relative weights of the organs and the testis of the bucks exposed to TFLM-contaminated diets are presented in Table 4. The results indicate that the diets neither have a significant effect ($p>0.05$) on the weights of the liver, spleen, lungs, heart, and right testis, nor on the right testis volume (RTV), left testis volume (LTV), left testis weight (LTW), left testis circumference (LTC) and right testis circumference (RTC). However, the average weights of the kidney and left testis were significantly affected ($p<0.05$).

Table 4: Relative weight of organs and testis morphometry

Parameters	T1(0% TFLM)	T2(5% TFLM)	T3(10% TFLM)	T4(15% TFLM)	SEM
Liver (%)	0.04	0.05	0.05	0.05	0.15
Kidney (%)	0.40^{b}	0.50^{ab}	0.45^{ab}	0.55^{a}	0.01
Spleen (%)	0.04	0.0	0.05	0.05	0.03
Lungs (%)	0.50	0.50	0.60	0.50	0.08
Heart (%)	0.20	0.31	0.25	0.24	0.06
Left Testis (%)	0.08^{b}	0.10^{a}	0.10^{a}	0.10^{a}	0.01
Right Testis (%)	0.09	0.12	0.10	0.10	0.01
LTV (ml)	1.75	1.90	2.00	2.00	0.12
RTV (ml)	1.75	2.00	2.00	2.00	0.24
LTW (g)	2.00	2.00	2.00	2.00	0.00
LTC (cm)	2.85	3.00	3.35	3.20	0.13
RTC (cm)	3.20	3.55	3.20	3.20	0.22

SEM: Standard Error of Mean

Discussion

Semen evaluation is an essential indicator of the fertility potential of semen and ensures effective reproductive performance (Quintero-Moreno *et al.*, 2007; Ajayi *et al.*, 2011; Maside *et al.*, 2023). In this study, it was revealed that none of the semen quantity and quality assessment parameters were significantly affected by the treatment, except abnormal sperm morphology. Many factors such as age, collection interval, plane of nutrition and environmental temperature can impact semen volume (Finzi *et al.*, 1994; Tohura *et al.*, 2018). As shown in Table 2, the semen volume was not significantly affected by ingestion of Talinum fruticosum, implying that at this level of inclusion, Talinum fruticosum does not impact the seminal accessory organs. A similar result was reported by Ogunlade *et al.* (2019) on the effects of Moringa oleifera to rabbit bucks. However, Adeyemi *et al.* (2022) reported that the inclusion of Vernonia amygdalina resulted in an increase in semen volume in rabbit bucks. A creamy white coloration of semen was witnessed across all treatments, which is considered to be normal (Campos *et al.*, 2014). Also, breeding success is determined by the number of viable spermatozoa available at fertilization.

Exposing the bucks to dietary TFLM was of no effect on the sperm concentration, a result supported by the report of Olatundun and Ogunlade (2022). Also, the low concentration of sperm cells recorded in this study contradicts the findings of Ajayi *et al.* (2009) who reported values that ranged from 126.00 to 154.00 x 106/ml. The difference may be as a result of exogenous factors like the feeding regime, breed, age and the environment. The sperm abnormalities recorded at high TFLM doses in this investigation may be due to the presence of phyto-chemicals (Ikechukwu *et al.*, 2017; Olukunle *et al.*, 2019).

This study has equally revealed that an appreciable level of hormones was recorded at 5% TFLM and that at higher inclusion level than 5% TFLM, a downward trend in the level of hormone production was observed. The increase in the hormone production might be attributed to higher relative weight of the left testis. This result has a nexus with the report of Malo *et al.* (2009) that there is connection between size of testes and levels of testosterone in Red deer. A downward trend in reproductive hormone production was reported in 2022 by Umamaheswari *et al.* (2022) when male Wistar rats were exposed to dietary Cleistanthus collinus. A similar reduction trend in the values of testosterone, luteinizing hormone, and follicle-stimulating hormone was also reported when Quassiaamara was administered to male albino rats (Raji & Bolarinwa, 1997). By implication, inclusion of dietary TFLM at 5% may be effective in improving hormone production.

The mean value of relative weight of the left testes in the rabbit bucks on TFLM was significantly higher compared to that of the bucks on the control diet. This result indicates that TFLM has the ability to enhance spermatozoa production capacity, since the testes size directly determines the amount of spermatogenic tissue they contain, thereby affecting the rate of sperm production (Brian *et al.*, 2011). A similar result was reported for breeder cocks that were placed on diets contaminated with fumonisin (Ogunlade, 2015).

This study also revealed that TFLM had no significant effect on the weight of the organs except the kidney. The result aligns with those reported by Isitua & Ibeh (2013) and Durunna *et al.* (2011) who discovered that administering aqueous extract from Moringa oleifera to indigenous chickens and feeding of bitter leaf meal to broilers chicken respectively, did not in any way affect their organ weights.

CONCLUSION

In conclusion, feeding Talinum fruticosum leaf meal at an inclusion level of up to 5% in the diet will enhance the male reproductive system of rabbits. As a result, it is therefore important to further carry out research on the effects of TFLM on the growth parameters of rabbits and its possible effects on the reproductive system of female rabbits.

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