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Impact of *Carica papaya* leaf meal on testes, epididymis and organ weights of rabbit bucks

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Abstract

Background The rabbit industry has embraced the use of unconventional feed ingredients; however, its impact on reproductive indices and other internal organs for optimum productivity has not been thoroughly investigated. This study was therefore carried out to assess the impact of *Carica papaya* leaf meal (CPLM) on testicular histology, epididymal sperm reserve and organ weights of rabbit bucks. Twenty-four male rabbits aged 7–8 months old were randomly allotted into four groups (CPLM0, CPLM5, CPLM10 and CPLM15) with six replicates each and fed pelletized diets containing 0, 50, 100 and 150 g of *Carica papaya* per kg of diet, respectively, at 120 g/rabbit/day for 8 weeks. The feed intake was recorded daily and weights of the animals taken weekly. At the end of the experiment, the rabbits were sacrificed, the testes, epididymis and some internal organs were carefully removed and weighed. The testes were subjected to histological assessment, and the epididymal sperm concentration was obtained using direct haemocytometric count.

Results Feed intake was higher in male rabbits fed diet with no CPLM. Bucks fed CPLM diets had higher number of testes with poor architecture compared with those fed diets without CPLM. Poor cytoarchitecture increased from 33.30 to 66.70% in the left testes while 33.30 to 100% was observed in the right testes as the dietary levels of CPLM increased from 5 to 15%. The liver, kidney and gall bladder weights of the rabbits fed 15% CPLM was significantly lower compared to that of rabbits fed no CPLM. No significant differences were recorded in the weight and sperm reserve of the epididymis.

Conclusion *Carica papaya* leaf meal is rich in nutrients but may possess some phytochemicals that can impair testicular development in rabbit bucks therefore should not be fed beyond 5% in breeder stock of male rabbits.

Keywords Cellular architecture, Epididymal reserve, Pawpaw leaf, Seminiferous tubules, Testes

Background

The relationship between nutrition and reproduction remains a focal point in livestock production as poor nutrition can have detrimental effects on the reproductive performance of animals (Meikle et al., 2018). Poor reproductive performance may be an indication of poorly developed reproductive organs and insufficient hormone synthesis which could be influenced by inadequate

nutrition and toxins. Fertility in males is associated with the development of the testes and synthesis of reproductive hormones (Li et al., 2024). The testis consists mainly of seminiferous tubules and interstitial tissue containing Leydig cells which serve as the site of spermatogenesis and testosterone synthesis, respectively (Eurell & Frappier, 2006). The tissue architecture of the testes plays a critical role in supporting and regulating spermatogenesis which can be analyzed through the structural and functional components that directly influence this process. Any disruption of these structures can negatively impact reproduction. Testicular Histology provides insights into the structural integrity of the testes, which is critical for spermatogenesis. Sperm reserve, a major

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indicator of reproductive potential in males can be influenced by diets either by improving hormone synthesis to enhance spermatozoa motility and count or reduce sperm parameters due to toxicity of some phytochemicals such as tannins and saponins (Shai et al., 2022).

The administration of plant extracts containing high levels of plant secondary metabolites such as saponins, alkaloids, essential oils, glucosides, flavonoids and tannins could improve or impair animal performance (Ivanova et al., 2024). There are several nutritious plants that can be fed to farm animals for optimum performance (Abdelsalam & Fathi, 2023; Ade et al., 2024; Wangui et al., 2024). Supplementing livestock diets with plants had positive impact on physiological processes in the body (Alem, 2024; Osei et al., 2024) and changes in organ weight often indicate phytochemical toxicity (Obode et al., 2020; Obike et al., 2024). Some plants have been identified as suitable for livestock; they are rich in compounds that have antioxidant and antimicrobial activities thus potent in improving fertility (Sarswat & Purohit, 2020). Several studies have explored the effects of plant-based diets on reproductive parameters in rabbits' traits (Adeyemi et al., 2022, 2024; Ogbuenu et al., 2024); however, limited reports exist on the impact of *Carica papaya* leaf meal (CPLM) in livestock production.

Carica papaya contains many biologically active compounds such as chymopapain, papain, caricain, and gly-cyl endopeptidase (Singh et al., 2022). *Carica papaya* is revered for its excellent antioxidant, digestive, and nutra-ceutical benefits (Koul et al., 2022). Fresh or wilted *Car-ica papaya* leaves can be fed to rabbits without adverse effect on reproductive parameters and hormonal assay (Henry et al., 2018). *Carica papaya* leaves can serve as an alternate feed ingredient in rabbit diet (Jiwuba, 2018) since they can thrive on forage-based diet. The dried form may contain lower concentration of anti-nutritional component hence may be suitable for feeding rabbit. However, there is a need to conduct further research to evaluate the impact of CPLM on rabbit diets, some phys-iological indices and anatomical structures. This study therefore assessed the impact of *Carica papaya* leaf meal on testicular histology, epididymal sperm reserve and organ weights of male rabbits.

Methods

Preparation of leaf meal, experimental animals and management

Fresh *Carica papaya* leaves were harvested, oven-dried at 40 °C for 2–3 days, ground and subjected to proximate and phytochemical analysis using the method of AOAC (2010). The *Carica papaya* leaf meal (CPLM) was there-after incorporated into the experimental diets at 0, 5, 10 and 15% levels (Table 1). Twenty-four male rabbits aged

Table 1 Composition of the experimental diets

Ingredients	Diets with CPLM levels			
	CPLM0	CPLM5	CPLM10	CPLM15
Maize	9	9	9	9
Corn bran	20	18	16	14
Soya bean	10	10	10	10
Rice bran	30	30	30	30
Wheat offal	28	25	22	19
<i>Carica papaya</i> leaf meal	0	5	10	15
Others	3.0	3.0	3.0	3.0
Total	100	100	100	100

CPLM—*Carica papaya* leaf meal, CPLM0—diet with no CPLM; CLM5—diet with 5% CPLM; CPLM10—diet with 10% CPLM; CPLM15—diet with 15% CPLM Others—Bone Meal = 1.5, Mineral/Vitamin Premix = 0.5, Salt = 0.5, Lysine = 0.25 and Methionine = 0.25

7–8 months from a heterogeneous stock were used for the study and assigned to dietary groups (CPLM0, CPLM5, CPLM10 and CPLM15) with six bucks per group housed individually as replicates in a Completely Randomized Design after balancing the weight for each group. The rabbits used for this study were carefully handled in compliance with the guidelines approved by the Institutional Animal Care Committee. The experimental diets were supplied to the animals at 120 g/rabbit/day and clean water was provided ad-libitum for 8 weeks. The feed intake was recorded daily, and the rabbits were weighed weekly using a digital weighing scale.

Organ evaluations

At the end of the experiment, all the rabbits were sacri-ficed. The testes and epididymis were carefully removed and weighed using an electronic digital scale. The vol-ume of the testes was estimated by placing each testes in a known volume of fluid, the volume of fluid displaced was recorded as the volume of the testes in centimeter cube (cm³). The testes were preserved in 10% formalde-hyde solution for histological assessment as described by Suvarna et al., (2019). The percentage of testes with normal cytoarchitecture was estimated by deducting the number of testes with poor cytoarchitecture from the total number of testes assessed; the difference was divided by the total number of testes and multiplied by 100. The value obtained was deducted from 100 as percentage of testes with poor cytoarchitecture. The epididymis was homogenized individually in 2 mL normal saline solu-tion and filtered using double layer of cheese cloth in funnels into a clean collecting tube. The filtrates obtained were thereafter diluted with normal saline at ratio 1:20 (Egbunike et al., 1975). The sperm cell concentrations

Table 2 Proximate composition of *Carica papaya* leaf meal and the experimental diets

Parameters (%)	Diets with CPLM levels				
	CPLM0	CPLM5	CPLM10	CPLM15	CPLM
Moisture	9.81	9.79	9.99	11.27	7.19
Ash	18.19	18.02	15.81	15.62	13.08
Ether Extract	4.38	3.74	3.62	3.45	8.49
Crude Fiber	6.87	9.96	12.06	10.45	6.84
Crude Protein	16.22	17.10	17.63	18.36	22.97

CPLM—*Carica papaya* leaf meal, CPLM0—diet with no CPLM; CPLM5—diet with 5% CPLM; CPLM10—diet with 10% CPLM; CPLM15—diet with 15% CPLM

Table 3 Phytochemical screening of *Carica papaya* leaf meal

Phytochemicals:	Presence/absence
Saponin	+
Alkaloids	+
Tannin	—
Flavonoid	+

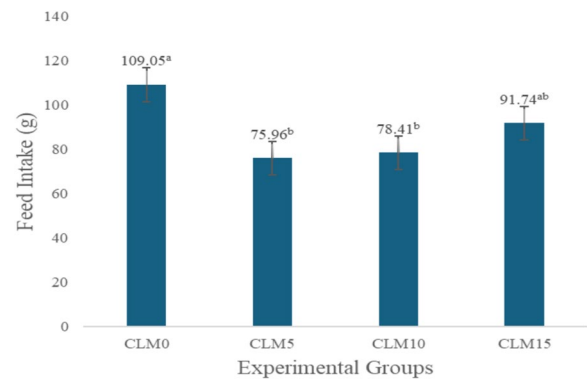
in the filtrate were obtained by direct haemocytometric count using the Neubauer haemocytometer.

Statistical analysis

Data obtained on testicular cytoarchitecture was analyzed using descriptive statistics while that of testes, epididymis and other organ weights were subjected to the General Linear Model procedure for Complete Randomized Design of Statistical Analytical System (SAS, 2009). The levels of *Carica papaya* leaf meal is the main effect in the model. The statistical significance of means was assessed using Duncan multiple range test at $p < 0.05$.

Results

The proximate composition of *Carica papaya* leaf meal and the experimental diets is shown in Table 2. The highest moisture content was recorded in CPLM15 diet. Ash and ether extract composition decreased with increasing CPLM levels. Crude fiber increased gradually in CPLM5, and CPLM10 but decreased again in CPLM15 group. The diet CPLM15 had the highest moisture and crude protein content, however, with low ash and ether extract. *Carica papaya* leaf meal is a rich source of plant protein (22.97%) feedstuff. Phytochemical screening of CPLM revealed the presence of some bioactive substances (Table 3). The rabbits fed diet with no CPLM had the highest average daily feed intake (109.05 g/day) while those fed CPLM diets

**Fig. 1** Average daily feed intake of male rabbits fed *Carica papaya* leaf meal**Table 4** Testicular cytoarchitecture (%) of rabbit fed diets with *Carica papaya* leaf meal

Experimental groups	Testes	Cellular cytoarchitecture	
		Normal	Poor
CPLM0	Right	100	0
	Left	66.70	33.30
CPLM5	Right	66.70	33.30
	Left	33.30	66.70
CPLM10	Right	33.30	66.70
	Left	33.30	66.70
CPLM15	Right	0.00	100
	Left	33.30	66.70

CPLM—*Carica papaya* leaf meal, CPLM0—diet with no CPLM; CLM5—diet with 5% CPLM; CPLM10—diet with 10% CPLM; CPLM15—diet with 15% CPLM

consumed less feed which ranged from 75.96 to 91.74 g/day (Fig. 1).

Testicular cytoarchitecture

A progressive alteration in the testicular cytoarchitecture of rabbits fed diets containing levels of *Carica papaya* leaf meal (CPLM) was recorded in this study (Table 4). Figures 2 and 3 also shows the cytoarchitecture and cellular assortment of the testis with indicators for Sertoli cells, myoid cells, Leydig cells, germinal epithelium and the lumen. In the group fed diet with no CPLM, the right testis showed 100% normal cellular cytoarchitecture, while the left testis had 66.7% normal and 33.3% poor cellular cytoarchitecture. Rabbits fed 5% CPLM had 66.7% normal and 33.3% poor cytoarchitecture for right testis and the reverse for the left testis. This shows a possible mild degeneration of the testicular tissues and alteration of testicular cellular integrity with the inclusion of CPLM at 5% level. With 10% CPLM, a further reduction in normal cytoarchitecture was recorded, both testes showed identical trends

(33.3% normal and 66.7% poor cytoarchitecture). The most severe alteration was observed in the group fed 15% CPLM, the right testis showed 0% normal and 100% poor cytoarchitecture, while the left testis had 33.3% normal and 66.7% poor cytoarchitecture.

Organ weights and epididymal parameters

The body weight and average weight of the internal organs (liver, heart, kidneys, gall bladder, spleen and lungs) of rabbit bucks fed *Carica papaya* Leaf Meal (CPLM) are presented in Table 5. The average body weight and weights of the heart, spleen, and lungs decreased numerically; however, the values were not

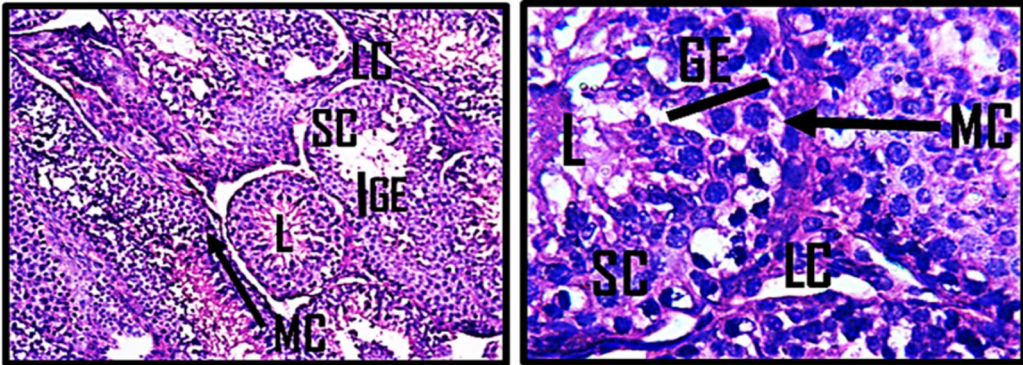


Fig. 2 Photomicrographs of rabbit testis showing normal testicular cytoarchitecture. L—Lumen, S—Sertoli cells, L—Leydig cells, GE—Germinal Epithelium, MC—Myoid Cells

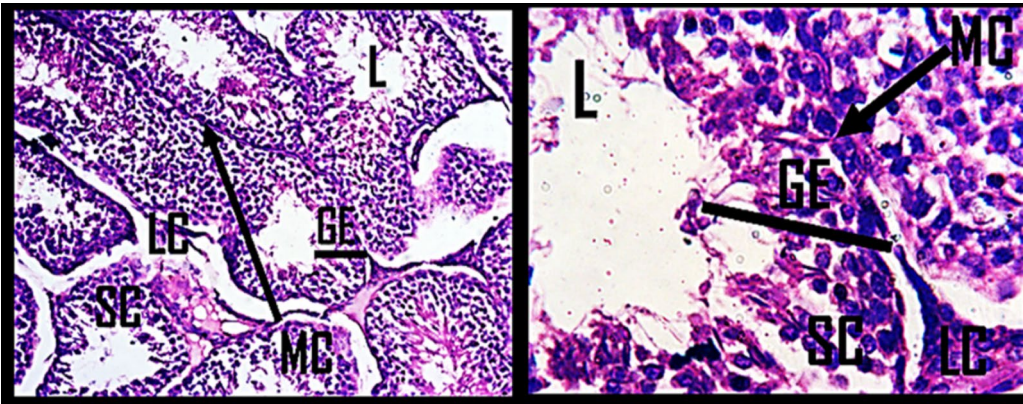


Fig. 3 Photomicrographs of rabbit testis showing poor testicular cytoarchitecture. L—Lumen, S—Sertoli cells, L—Leydig cells, GE—Germinal Epithelium, MC—Myoid Cells

Table 5 Body and relative organ weights of rabbit bucks fed diets with *Carica papaya* leaf meal

Parameters	CPLM0	CPLM5	CPLM10	CPLM15	SEM	p value
Body weight (g)	2391.23	2213.44	2159.32	2112.17	73.12	0.6282
Liver (%)	3.68 ^a	2.66 ^b	2.48 ^b	2.34 ^b	0.053	0.0051
Heart (%)	0.23	0.25	0.21	0.22	0.003	0.4153
Kidney (%)	0.49 ^a	0.51 ^a	0.42 ^b	0.42 ^b	0.005	0.0338
Gall bladder (%)	0.04 ^a	0.03 ^b	0.03 ^b	0.02 ^c	0.0007	0.0351
Spleen (%)	0.04	0.04	0.04	0.03	0.0007	0.1315
Lungs (%)	11.29	10.94	8.41	8.74	0.681	0.3502

CPLM—*Carica papaya* leaf meal, CPLM0—diet with no CPLM; CPLM5—diet with 5% CPLM; CPLM10—diet with 10% CPLM; CLM15—diet with 15% CPLM

SEM=standard error of mean. a, b, c—means with different superscripts differ significantly at $p < 0.05$

significantly ($p > 0.05$) different among the experimental groups. A significant ($p < 0.05$) decrease was recorded for liver, kidney and gall bladder weights with the highest in rabbits fed CPLM0 (3.68, 0.49, 0.04%, respectively) and the lowest in CPLM15 group (2.34, 0.42, 0.02%, respectively). The liver weights of rabbits fed CLM diets were 15 to 35% lower than the CLM0 group. The kidney weights reduced by 14 to 15% in the CLM10 and CLM15 group compared with that of CLM0 and CLM 5. Also, the gall bladders weighed half its size in rabbits fed CLM15 compared to CLM0 group. Table 6 shows the testicular weight and volume, epididymal weight and sperm reserve of rabbit bucks fed levels of *Carica papaya* Leaf Meal. A non-significant ($p > 0.05$) effect of CPLM was recorded on the parameters assessed among the groups.

Discussion

A knowledge of the composition of feedstuff helps to know its nutritive value and suitability for productivity of livestock (Baris, 2023). Protein, a macronutrient essential for body building and reproductive function was relatively high in CPLM which resulted in a gradual increase in the crude protein content of CPLM diets compared to the CPLM0 group. This implies that CPLM can be a good source of plant protein feedstuff in rabbit feeding. The diet with 15% CPLM had the crude fiber and crude protein content, with low ash and ether extract. This may be due to the different amount of nutrients contributed to the diet by CPLM compared to the ingredients (corn bran and wheat offal) it replaced in the diets. Despite similar drying method used in processing the CPLM, higher moisture content was recorded in CPLM10 and CPLM15 diets. This might be associated to factors related to the hygroscopic nature of leaves (Celino et al., 2014, Mustapha & Mahfoud, 2022) indicating that diets

with higher level of *Carica papaya* leaves might have absorb moisture from the environment even after drying or when incorporated into the experimental diets before the samples were subjected to proximate analysis. Feed intake directly impacts nutrient utilization, growth performance and overall productivity of farm animals (Baris, 2023). The highest feed intake was recorded in the control group (CPLM0) with a decline in rabbits fed CPLM5 and CPLM10 which was statistically similar to that of CPLM15 group. The reduced feed intake at these levels may be attributed to the presence of anti-nutritional factors such as alkaloids (like carpaine) and papain in papaya leaves (Damter et al., 2024; Ugbogu et al., 2023). Also, diets with higher protein content from plant feedstuff like the CPLM may be less consumed by animals once their protein requirement is met. Although CPLM has shown to be a protein-rich feedstuff, its inclusion must be optimized to prevent detrimental effect on feed intake.

Histological assessment showed that CPLM influenced the testicular architecture of rabbits in this study. Testes with good cytoarchitecture showed a well centralized lumen, Sertoli cells with oval nucleus and dark nucleolus filled with abundant cytoplasm that extend from the basement membrane to the lumen. Also, the Leydig cells were properly arranged and surrounded by connective tissues with interstitial space. The germinal epithelia were also well arranged with lots of spermatid within it. Furthermore, the myoid cells show nucleus with dark pigmentation. This implies that the integrity of the cells with good architecture was maintained despite the levels of CPLM consumed by the rabbit. For the testes with poor architecture, vacuolated and decentralized lumen, with necrotic Leydig and myoid cells and pyknotic Sertoli cells were observed. The germinal epithelium was also fragmented and disarrayed. The percentage of cells with

Table 6 Testis, epididymis and sperm reserve of male rabbits fed levels of *Carica papaya* leaf meal

Parameters	CPLM0	CPLM5	CPLM10	CPLM15	SEM	p value
Testicular weight (g)						
Right	2.32	2.61	2.73	2.19	0.164	0.6857
Left	2.29	2.61	2.91	2.21	0.188	0.6012
Testicular volume (cm ³)						
Right	2.00	2.67	2.67	2.00	0.225	0.5957
Left	2.33	2.33	2.67	1.67	0.025	0.6140
Epididymal weight (g)						
Right	1.20	1.51	1.41	1.18	0.099	0.6224
Left	1.08	1.56	1.40	1.35	0.088	0.3015
Epididymal sperm reserve (10 ⁶ sperm cells)						
Right	163.35	155.00	147.50	128.35	33.01	0.9724
Left	150.85	175.85	170.85	115.15	23.77	0.8894

CPLM—*Carica papaya* leaf meal, CPLM0—diet with no CPLM; CPLM5—diet with 5% CPLM; CPLM10—diet with 10% CPLM; CPLM15—diet with 15% CPLM

poor architecture (100% in the right testes and 66.7% in the left testes) was higher in the CPLM15 group. These findings align with earlier studies that showed that 15% CPLM decreased testosterone concentration in rabbits (Adeyemi et al., 2024) which indicate possible toxicity that can alert cellular activities in reproductive structures. Poor structure of the germinal epithelium indicates that the lining of the seminiferous tubules may not support efficient spermatogenic as the activities of Sertoli cells and spermatogonia may be impaired. Developing sperm cells usually fill the lumen of the seminiferous tubules which is essential for sperm maturation, storage and transportation. Decentralized lumen signals structural disintegration of the epithelial tissue and cells. Fertility can be impaired in the absence of developing sperm in the testicular lumen (Dostalova et al., 2017, Uwejigbo et al., 2025). A disruption in Sertoli cell structure or function can compromise the blood-testis barrier; and any alteration in the architecture of the Leydig cell can reduce androgen synthesis thus resulting in impaired spermatogenesis (Hentrich et al., 2011; Mruk and Chen, 2015). A distortion in the components of the testes can result in reproductive dysfunction and possibly infertility (Ali et al., 2022; Wu et al., 2023). Disruptions in testicular architecture often result in reduced sperm counts, motility and abnormal morphology; all contribute to male infertility. The changes in the percentages of normal and poor cellular cytoarchitecture across the groups showed the potential impact of CPLM inclusion in the diet of male rabbits on their testicular health. Nutrition impacts testicular structures and male fertility (Imik et al., 2024; Uno et al., 2024), thus a progressive decline in normal testicular cytoarchitecture with increasing levels of CPLM suggests that high dietary inclusion of *Carica papaya* leaf meal may negatively affect testicular health. This may be attributed to several factors which includes the presence of alkaloids, e.g., carpaine—known to be cytotoxic, exerting toxic effects on reproductive tissues (Shai et al., 2022). The presence of alkaloids was confirmed from the phytochemical screening in this study therefore carpaine and some other toxic bioactive substances may be higher in the CPLM10 and CPLM15 diets. This may disrupt the oxidative balance in tissues leading to cellular damage and impaired testicular function (Gad et al., 2021).

The reduction in the liver, kidney and gall bladder weights of the rabbits in this study indicates a physiological impact of higher CPLM levels on the function and size of these organs. This may be due to the presence of anti-nutritional factors in CPLM suggesting a dose-dependent effect. This may be linked to toxin excretion from CPLM indicating that *Carica papaya* leaf inclusion may influence bile production due to its impact on the liver (Dile et al., 2024) and subsequently on the gall

bladder which is the storage organ. The liver and kidney are metabolically active organs, and the observed reductions could suggest physiological stress or adaptation which may be linked anti-nutritional factors, toxins, or altered nutrient absorption of *Carica papaya* leaf meal.

A non-significant effect of CPLM on testicular and epididymal parameters in this study suggests that dietary CPLM inclusion within the range fed did not adversely affect testicular or epididymal development, nor did it impact sperm production or storage. However, there is tendency for weights and volumes to increase at moderate levels of CPLM (5 and 10%) but decrease at the highest level (15%). Also, the numerical decline in the sperm reserve in right and left epididymis of rabbits fed 15% CPLM is an indication of its detrimental potential at this level of inclusion. This may indicate that lower CPLM levels may be tolerable but with possible adverse effects at higher levels. The insignificant effect of CPLM on the testicular and epididymal parameters could also be attributed to individual animal response as the genetic makeup or adaptive traits acquired by an animal over a period may influence the overall effect of CPLM on the physiology of body structures. *Carica papaya* leaf meal has nutritional benefits (Banjoko et al., 2020); however, increase in the proportion of testes with poor cellular architecture relative to increasing dietary level showed its depressing effect on testicular structures and epididymal sperm reserve. However, feeding a higher quantity of *Carica papaya* leaf meal to rabbit bucks may have a toxic effect on reproductive structures.

Conclusions

It can be concluded that *Carica papaya* leaf meal can be adopted as an unconventional feed ingredient; however, consumption of CPLM beyond 5% may result in infertility in breeding male rabbits. Balancing its nutritional advantages against potential reproductive risks is essential for its sustainable use in rabbit production systems.

Abbreviation

CPLM *Carica papaya* Leaf meal

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Author contributions

A.A.A. designed and supervised the study, monitored the experimental protocol and wrote the first draft of the manuscript. O.O. A. and O.B.O. carried out the feeding trial, sample collection, and data analysis. All authors read, edited and approved the final manuscript.

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Data availability

No datasets were generated or analyzed during the current study.

Declarations

Ethics approval

The study was approved by the research committee on the care and use of animals for the experiment in the Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria in line with NIH guide for the care and use of laboratory animals.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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