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Chemical composition of *Carpolobia lutea* leaf meal and its inclusion effect in the diets on semen quality of rabbit bucks

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Abstract

Carpolobia lutea leaf meal, a potential phytogenic feed resource rich in nutrients and bioactive compounds, has received limited attention in rabbit production. Therefore, this study was conducted to investigate the chemical composition of Carpolobia lutea leaf meal (CLLM) and its supplementation effect on semen quality of rabbit bucks. Thirty-six rabbit bucks of average age of 10 months with mean initial weight of 1611.83 ± 5.81g were randomly assigned to four treatments of 9 rabbits each. Rabbits in each treatment group were replicated three times, with 3 rabbits in a replicate group arranged in a completely randomized design. Experimental diets fed to the rabbits were included with CLLM at the levels of 0 CLLM at5 g (T2), 10 g (T3), and 15 g (T4) per kg feed and then fed ad libitum for 12 weeks. Chemical composition of CLLM was assessed, and semen samples from the rabbit bucks fed the experimental diets were obtained for analysis from one buck in each replicate. The data obtained from the chemical composition of CLLM, and semen quality were analysed statistically. Results revealed that *CLLM contain crude protein (14.31%), crude fibre (15.25%) and ash (2.96%). The phytochemical analyses showed that CLLM contain phenols, flavonoids, tannins, oxalates, carotenoids, terpenoids, alkaloids, saponins, phytate and cyanogenic glycosides. The result on semen quality characteristics revealed that rabbit bucks in the T2 had highest ($p<0.05$) semen volume and active motile sperm compared with corresponding results in T1, T3, and T4. Rabbit bucks in T1 and T2 had significantly ($p<0.05$) higher sperm concentration and sperm motility than those in the T3 and T4. Rabbit bucks in T2 recorded significantly ($p<0.05$) lower numbers of sperm with big heads and coiled tails than those in the T3 and T4 groups. Sluggish motile sperm value was significantly ($p<0.05$) lower in T4 than those of other treatments. Rabbit bucks in the T2 and T3 had significantly ($p<0.05$) lower non-motile sperm than those in the T1 and T4. From this study, it was observed that CLLM is rich in nutrients and phytochemicals, and its dietary supplementation at 5 g/kg feed improved semen quality parameters in rabbit bucks.*

***Key words**: Leaf meal, chemical composition, rabbit bucks, semen quality characteristics*

Composition chimique de la farine de feuilles de Carpolobia lutea et effet de son incorporation dans l'alimentation sur le sperme

Résumé

La farine de feuilles de Carpolobia lutea, une ressource alimentaire d'origine végétale potentielle riche en nutriments et en composés bioactifs, n'a fait l'objet que d'une attention limitée dans le domaine de l'élevage des lapins. Par conséquent, cette étude a été menée afin d'étudier la composition chimique de la farine de feuilles de Carpolobia lutea (CLLM) et son effet sur la qualité du sperme des lapins mâles. Trente-six lapins mâles d'un âge moyen de 10 mois et d'un poids initial moyen de 1 611,83 ± 5,81 g ont été répartis aléatoirement en quatre groupes de 9 lapins chacun. Les lapins de chaque groupe de traitement ont été reproduits trois fois, avec 3 lapins par groupe de réplication, selon un plan d'expérimentation complètement aléatoire. Les régimes alimentaires expérimentaux donnés aux lapins comprenaient de la CLLM à des doses de 0, 5 g (T2), 10 g (T3) et 15 g (T4) par kg d'aliment, et les animaux ont été nourris à volonté pendant 12 semaines. La composition chimique de la CLLM a été évaluée, et des échantillons de sperme provenant des mâles nourris avec les régimes expérimentaux ont été prélevés pour analyse sur un mâle de chaque réplicat. Les données obtenues concernant la composition chimique de la CLLM et la qualité du sperme ont fait l'objet d'une analyse statistique. Les résultats ont révélé que le CLLM contient des protéines brutes (14,31 %), des fibres brutes (15,25 %) et des cendres (2,96 %). Les analyses *phytochimiques ont montré que le CLLM contient des phénols, des flavonoïdes, des tanins, des oxalates, des caroténoïdes, des terpénoïdes, des alcaloïdes, des saponines, du phytate et des glycosides cyanogéniques. Les résultats concernant les caractéristiques de la qualité du sperme ont révélé que les lapins mâles du groupe T2 présentaient un volume de sperme et un nombre de spermatozoïdes mobiles actifs plus élevés ($p < 0,05$) que ceux des groupes T1, T3 et T4. Les lapins mâles des groupes T1 et T2 présentaient une concentration et une motilité des spermatozoïdes significativement ($p < 0,05$) plus élevées que celles des groupes T3 et T4. Les mâles du groupe T2 présentaient un nombre significativement ($p < 0,05$) plus faible de spermatozoïdes à grosse tête et à queue enroulée que ceux des groupes T3 et T4. La valeur de la motilité lente était significativement ($p < 0,05$) plus faible dans le groupe T4 que dans les autres groupes. Les lapins mâles des groupes T2 et T3 présentaient une proportion de spermatozoïdes immobiles significativement ($p < 0,05$) plus faible que ceux des groupes T1 et T4. Cette étude a permis d'observer que la farine de feuilles est riche en nutriments et en composés phytochimiques, et que son ajout à raison de 5 g/kg d'aliment a amélioré les paramètres de qualité du sperme chez les lapins mâles.*

Mots-clés: Farine de feuilles, composition chimique, lapins mâles, caractéristiques de la qualité du sperme

Introduction

Rabbits have gained considerable attention in recent decades, as it has become one of the quickest ways to improve food security by providing quality animal protein to ever increasing world population (Bamanga *et al.,* 2020). Rabbits are efficient in converting agro-industrial by-products and forages to meat than other traditional livestock (Popoola *et al.,* 2023). Some outstanding attributes that promote rabbit production in Nigeria include short gestation interval, high growth rate, and the ability to reproduce immediately after parturition (Ayo-Ajasa *et al.*, 2018).

Despite these numerous advantages of rabbit production over other traditional livestock, rabbits have not achieved their full potential as a cheap animal protein source in the tropics. The inability of the rabbits reared in tropical conditions to achieve their full genetic potentials could be attributed to poor nutrition and harsh environmental rearing conditions, leading to poor reproductive performance (Sabry *et al.,* 2021). Several attempts have been made to improve the reproductive performance of rabbits using synthetic fertility drugs (Svoradová *et al.,* 2022). However, the technology has not received the needed attention from smallholder farmers who are drivers of rabbit production in developing countries due to the absence of the requisite skills and equipment required for their administration Therefore, there is a need to explore the potential of indigenous medicinal plants with proven fertility-enhancing effects.

Carpolobia lutea G. Don is a tropical plant belonging to the family polygalaceae. The plant is commonly known as cattle stick or poor man's candle. It is called *Ikpafum* in Ibibio, *Agba* in Igbo, and *Egbo* Oshunshun in Yoruba. *C. lutea* is readily available and widely utilised in traditional medicine. This plant has high nutritional and medicinal value in its leaves, fruits, and bark (Nwidu *et al*., 2012). Its juicy fruits are consumed by the local population of Southern Nigeria. Literature is dotted with the fact that the plant contains a wide range of bioactive constituents, such as flavonoids, carotenoids, phenolic acids, and soluble and insoluble dietary fibre and essential minerals, which make the plant pharmacologically and therapeutically active (Nwidu *et al*., 2012). The roots contain proteins, fats, fibre, carbohydrates, and minerals. Studies revealed that *C. lutea* roots contained phosphorus (60 mg/100 g), calcium (75.00 mg/100 g), iron (6.3 mg/100 g), manganese (0.02 mg/100 g), magnesium (4.50 mg/100 g), zinc (0.05 mg/100 g) (Gbadamosi and Oloyede, 2014). In animals other than rabbits, Akintude *et al.* (2020) found enhanced sperm count and sperm motility in rats administered 94 mg/kg body weight and 141 mg/kg body weight of aqueous extract of *C. lutea* stem with no significant changes in morphological grading. The authors attributed their observations to the antioxidant activities of *C. lutea* on the reproductive cells. The positive effects of CLLM on the semen quality of rats have also been reported by Akinola et al. (2020). However, there are few studies on the effect of CLLM supplementation on the semen quality of rabbit bucks. This study was therefore designed to assess the chemical composition of CLLM and its supplementation effect on the semen quality of rabbit bucks.

Materials and methods

Experimental site

The study was conducted at the Rabbitry Unit of the Teaching and Research Farm of Federal University of Technology Owerri (FUTO) (5°29'N and 7°03'E), Imo State, Nigeria. The annual rainfall, temperature range, and relative humidity are 234.25 mm, 23.71 – 32.93ºC, and 76.67%, respectively. Dry season duration (i.e., months with less than 65mm rainfall) is 3 months (January, February, and March) (Global Historical Weather and Climate Data, 2022).

Preparation of CLLM

The *Carpolobia lutea leaves* used in this study were harvested from Ogba Zoological Nature Park in Benin City, Edo State, Nigeria. The plant leaves were confirmed and authenticated by a Botanist at Herbarium Laboratory of the Department of Botany, University of Benin, Benin City, Nigeria. The matured *C. lutea* leaves were air-dried until they became crispy while retaining their greenish colouration. The dried leaves were milled using a hammer mill to crush them to powder.

Experimental animals and management

Thirty-six, 10-month-old mature crossbred rabbit bucks were used for the study. The experimental animals were housed in wooden hutches and galvanized wire mesh cages measuring 70 cm (length) × 60cm (breadth) × 45 cm (height), elevated 90cm above the floor. All cages and pens were washed and disinfected before the animals were introduced. Water and feed were provided *ad libitum*. A basal diet (Table 1) was formulated to meet the nutrient requirements of the rabbit bucks. The animals were randomly assigned to four treatments in a completely randomized design (CRD). The four treatments comprised a basal diet supplemented with CLLM at 0 (T1), 5 (T2), 10 (T3), and 15 (T4) g/kg feed, respectively.

Table 1 Percentage gross composition of eexperimental diet

Ingredients	Percent (%)
Yellow maize	40.00
Soyabean meal	10.00
Wheat offal	15.00
Fish meal	3.00
Brewer’s spent grains	14.00
Rice husk	13.00
Bone meal	3.00
Oyster shell	1.00
Common salt	0.50
Vitamin-mineral premix	0.50
Total	**100.00**
Calculated nutrient composition	
Metabolisable energy (Kcal/kg)	2592.00
Crude protein (%)	16.14
Crude fibre (%)	11.93
Calcium (%)	1.64
Phosphorus (%)	1.23

*To provide the following per kg of premix; Vit A, 10,000 IU; Vit D; 1,500 IU; Vit E, 3 IU; Vit K, 2 mg; Riboflavin, 3 mg; Vit $B_{12,}$0.8 mg; Folic acid, 4 mg; Mn, 8 mg; Zn, 0.5 mg; Iodine, 1.0 mg; Co, 1.2 mg; Cu, 10 mg; Fe, 20 mg

Data collection

Proximate composition of CLLM

Proximate analysis of CLLM followed the methods of AOAC (2012). The parameters measured include moisture content, ash, crude protein (CP), crude fibre (CF), ether extract (EE), and nitrogen-free extract (NFE). The fibre fractions [neutral detergent fibre (NDF), acid detergent lignin (ADL), and acid detergent fibre (ADF)] of CLLM were determined by the anthracene sulfate colorimetry method as described by Xiong *et al.* (2005). Hemicellulose content was determined by the 2% hydrochloric acid hydrolysis method combined with the 3, 5- dinitrosalicyxlic acid (DNS) reducing sugar content determination method, and concentration of cellulose was determined by comparing the absorbent value of the sample solution at 620 nm on a spectrophotometer with that of a standard curve as described by Xiong *et al*. (2005).

Phytochemical analysis of CLLM

The phytochemical assay for cyanogenic glycosides, alkaloids, flavonoids, phenols, terpenoids, and saponins in the samples were determined using the procedures described by Harbone (1973) and Sofowora (1993). The oxalate level in the sample was determined using the permanganate titrimetric method as stated by Onwuka (2005). Carotenoids were quantified using the high-performance liquid chromatography (HPLC) system method described by Alamu *et al*. (2014). Tannins were determined using the Folin-Denis spectrophotometric method as described by Shabbir *et al*. (2013). Phytate levels were determined using the method described by Tyohemba *et al*. (2019).

Semen collection and analysis

Semen was collected weekly during the 12-week feeding trial between 9 am and 10 am to obtain quality semen samples. A mature cycling doe was used to tease the buck (teaser doe), and an artificial vagina (AV) was utilized for collection. The AV was designed and constructed from a reinforced polyvinyl chloride (PVC) tube (50 mm thick) cut to a length of 2.5 cm, with a latex material forming the inner surface, as described by Herbert and Adejumo (1995). Before semen collection, the AV was warmed by leaving it in a warm bath at 60°C for 10–15 minutes, dried with a serviette, and lubricated with glycerol, a heavy organic solvent that retains heat, reduces friction, and does not contaminate semen. The AV was strategically placed under the belly of the teaser doe to allow the buck's penis to enter it. Precautions were taken to ensure the AV was not excessively hot, as this could scald the penile organ, reduce collection efficiency, or contaminate semen with urine, following the method described by Ezea and Ibeh (2021). Semen volume was measured in milliliters directly from a calibrated glass collection tube attached to the AV. Sperm motility was determined subjectively by examining a drop of fresh semen on a pre-warmed glass slide covered with a slip under a microscope. The percentage of progressively motile spermatozoa was estimated at ×400 magnification, as outlined by Jimoh *et al*. (2021). Evaluation of sperm cell concentration was measured using an improved Neubauer Haemocytometer Method using a formal saline solution mixed with semen at a v/v dilution according to the procedure outlined by Jimoh *et al*. (2021). Sperm morphology was determined by differential counts of normal and abnormal sperm shapes using Eosin-Nigrosin stain, following the procedure described by Ekuma *et al.* (2017).

Statistical analysis

The data generated on proximate, fibre fractions, and phytochemical contents were analyzed and presented as means ± standard deviation and coefficient of variation (CV) using the Statistical Package for Social Sciences (SPSS, 2012) version 20. Furthermore, data obtained on semen quality parameters were subjected to analysis of variance (ANOVA) in the general linear model. Significantly ($p < 0.05$) different means were separated using Duncan Multiple Range Test of the same statistical software according to the model:

$$Y_{ij} = \mu + T_i + E_{jj}$$

Where Y_{ij} represents the semen quality; μ is the population mean; T_i is the effect of CLLM supplementation; and E_{ij} is the random error. The model assumes normality of observations and homogeneity of variance across treatment groups.

Results

The proximate composition of CLLM, as presented in Table 2, indicates that CLLM contains 7.86% moisture, 14.31% CP, 15.25% CF, 4.36% EE, 2.96% ash, and 55.26% NFE. The coefficient of variation (COV) ranged from 5.80 to 14.90%

Table 2. Proximate composition of *Carpolobia lutea* leaf meal

Parameters (%)	Mean ± SD	COV (%)
Moisture content	7.86 ± 0.94	12.00
Crude protein	14.31 ± 1.69	11.80
Crude fibre	15.25 ± 2.25	14.80
Ether extract	4.36 ± 0.40	9.20
Ash	2.96 ± 0.44	14.90
Nitrogen-free extract	55.26 ± 3.22	5.80

SD – standard deviation; COV – coefficient of variation

Table 3 presents the results of the fibre fractionating analysis of CLLM. The result showed that CLLM contained 53.63% NDF, 49.40% ADF, 6.52% ADF, 42.88% cellulose, and 4.23% hemicellulose. The COV ranged from 0.30 to 8.10%

Table 3. Fibre composition of *Carpolobia lutea* leaf meal

Parameters (%)	Mean ± SD	COV (%)
Cellulose	42.88 ± 0.12	0.30
Hemicellulose	4.23 ± 0.18	4.30
Neutral detergent fibre (NDF)	53.63 ± 1.45	2.70
Acid detergent fibre (ADF)	49.40 ± 0.70	1.40
Acid detergent lignin (ADL)	6.52 ± 0.53	8.10

SD – standard deviation; COV – coefficient of variation

Table 4 shows that CLLM contains tannins (295.00 mg/100 g), phenols (477.50 mg/100 g), flavonoids (773.71 mg/100 g), terpenoids (12.65 mg/100 g), and phytates (4.19 mg/100 g). Phytochemical analysis also revealed that CLLM contains oxalates (83.46 mg/100 g), cyanogenic glycosides (1.09 mg/100g), carotenoids (22.80 %), alkaloids (19.72 mg/100 g), and saponins (11.90 %).

Table 4 Phytochemical composition of *Carpolobia lutea* leaf meal

Parameters	Mean ± SD	COV (%)
Tannins (mg/100 g)	295.28 ± 4.74	1.60
Phenols (mg/100 g)	477.50 ± 11.51	2.40
Flavonoids (mg/100 g)	773.71 ± 6.85	0.90
Terpenoids (mg/100 g)	12.6 5 ± 1.11	8.77
Phytate (mg/100 g)	4.19 ± 0.93	22.20
Oxalate (mg/100 g)	83.46 ± 3.21	3.80
Cyanogenic glycoside (mg/100 g)	1.09 ± 0.91	8.50
Carotenoids (%)	22.80 ± 1.30	5.70
Alkaloids (%)	19.72 ± 1.56	7.90
Saponins (%)	11.90 ± 1.52	12.80

SD – standard deviation; COV – coefficient of variation

Table 5 presents the results of semen quality characteristics of rabbit bucks fed CLLM-supplemented diets. Semen pH and pus cells among the dietary groups were not significantly ($p > 0.05$) affected by dietary CLLM supplementation. In contrast, rabbit bucks in the T1 and T2 groups recorded significantly ($p<0.05$) higher semen volume, sperm concentration, and sperm motility than those in the T3 and T4 groups. Rabbit bucks in the T2 and T3 groups recorded significantly ($p<0.05$) higher active motile sperm, than those in the T1 and T4 groups. Results showed that rabbit bucks in T4 had significantly ($p<0.05$) highest sluggish motile sperm than those in T1, T2, and T3. Rabbit bucks in T2 produced sperm with significantly ($p < 0.05$) lower big head than the rabbit bucks in the other groups. Additionally, rabbit bucks in T2 produced sperm with significantly ($p<0.05$) lowest coiled tails than those in the T3 and T4 groups.

Table 5. Semen quality characteristics of rabbit bucks fed diets supplemented with *Carpolobia lutea* leaf meal

Parameters	Supplementation levels of CLLM				SD	SEM	P-value
	T1 (0 g/kg feed)	T2 (5 g/kg feed)	T3 (10 g/kg feed)	T4 (15 g/kg feed)			
Semen pH	6.90	7.10	7.12	6.65	0.55	0.16	0.170
Semen volume (mL)	0.70^{b}	0.75^{a}	0.56^{c}	0.55^{c}	10.51	0.05	0.020
SC (x 10^6/mL)	132.20^{a}	137.00^{a}	117.80^{b}	118.40^{b}	12.50	4.50	0.043
Sperm motility (%)	72.30^{a}	79.00^{a}	63.00^{b}	62.70^{b}	3.35	2.01	0.040
Active motile sperm (%)	53.63^{b}	69.50^{a}	60.00^{b}	39.00^{c}	9.32	4.12	0.035
SMS (%)	8.67^{b}	7.50^{b}	9.00^{b}	13.00^{a}	4.20	1.01	0.011
Non-motile sperm (%)	37.7^{a}	23.00^{b}	31.00^{b}	48.00^{a}	8.32	2.75	0.030
Big head (%)	7.00^{a}	2.00^{b}	8.33^{a}	13.00^{a}	4.74	1.37	0.010
Coiled tail (%)	5.00^{bc}	2.50^{c}	8.00^{b}	12.00^{a}	3.78	1.09	0.001
Pus cells	3.5	3.50	4.50	5.00	1.21	0.3	0.130

^{abc}Means within rows with different superscripts differed significantly ($p<0.05$). SC – sperm concentration; SMS – sluggish motile sperm; SD - Standard deviation; SEM - Standard error or mean.

Discussion

Proximate studies are commonly employed in research to give a clue to the nutrient contents of feedstuffs. The CP content of CLLM observed in this study was higher (14.31 ± 1.69%) than the values of 8.39% and 8.35% reported for the same leaf by Olayinka *et al*. (2019) and Olusola *et al.* (2022), respectively. This could be attributed to the age of the leaf during harvesting, soil type, processing and analytical methods. However, this CP value was lower than the values reported for soybean meal (40-49%) and groundnut cake (40-50%), which are the main plant protein source in livestock feed (Shehu *et al.*, 2021). The CP is also lower than the 28% reported for moringa leaf by Okiki *et al*. (2015), suggesting that CLLM can be used as a protein source supplement in rabbit diets. The crude fibre content value of 15.25% recorded for CLLM was much higher than the values of 1.10 and 1.05% reported for *Carpolobia* root (Olayinka *et al*., 2019; Olusola *et al*., 2022). This result suggests that the leaves have a higher CF content than the roots. This difference can be attributed to the fact that roots primarily function as a storage for carbohydrates, whereas leaves contain more structural tissues required for photosynthesis and structural support.

The moisture level of 7.86% found in the current study is slightly lower to the 8.84% reported by Olayinka *et al*. (2019). Low moisture level is beneficial for long storage of the leaf meal, since high moisture contents in feedstuff make them susceptible to microbial spoilage during storage (Gqaza *et al*., 2013). The present result showed that CLLM contain 15.25 ± 2.25% CF, which can be utilized in rabbit diets to enhance digestion and gut health (Amadi *et al*., 2018). This finding also tends to be advantageous to rabbit production, since rabbits have the potential to handle fibrous materials (Okonkwo *et al*., 2010). In contrast, the CF obtained in this study is higher than the value of 12.57% obtained in moringa leaves by Okiki *et al.* (2015). However, the observed disparity in the CF content of CLLM in the present study and those previously reported by Olayinka *et al*. (2019) and Olusola *et al*. (2022) may be due to variations in the growth stage of the leaf before processing into a meal.

The EE content of 4.36% reported in this study is lower than the values reported for other tropical leaf meals (5.4 – 6.05%) by Ogbuewu and Mbajiorgu (2024). This suggests that CLLM may serve as the storehouse for bioactive ingredients. The NFE value of 55.26% obtained in the present study indicates that CLLM is high in soluble carbohydrates when compared to the values of 38.2 and 52.3% reported for *Acalypha spp.* and *Tithonia diversifolia* (Iniaghe *et al*., 2009; Olayinka *et al*., 2015). However, the value recorded for NFE in the current study is lower than the value of 75.74% reported for hairy indigo leaves (Gafar *et al.*, 2011). This observed difference could be attributed to variations in plant species.

Ash reflects the mineral elements preserved in food materials (Iniaghe *et al*., 2009). The ash content of 2.96 ± 0.44% recorded for CLLM in this study is lower to the value of 3.65% previously reported for the same plant by Olayinka *et al*. (2019) for the same plant. This value is also considerably lower than the 10.30% reported for *Tithonia diversifolia* leaf meal (Olayinka *et al*., 2015). The observed differences in ash content obtained in the present study and those reported in the literature could be due to factors such as leaf age, environmental factors, plant species, and soil type (Abubakar *et al*., 2021).

The results showed that CLLM contains Neutral detergent fibre (NDF), Acid detergent fibre (ADF), Acid detergent lignin (ADL), cellulose, and hemicellulose. The NDF value of 53.63% recorded in this study indicates that fibre accounts for more than half of the dry weight of CLLM, which may contribute to bulkiness in the diet. The ADF value of 49.40% observed in this study suggests that a significant portion of the fibre in CLLM is less digestible, indicating potential limitations in nutrient availability. However, the ADL content of 6.52% recorded for CLLM in the present study suggests that CLLM is not rich source of ADL. The cellulose level of 42.88% in CLLM indicates a substantial amount of structural fibre (Olayinka *et al*., 2019). The hemicellulose value of 4.23 ± 0.18% further confirms that cellulose is the dominant fibre component in CLLM.

The results of phytochemical analysis revealed that CLLM contains flavonoids, phenols, tannins, alkaloids, oxalates, terpenoids, carotenoids, and saponins. Likewise, the current study shows that CLLM contains cyanogenic glycosides and phytate. This finding is consistent with Yakubu and Jimoh (2014), who noted that the extract of *C. lutea* root contained saponins, alkaloids, flavonoids, tannins, and cyanogenic glycosides. The high flavonoid content (477.50 ± 11.51 mg/100g) recorded for CLLM from this study supports its strong antioxidant properties and justifies its use in traditional medicine practice to treat several ailments. This present finding agrees with Oluruntola *et al*. (2020), who reported that fortification of diets with herbs rich in natural antioxidants helps to mitigate the accumulation of free radicals in the animal's body. The flavonoid level obtained in the present study was higher than the values reported for *Securidaca longipedunculata* leaf extract (17.09 mg), a plant belonging to the same family as *C. lutea* (Ufuoma *et al.*, 2020). The *C. lutea* plant has also been found to have anti-inflammatory and antibacterial properties due to the presence of flavonoids, terpenoids, carotenoids, and phenols (Olayinka *et al*., 2019). The current results revealed that the phenol content of CLLM was higher than the values reported for *S. longipedunculata* leaf (22.73 mg) and sunflower leaf (18.17 mg) by Ufuoma *et al.* (2020). However, it was lower than the values reported for *Polygala sabulosa* (64.92 mg) and *P. cyparissia* (33.97 mg) by Brighente *et al*. (2007). The phenol content (477.50 ± 11.51 mg/100 g) recorded in CLLM in this study could be attributed to its well documented antioxidant properties (Nwidu *et al*., 2017).

The alkaloid content of 19.72 ± 1.56% recorded for CLLM in the present study suggests that supplementation of CLLM in the rabbit diet has the potential to enhance reproductive performance in rabbits (Hou *et al.,* 2018). Saponins are considered adaptogens or anti-stress agents (Abbas *et al*., 2015). Reports also suggest that saponins have cholesterol-lowering, anti-stress, and antioxidant properties (Singh *et al.*, 2023). The levels of phytates (4.19 ± 0.93 mg/100 g) and cyanogenic glycosides (1.09 ± 0.93 mg/100 g) obtained for CLLM in this study were considerably lower than the critical thresholds (> 40 mg/100 g and 50 mg/100 g, respectively) considered high in leaf meals (Abbas *et al*., 2014). This indicates that supplementation of CLLM in rabbit diets is unlikely to have any adverse effects on rabbit performance. However, the tannins content of 295.28 ± 4.74 mg/100 g recorded in CLLM was higher than the level > 40 mg/100 g considered high in leaf meals (Tuli *et al.*, 2016), Since tannins are known to impair digestive enzyme activities and nutrient absorption by binding and precipitating proteins (Adejoro e*t al*., 2013), high levels of CLLM supplementation in rabbit diets may limit nutrient utilisation and consequently result in poor performance.

The main phytochemicals in CLLM include phenols and flavonoids, which have been implicated as antioxidant agents that can enhance reproductive performance in farm animals. There were significant differences in aspects of semen quality parameters of rabbits fed CLLM-supplemented diets in the current study. The semen pH recorded in the present investigation was the same among the experimental groups, implying that supplementation of up to 15 g CLLM/kg feed did not affect semen acid-base equilibrium in rabbits. The present study showed that supplementation of rabbit diets with 5 g CLLM/kg feed increased semen volume and sperm concentration, whereas supplementation levels of 10-15 g/kg feed reduced both semen volume and sperm concentration. The adverse effect of plant metabolites on the secretory activity of the accessory sex glands, which produce the seminal plasma, has been reported in rabbits (Ogbuewu *et al.*, 2020). The significantly high sperm concentration at 5 g CLLM/kg feed supplementation implies that this level could be the acceptable threshold level for the rabbits. However, the exact mechanism by which CLLM at 10 and 15 g/kg feed reduces sperm concentration and semen volume in rabbits is not clear but could be attributed to high tannin content in CLLM. The decline in semen volume may be due to degenerative changes in the inner lining of the seminiferous tubules due to CLLM supplementation. The present result agrees with the findings of Adeyemi *et al*. (2024), who reported a decline in semen volume and sperm concentration in rabbit bucks fed a diet supplemented with high levels (50, 100, and 150 g/kg feed) of *Carica papaya* leaf meal.

The high sperm motility and active motile sperm in rabbit bucks fed a diet supplemented with 5 g CLLM/kg feed support the findings of El-Desoky *et al*. (2017) that administration of lower doses of moringa leaf extract (50 and 100 mg/kg body weight) increased sperm motility in rabbits. The increased sperm motility and active motile sperm imply that 5 g CLLM/kg feed enhanced the activities of AMP-activated protein kinase (AMPK), an enzyme that assists in maintaining cellular energy balance. The significantly high sluggish motile and non-motile sperm counts recorded at 15 g/kg CLLM supplementation indicate the reduced ability of the CLLM-supplemented diet to support the formation of active sperm, which can lead to improved sperm fertilization ability. The production of sperm with reduced sluggish motile and non-motile cells in the 5 g CLLM-treated rabbits suggests the potential of antioxidant components (phenols and flavonoids) in CLLM, as evidenced in phytochemical results to protect sperm membranes against oxidative stress (Jimoh *et al*., 2021). The significantly reduced sperm with big heads, as obtained in the rabbits fed 5 g CLLM/kg feed, suggests the ability of the treatment to support the production of sperm with normal morphology, while higher levels of CLLM (10 and 15 g/kg feed) encourage the production of sperm with abnormal sperm morphology in rabbits. These results agreed with the findings of Ogbuewu *et al.* (2020), who discovered that as sperm motility increased, the number of sperm with abnormal cells decreased.

Conclusion

The results of the present study show that CLLM is moderate in crude protein, crude fibre, carbohydrates, and ether extracts. In addition, CLLM is moderate in neutral detergent fibre, acid detergent fibre, and cellulose, and low in acid detergent lignin and hemicellulose. The results also reveal that CLLM is a rich source of flavonoids, phenols, carotenoids, and tannins, moderate in terpenoids, alkaloids, oxalate, saponins, and low in phytate and cyanogenic glycosides. Dietary supplementation of CLLM at 5 g/kg feed improved semen volume, sperm motility, sperm concentration, active motile sperm, and reduced sperm with abnormal morphology (coiled tail, big head, sluggish, and non-motile cells). The findings show that dietary supplementation of CLLM at 5 g/kg feed enhanced sperm quality of rabbit bucks

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