# Semen characteristics and hormonal profile of Yankasa rams fed a mixed ration of cowpea husk (*Vigna unguiculata* L. Walp) and tiger nuts (*Cyperus esculentum* L.) residue

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**Abstract**

Thirty growing and healthy Yankasa rams were randomly allotted five dietary treatments with six animals per treatment to ascertain the effect of diet on their reproductive potential and hormonal profile. Treatments compared were $T_1$ (cowpea husk 40% + tiger nuts residue 0%), $T_2$ (cowpea husk 30% + tiger nuts residue 10%), $T_3$ (cowpea husk 20% + tiger nuts residue 20%), $T_4$ (cowpea husk 10% + tiger nuts residue 30%), and $T_5$ (cowpea husk 0% + tiger nuts residue 40%). The results of the chemical composition of the diets showed that the dry matter was high for all treatments. The semen characteristics and hormonal profile showed significant differences ($p < 0.05$) in all the parameters observed but were within normal ranges. However, the group fed a high percent tiger nut mixed ratio had a depreciating effect on semen characteristics, and LH, FSH, and testosterone levels. There is no deleterious effect on the reproductive potential of growing Yankasa rams fed varying levels of cowpea husk and tiger nut residue at the inclusion levels in this study. Hence, it is safe to feed breeding rams the ration mix. However, the authors advise caution of including tiger nuts levels of up to 40% in a mixed ration. further investigation may be conducted with ewes to determine the effect of a mixed ration of cowpea husk and tiger nut residue in reproduction.

**Keywords:** semen, hormone, Yankasa rams, cowpea husk, tiger nut residue

# Características do sêmen e perfil hormonal de carneiros Yankasa alimentados com ração mista de casca de feijão-caupi (*Vigna unguiculata* L. Walp) e resíduo de chufa (*Cyperus esculentum* L.)

**Resumo**

Trinta carneiros Yankasa em crescimento e saudáveis foram distribuídos aleatoriamente em cinco tratamentos dietéticos com seis animais por tratamento para verificar o efeito da dieta em seu potencial reprodutivo e perfil hormonal. Os tratamentos comparados foram T1 (casca de feijão-caupi 40% + resíduo de castanha-de-caupi 0%), T2 (casca de feijão-caupi 30% + resíduo de castanha-de-caupi 10%), T3 (casca de feijão-caupi 20% + resíduo de castanha-de-caupi 20%), T4 (casca de feijão-caupi 10 % + resíduo de castanha-de-tigre 30%) e T5 (casca de feijão-caupi 0% + resíduo de castanha-de-tigre 40%). Os resultados da composição química das dietas mostraram que a matéria seca foi elevada para todos os tratamentos. As características do sêmen e o perfil hormonal apresentaram diferenças significativas ($p < 0,05$) em todos os parâmetros observados, mas estavam dentro da normalidade. No entanto, o grupo alimentado com uma proporção elevada de castanhas de tigre teve um efeito depreciativo nas características do sêmen e nos níveis de LH, FSH e testosterona. Não há efeito deletério sobre o potencial reprodutivo de carneiros Yankasa em crescimento alimentados com níveis variados de

casca de feijão-caupi e resíduo de castanha de tigre nos níveis de inclusão neste estudo. Portanto, é seguro alimentar carneiros reprodutores com a mistura de ração. No entanto, os autores aconselham cautela ao incluir níveis de nozes de tigre de até 40% em uma ração mista. investigações adicionais podem ser realizadas com ovelhas para determinar o efeito de uma ração mista de casca de feijão-caupi e resíduo de castanha de tigre na reprodução.

**Palavras-chave:** sêmen, hormônio, carneiros Yankasa, casca de feijão-caupi, resíduo de noz-de-tigre.

## 1. Introduction

Evaluation of semen quality and reproductive hormones in rams is essential for examining reproductive health and fertility in livestock animals (Osinowo et al., 1988; Ihulwumere; Okere, 1990; Mozo et al., 2015). Nutrition plays a significant role in influencing reproductive performance, and the inclusion of alternative feed resources in animal diets has gained attention due to their potential impact on semen quality and hormonal regulation (Akinlade et al., 2020). More so, the spermatogonic cycle is chiefly controlled by hormones (O'Shaughnessy, 2014).

Yankasa rams are a breed of sheep known for their robustness and adaptability to various environmental conditions. However, optimizing their reproductive performance is essential for sustainable breeding and genetic improvement programs. Semen characteristics, such as sperm count, motility, morphology, and concentration of seminal plasma hormones, are reliable indicators of male fertility (Adebayo et al., 2014).

Understanding the relationship between nutrition, semen characteristics, and hormonal profiles is crucial for optimizing reproductive performance (Abubakar, et al., 2014) in male animals. The utilization of alternative feed resources, such as cowpea husk and tiger nut residue, has economic and ecological advantages, as they can serve as sustainable feed options while reducing waste in agricultural systems. Dietary components have a profound influence on reproductive function in rams. The inclusion of cowpea husk and tiger nut residue in the ration holds promise due to their nutritional composition and potential benefits for livestock production (Ayim-Akonor, et al., 2015).

Cowpea husk, a by-product of the legume crop, is rich in protein, fiber, and minerals, while tiger nut residue is a by-product of the tuberous crop and contains carbohydrates, fiber, and essential nutrients (Enyuikwu et al., 2018; Carneiro da Silva et al., 2019). Tiger nut residue is obtained after milk extraction from the nut and is discarded as waste. The tiger nut residue is reported to have high energy content (Archibong et.al., 2018). Despite the numerous nutritional, and health benefits, potentials, and prospects, tiger nut residue as a nutrient source for livestock has not been studied extensively.

The present study was designed to investigate the effects of the mixed ration of cowpea husk and tiger nut residue on semen characteristics and hormonal profiles of Yankasa rams. The research aimed to assess parameters such as sperm concentration, motility, viability, and morphology, as well as the levels of some reproductive hormones, including testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH). By evaluating these parameters, the study sought to determine the impact of the experimental diet on semen characteristics and the concentration of some reproductive hormones in the growing rams.

## 2. Materials and Methods

### *2.1 Experimental site*

The study was conducted at the small ruminant experimental unit of the National Open University of Nigeria (NOUN), Kaduna farm located at Latitude 10.61362 N$10^{0}36^{1}47.692$", Longitude 7.46852 E $7^{0}28^{1}23.667$" at an altitude of 612.65 m. The climate is tropical wet and dry, classified as Koppen Aw. The wet season lasts from April through to mid-October with a peak in August; while the dry season extends from mid-October of one calendar year to April the next year (Abaje et al., 2018). The spatial and temporal distribution varies, decreasing from an average of about 1,203 mm. The highest average air temperature occurs in April (28.9 ºC) and the lowest in December (22.9 ºC) through January (23.1 ºC) (Abaje et al., 2016). The mean atmospheric relative humidity ranges between 70-90% and 25-30% for the rainy and dry seasons respectively (Abaje et al., 2018).

### *2.2 Experimental Animals and Diet*

Thirty (30) growing Yankasa rams aged six to eight months with an initial weight of 15.50 ± 0.67 kg were used

for this study. The animals were purchased from an open livestock market in Makarfi LGA of Kaduna State. The pens and the surroundings were cleaned and disinfected with a strong antiseptic (Morigad) two weeks before the arrival of the animals. Upon arrival, the animals were administered a prophylactic treatment consisting of a long-acting antibiotic (Oxytetracycline 20% LA at 1.0 ml/10kg body weight) intramuscularly, and Ivomec injection at 200 µg/kg (0.3ml/10kg) to control endo and ectoparasites (subcutaneous). The animals were housed in their cages and allowed to adapt to the environment for 14 days before the commencement of the experiment. The cowpea husks used for the study were from the previous harvest from nearby farms that have been allowed to dry in the sun while the tiger nuts residue used was obtained from a local processor who produces local drink, and the residue was dried under shade.

*2.3 Experimental Design and Treatment*

The experimental animals (Yankasa rams) were allocated to five dietary treatments with six animals per treatment in a complete randomized design as presented below:

$T_1$ = Cowpea husk 40% + Tiger nuts residue 0%

$T_2$ = Cowpea husk 30% + Tiger nuts residue 10%

$T_3$ = Cowpea husk 20% + Tiger nuts residue 20%

$T_4$ = Cowpea husk 10% + Tiger nuts residue 30%

$T_5$ = Cowpea husk 0% + Tiger nuts residue 40%

Table 1. Ingredients and composition of the experimental diets (%).

| Ingredient | Treatment Diets | | | | |
|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ |
| Tiger nut | 0.00 | 10.00 | 20.00 | 30.00 | 40.00 |
| Cowpea husk | 40.00 | 30.00 | 20.00 | 10.00 | 0.00 |
| Sorghum stover | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 |
| Wheat bran | 24.00 | 24.00 | 24.00 | 24.00 | 24.00 |
| Groundnut cake | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Premix | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Calculated Crude Protein (%) | 15.81 | 14.95 | 14.10 | 13.24 | 12.38 |

Source: Authors, 2023.

*2.4 Feeding and Management*

The study comprised 14 days of the feed adaptation period and 13 weeks (91 days) of the measurement period. Feed was weighed and fed twice daily at 8 a.m. and 2 p.m. Water was provided ad libitum. The quantity of feed provided, and the residue of the previous day were weighed to determine the feed intake of each animal. The rams were weighed at the beginning of the experiment at weekly intervals in the morning before feeding.

*2.5 Chemical analysis*

The feed samples of experimental diets were collected and dried in an air-draft oven at 600 ºC for 96 h, ground separately to pass through a 1 mm sieve in a Wiley mill and sampled for chemical analysis using the standard methods of the Association of Official Analytical Chemists (AOAC, 2005). Dry matter was determined by drying at 1000 ºC for 24 h, ash concentration was determined after ignition at 550 ºC for 4 h in a muffle furnace and used to calculate organic matter (OM). Fiber fraction analysis was done by the methods of Van Soest et al. (1991). Hemicellulose and cellulose were estimated as differences between neutral detergent fiber (NDF) and acid detergent fiber (ADF) and ADF and lignin, respectively.

*2.6 Semen collection*

Semen samples used in this study were collected from rams within the last week of the experiment using electro-ejaculator techniques as described by Garba et al. (2022). Before the semen collection, preliminary training was given to the rams. The animals were gently restrained and placed on their side and the penis extended from the sheath by stretching the sigmoid flexure. The penis was then grasped with the sterile gauze and the gland penis was diverted into a 50 mL disposable tube that was insulated by the hand of the collection technician. The animals were gently massaged around the hind region by exerting downward pressure for 10 to 15 s before the insertion of the electro-ejaculator. The lubricated electro-ejaculator probe was then inserted into the rectum of the animal and turned on where the voltage increased manually for three to eight seconds and then the animal was allowed to rest for 15-20 s and repeated till ejaculation (Not exceeding six pulses per animal). After ejaculation, the semen was covered to maintain its temperature and taken to the laboratory for processing.

*2.7 Semen volume and color*

Ejaculate volume was measured immediately after collection from the ram using a graduated collecting tube while semen color was determined by visual observation and compared with a color chart.

*2.8 Semen pH*

Initial semen pH was obtained using a comparative pH paper as described by Garba et al. (2022). The pH paper was calibrated from 1 to 14 in different colors. One inch of the paper was inserted into each sample of the semen then removed and checked for the color match on the pH paper chart. A pH meter was also used to validate semen pH in the laboratory.

*2.9 Percentage sperm cell motility*

A drop of semen diluted with a drop of sodium citrate was placed on a warm glass slide covered with a clover slip. This was viewed using a 40x objective of a light microscope and the percentage of active, progressively motile cells were estimated as described by Chenoweth (2005).

*2.10 Semen concentration*

Spermatozoa concentration was determined with the aid of an improved Neubauer hemocytometer (BLAUBRAND® Neubauer improved hemocytometer, UK). Semen from each sample was diluted with 10% formol-saline solution at the ratio 1:100 (*w/v*). A cover slip was put on the hemocytometer and about 8 µl of the diluted semen sample was introduced into the groove of the hemocytometer underneath the cover slip. The sperm cell heads in five large squares of the hemocytometer were counted under the view of a microscope at x 40 objective. The number of cells counted was expressed as x $10^6$ sperms/mL of semen.

*2.11 Percentage of morphologically normal sperm cell*

One drop of the diluted semen sample was added to two drops of eosin-nigrosine stain, and mixed thoroughly before a smear was made on a clean glass slide. The smear was allowed to dry and examined under the microscope at x 100 magnification. Sperm cell with morphological aberrations was determined from a total count of 100 spermatozoa and the percentage number of normal cells were determined (Chenowoth, 2005).

*2.12 Hormonal Assay*

Jugular venipuncture was employed to get blood samples, which were then placed in heparinized tubes. The samples (in triplicate) were immediately centrifuged at 3000 g for 10 min after collection, and the serum obtained was kept at -20 ℃ until analysis. Serum testosterone, LH, and FSH were determined using testosterone, LH, and FSH enzyme-linked immunosorbent assay (ELISA) kits for sheep (Diagnostic Automation/Cortes Inc. Immuno Diagnostics CA, Woodland Hills, California) as described by Micallef et al. (1995). Cortisol level was estimated using AccuDiagTM sheep cortisol ELISA kits.

*2.13 Validation of samples*

ELISA kits for sheep were evaluated using analytical validation. The procedure for assay validation was conducted as described by the manufacturer (Diagnostic Automation/Cortes Inc. Immuno Diagnostics CA, Woodland Hills, California). The analytical validation comprised accuracy (that is, adding known quantities of the hormones and calculating the percentage of recovery). The test was carried out in triplicates and the sensitivity was reported as provided by the manufacturer.

*2.14 Statistical Analysis*

Data collected were subjected to analysis of variance (ANOVA) in a completely randomized design using the SAS software (SAS, 2005) where significant differences were observed, and means were separated using *Duncan's* Multiple Range Test (DMRT).

**3. Results and Discussion**

*3.1 Chemical composition of experimental diets*

The chemical composition of the experimental diets is shown in (Table 2). From the Table, the dry matter content of the experimental diets ranged from 895.7 g $kg^{-1}$ DM in $T_4$ (10% cowpea husk and 30% Tiger nuts residue ration mix) to 920.1 g $kg^{-1}$ DM in $T_3$ (20% Cowpea husk and 20% Tiger nuts residue ration mix). $T_1$ was observed to have the lowest ash content compared to other treatments. The pattern of significant differences ($p < 0.05$) for Lignin and NDF were similar with the highest values (122 g $kg^{-1}$ DM and 453.7 g $kg^{-1}$ DM respectively) recorded in $T_1$ and lowest values (113 g $kg^{-1}$ DM and 420.6 g $kg^{-1}$ DM respectively) in $T_5$. ADF was significantly different with $T_1$ recording the highest value of 265.1 g $kg^{-1}$ DM and the lowest in $T_5$ 232.0 g $kg^{-1}$ DM.

Crude protein values for dietary treatments were significant ($p < 0.05$) with $T_1$ higher (155.1 g $kg^{-1}$ DM), lowest in $T_5$ (145.1 g $kg^{-1}$ DM), and 150.3 g $kg^{-1}$ DM, 149.2 g $kg^{-1}$ DM, 148.0 g $kg^{-1}$ DM respectively compared to other treatments. A similar trend was observed with the energy content of feeds with $T_1$ having the highest energy of 10.68 MJ and the lowest for $T_5$ (10.13 MJ). The crude protein content was observed to decrease as the level of Cowpea husks decreased and the level of Tiger nuts residue increased.

The crude protein level in this study fell within the range of 139 g $kg^{-1}$ DM to 220 g $kg^{-1}$ DM reported by Olafadehan et al. (2022) who used cowpea husks to feed goats but lower than the range of 162.1-203.3 g $kg^{-1}$ DM and 354 g $kg^{-1}$ DM to 383 g $kg^{-1}$ DM reported by Njidda et al. (2018) and Nasir et al. (2014) respectively. Abdu et al. (2012) and Okafor et al. (2012) reported 102.5 and 137.3 g $kg^{-1}$ DM which is lower than that obtained from this study. The variation observed between previous reports and results from this study could be attributed to the difference in the composition of diets offered to the experimental animals.

The crude protein content in this study is above 7% CP recommended for rumen microbes of tropical livestock by Minson (1990) below which performance will be deficient. The NDF and ADF were highest at $T_1$ (453.7 g $kg^{-1}$ and 265.1 g $kg^{-1}$ DM respectively) and lowest at $T_5$ (420.6 g $kg^{-1}$ and 232.0 g $kg^{-1}$ DM) which implies that increasing the inclusion of Tiger nuts residue decreases and the values reported from this experiment is lower than the range of 527 g $kg^{-1}$ DM – 540.31 g $kg^{-1}$ DM for NDF and 321.31 g $kg^{-1}$ DM – 325 g $kg^{-1}$ DM for ADF as reported by Njidda et al. (2018).

Meissner et al. (1991) posited that NDF level of forages beyond 65% can limit feed intake. The EE obtained from this study fell within the range of 22.10 g $kg^{-1}$ DM - 42.21g $kg^{-1}$ DM reported by Njidda et al. (2018). The Lignin content reported from this study is higher than that reported by Njidda et al. (2018) and Nasir et al. (2014). Differences observed in the composition of the feed are attributed to the ration used as Njidda et al. (2018) used a mix of soyabean meal and *Gmelina arborea* leaves, Nasir et al. (2014) used cotton seed cake while a mixed ration of cowpea husk and tiger nuts residue was used for this study.

Table 2. Chemical Composition of experimental diets on dry matter (DM) (g $kg^{-1}$ DM).

| Parameter | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | SEM |
|---|---|---|---|---|---|---|
| DM | $904.70^{ab}$ | $908.00^{a}$ | $920.10^{a}$ | $895.70^{b}$ | $910.10^{a}$ | 1.80 |
| CP | $155.10^{a}$ | $150.30^{ab}$ | $149.20^{ab}$ | $148.00^{ab}$ | $145.10^{b}$ | 0.70 |
| EE | $41.00^{a}$ | $39.40^{a}$ | $36.70^{ab}$ | $35.40^{ab}$ | $34.00^{b}$ | 0.60 |
| Ash | $68.50^{b}$ | $71.10^{a}$ | $71.80^{a}$ | $72.60^{a}$ | $73.30^{a}$ | 0.40 |
| ADF | $265.10^{a}$ | $244.70^{b}$ | $240.60^{bc}$ | $235.90^{bc}$ | $232.00^{c}$ | 2.60 |
| NDF | $453.70^{a}$ | $433.60^{b}$ | $429.00^{b}$ | $422.00^{b}$ | $420.60^{b}$ | 2.70 |
| Lignin | $122.00^{a}$ | $117.70^{b}$ | $115.00^{b}$ | $114.20^{b}$ | $113.30^{b}$ | 0.70 |
| NFE | $470.30^{c}$ | $494.50^{b}$ | $501.70^{b}$ | $508.10^{ab}$ | $515.60^{a}$ | 3.50 |
| Cellulose | $143.10^{a}$ | $127.00^{b}$ | $125.60^{bc}$ | $121.70^{c}$ | $118.70^{c}$ | 1.90 |
| Hemicellulose | $188.60^{a}$ | $188.90^{a}$ | $188.40^{a}$ | $186.10^{b}$ | $188.60^{a}$ | 0.20 |
| Energy (MJ) | $10.68^{a}$ | $10.44^{ab}$ | $10.26^{b}$ | $10.14^{c}$ | $10.13^{c}$ | 0.05 |

Note: $^{a,b,c}$ means in the same row with different superscripts are significantly ($p < 0.05$) different, DM = Dry matter, CP = Crude protein; EE = Ether Extract; ADF = Acid detergent fibre; NDF = Neutral detergent fibre; NFE = Nitrogen free extract; MJ=Megajoule; SEM= Standard error of mean. Source: Authors, 2023.

*3.2 Semen characteristics*

Semen volume differed significantly ($p < 0.05$) with the highest volume (0.60 mL) reported from Yankasa rams in $T_4$ while the least (0.20 mL) was recorded from rams fed dietary treatment 5 ($T_5$). Semen colour did not vary significantly among rams and indicated it is of good quality (milky to creamy). Semen motility varied significantly ($p < 0.05$) among Yankasa rams with $T_1$ having the highest motility (90%) and $T_5$ indicating the lowest motility of 60%. The sperm concentration ($x10^6$ sperm/mL) showed a significant difference ($p < 0.05$) with $T_1$ having the highest concentration of 286.00 and $T_5$ with the lowest concentration of 111.00. The highest proportion of live cells was obtained from $T_1$ (90) while the lowest was observed in $T_5$ (60).

Semen volume is one of the important factors in semen evaluation and reproductive performance in male animals (Jha et al., 2018). The semen volume in our study is lower than the report by Malama et al. (2013) and Jha et al. (2018) who obtained between 0.59ml to 0.99 mL. this could be due to the differences in breed and age of rams utilized during the study. Bangladeshi rams above 1.5 years were used in the previous studies while growing Yankasa rams (< 1year) were used for this study. Ejaculate volume is also affected by the method of semen collection. Semen collection by electro-ejaculation results in larger volumes of ejaculate compared to semen collected using an artificial vagina. This could be due to electrical stimulation on accessory glands that provokes additional secretion of seminal plasma. Semen volume decreases (by 25 to 53%) with frequency and interval of collections (Jha et al., 2018).

Semen colour in this study (milky to creamy) was consistent with the reports of Azizunnesa et al. (2013) and Jha et al. (2018). Colour may be an indicator of injury or infection in the reproductive tract. The presence of blood or pus flakes may indicate infection in the reproductive tract (Nabil et al., 2006). Sperm motility provides evidence of sperm maturation. Sperm motility is a reliable indication of sperm viability (Jha et al., 2018). Sperm motility from this study is higher than the range of 60-85% reported by Khalifa et al. (2013), Azizunnesa et al. (2014), and Jha et al. (2018). The higher motility observed from this study could be attributed to the fact that semen collection was made on growing Yankasa rams for the first time and the experimental diet rams were placed on was nutritionally balanced. Sperm motility is affected by the frequency of semen collection and the nature of diet (Jha et al., 2018).

The sperm motility decreases by 19-36% with successive and frequent ejaculation (Kaya et al., 2002; Jha et al., 2018). It has been documented that there is a decrease in sperm motility in feed-restricted rams which is due to low seminal plasma fructose concentration and depressed activity of the pituitary gland (Jha et al., 2018). The composition of the diet may also affect semen characteristics indirectly by influencing the secretion of reproductive hormones. For instance, the ingestion of Ginseng increases testosterone levels in female subjects (Al-Dujaili, 2020). Semen pH in this study is below the range of 6.9 – 7.2 reported by Al-Samarrae (2009). Good-quality semen is always slightly acidic (Madhuri et al., 2012), therefore the acidic semen pH obtained

from this study is an indication that the semen from the growing Yankasa rams is of good quality.

The sperm concentration obtained from this study is lower than the reports of Jha et al. (2018). The sperm concentration increases with age and decreases with successive frequent ejaculations by 19-55% (Jha *et al.*, 2018). The quality of semen is generally influenced by age, body weight, and testicular size. The size of the testicles increases with the advancement in age (Toe et al., 2000). Body weight is vital than age in determining testicular growth and development, and its effect on semen quality (Jha et al., 2018). The higher sperm concentration allows more dilution and production of a higher number of insemination doses, ultimately providing an opportunity to inseminate many ewes.

Table 3. Semen Characteristics of Yankasa rams fed a mixed ration of cowpea husk and tiger nuts residue.

| | Treatment | | | | | |
|---|---|---|---|---|---|---|
| Parameter | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | SEM |
| Volume (mL) | $0.40^{a}$ | $0.30^{b}$ | $0.30^{b}$ | $0.60^{a}$ | $0.20^{c}$ | 0.07 |
| Colour | Creamy | Milky | Milky | Creamy | Milky | - |
| Motility (%) | $90.00^{a}$ | $80.00^{b}$ | $60.00^{d}$ | $85.00^{ab}$ | $75.00^{c}$ | 5.15 |
| PH | $7.00^{a}$ | $6.00^{b}$ | $6.50^{ab}$ | $7.00^{a}$ | $6.00^{b}$ | 0.22 |
| Concentration ($x10^6$/mL) | $286.00^{a}$ | $176.00^{b}$ | $128.00^{b}$ | $279.00^{a}$ | $111.00^{c}$ | 36.90 |
| Live cells (%) | $90.00^{a}$ | $80.00^{b}$ | $70.00^{c}$ | $90.00^{a}$ | $60.00^{d}$ | 5.83 |
| Dead cells (%) | $10.00^{d}$ | $20.00^{c}$ | $30.00^{b}$ | $10.00^{d}$ | $40.00^{a}$ | 5.83 |

NOTE: $^{a,b,c,d}$ Means within the same rows with different superscripts differed significantly ($p < 0.05$); SEM = Standard error of mean. Source: Authors, 2023.

*3.3 Semen morphological abnormalities*

On morphological abnormalities, the normal sperm morphology varied significantly ($p < 0.05$) with the highest proportion of normal cells observed from rams fed dietary Treatment 1 (98) and the least in $T_{5.}$ (79). Of the morphological abnormalities observed, the bent tail was the only abnormality from rams in Treatment 1, while other semen from Treatments 3, and 5 had coiled tails, detached heads, free tails, and bent tails. Semen from rams fed dietary Treatment 2 did not have a detached head, while Treatment 4 did not have a free tail abnormality. There were no mid-piece droplets abnormalities observed across all treatments (Table 4).

Breeding rams should have a proportion of at least 70% of live cells (Jha et al., 2018). Semen collected from most rams from this study contained some abnormalities. Sperm quality improves with age in the matured rams (Jha et al., 2018). It has been shown that ejaculates from growing rams contain more abnormal cells compared to adult rams, which signifies incomplete spermatogenic activity and incomplete epididymal maturation (Colas, 1983). Also, the presence of certain nutrients in animal diet, such as Vitamin E, could exert a positive effect in improving semen quality and quantity (Yue, et al., 2010).

Table 4. Semen Morphological abnormalities of Yankasa rams fed a mixed ration of cowpea husk and tiger nuts residue.

| Parameter | Treatment | | | | | |
|---|---|---|---|---|---|---|
| (%) | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | SEM |
| Mid piece droplets | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Coiled Tail | 0.00 | $3.00^{b}$ | $2.00^{bc}$ | $1.00^{c}$ | $6.00^{a}$ | 1.03 |
| Detached Head | 0.00 | 0.00 | $6.00^{a}$ | $2.00^{b}$ | $3.00^{b}$ | 1.11 |
| Free tail | 0.00 | $1.00^{b}$ | $1.00^{b}$ | 0.00 | $5.00^{a}$ | 0.93 |
| Bent tail | $2.00^{c}$ | $6.00^{a}$ | $3.00^{b}$ | $4.00^{b}$ | $7.00^{a}$ | 0.92 |
| Normal cells | $98.00^{a}$ | $90.00^{ab}$ | $88.00^{b}$ | $93.00^{a}$ | $79.00^{c}$ | 3.14 |

Note: $^{a,b,c}$ Means within the same rows with different superscripts differed significantly ($p < 0.05$); SEM = Standard error of mean. Source: Authors, 2023.

*3.4 Hormonal assay*

Hormonal levels significantly differed in rams across the dietary treatments (Table 5). FSH was higher in $T_5$ (0.96 ng/mL) and lowest in $T_1$ (0.28ng/ml). Similarly, LH was higher in $T_5$ (1.81 ng/mL) and lowest in $T_1$ (0.47 ng/mL). For testosterone levels, rams raised with dietary treatment 5 had high testosterone levels (2.87 ng/mL), and rams in treatment 3 had the lowest testosterone concentration of 2.40 ng/mL. Cortisol concentration was higher in $T_5$ (8.28 µg/dL) and least in $T_2$ (4.50 µg/dL).

The concentration of reproductive hormones in growing rams can vary depending on various factors such as age, breed, season, feed, and individual differences. Testosterone levels generally increase during puberty and reach peak levels during sexual maturity. The testosterone levels obtained from this study fall within the range reported for prepubertal (< 0.5 ng/mL) and pubertal rams (0.5-4 ng/mL) reported by Estienne et al. (1993) and Silva et al. (2008) respectively. Carlos et al. (2016) reported 3.08 ng/mL and 3.22 ng/mL testosterone concentration in rams during the inactive (no sexual activity) and active (mounting of ewes) periods respectively from Castellana, Churra, and Assaf rams. The lower concentration of testosterone obtained from this study could be attributed to differences in breed and stage of maturity of Yankasa rams.

Similarly, the concentration of follicle-stimulating hormone (FSH) from the Yankasa rams fed a mixed ration of cowpea husks and tiger nuts residue is within the range of 0.2 to 2.0 ng/mL reported from previous studies (Olsen et al., 1985; Barnes et al., 1988; Schanbacher, 1989). There is a paucity of information on the concentration of FSH in growing Yankasa rams-fed cowpea husk and tiger nuts residue in a mixed ration.

LH obtained from this study is lower compared to the reports from Nagatani et al. (2000) who gave a range of 1.10 ng/mL to 5.60 ng/mL. They used gonadectomized post-pubertal Suffolk rams while growing Yankasa rams were used for this study. There is limited information on the concentration of LH in Yankasa rams-fed cowpea husk and tiger nuts residue.

Cortisol levels from this study were high compared to the results obtained by Hantzopoulou et al. (2022) who obtained a range of 0.72-2.3 ng/mL in Australian Merino lambs. The variation observed could be because cortisol levels were determined via blood serum from this study while cortisol levels were determined in the wool of Merino lambs, breed differences, and study location. Animals that are restrained could exhibit high levels of cortisol as reported by Carlos et al. (2016).

However, the current study showed significant differences in semen parameters and serum hormonal levels between the treatment groups. This could indicate that the type and quantity of the variable components in each treatment may have endocrine effects and subsequently affect spermatogenesis. For instance, T5 (high tiger nuts mixed ratio diet) treated groups had the highest serum LH, FSH, testosterone, and cortisol levels but had the lowest semen volume, pH, concentration, live cells, and normal cell morphology. Tiger nuts increase testosterone levels in male Wistar rats (Allouh et al., 2015).

Zhao et al. (2020) associated depressed semen characteristics with high levels of FSH, LH, and testosterone levels in men. Tiger nuts contain quercitin a phytoestrogen that induces a stimulatory effect on steroidogenesis (Chen et al., 2007). No wonder the high levels of testosterone and cortisol in the high tiger nut-mixed ratio treated rams when compared to levels in rams from other groups. It is interesting to note in the current study that

rams fed with a 40% cowpea husk rationed diet had the lowest testosterone levels but the best semen characteristics. These findings were contrary to the expectation that testosterone aids spermatogenesis and consequently lower levels would impair semen characteristics. Umapathy, (1992) showed that cowpea seeds decrease testosterone levels when fed to Wistar rats but cause epididymal dysfunction. The signs of epididymal dysfunction observed by Umapathy, (1992) may be due to anti-nutritional factors present in cowpea seeds but absent in the husks used for the current study.

Table 5. Effect of cowpea husk and tiger nuts residue in a mix ration on hormonal assay of Yankasa rams.

| | Treatment | | | | | |
|---|---|---|---|---|---|---|
| Parameter | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | SEM |
| FSH (ng/mL) | $0.28^{d}$ | $0.55^{b}$ | $0.41^{c}$ | $0.93^{a}$ | $0.96^{a}$ | 0.14 |
| LH (ng/mL) | $0.47^{c}$ | $0.42^{c}$ | $0.45^{c}$ | $1.15^{b}$ | $1.81^{a}$ | 0.27 |
| Testosterone (ng/mL) | $2.63^{a}$ | $2.43^{b}$ | $2.40^{b}$ | $2.50^{b}$ | $2.87^{a}$ | 0.09 |
| Cortisol (µg/dL) | $4.69^{d}$ | $4.50^{e}$ | $6.81^{b}$ | $5.32^{c}$ | $8.28^{a}$ | 0.72 |

Note: $^{a,b,c,d,e}$ Means within the same rows with different superscripts differed significantly ($p < 0.05$); FSH = Follicle Stimulating hormone; LH = Luteinizing hormone; SEM = Standard error of mean. Source: Authors, 2023.

## 4. Conclusions

It is concluded that there is no deleterious effect on the reproductive potential (semen characteristics and hormonal concentration) of growing Yankasa rams fed a mixed ration of cowpea husk and tiger nuts residue, hence, it is safe to feed breeding rams the ration mix.

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## 6. Authors' Contributions

*Ahmed Amin1 Njidda*: study design, study animals, research farm, writing, corrections, and publication. *Isaac Sammani Butswat*: corrections, and publication. *Hosea Yakubu:* study design, collection, and processing of cowpea husk and tiger nut residue, laboratory analysis, animal testing, data analysis, study writing, corrections, submission, and publication. *Ijeoma Chika Chibuogwu*: writing, corrections, and publication. *Abayomi Samuel Bankole:* corrections and publication.

## 7. Conflicts of Interest

No conflicts of interest.

## 8. Ethics Approval

Not applicable.

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