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EFFECT OF FERMENTEDCINNAMON JUICE EXTRACT ON RED SOKOTO BUCKS TESTICULAR HISTO-MORPHOLOGY AND SEXUAL ETHOGRAM

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ABSTRACT

This study was aimed to determine the effect of fermented cinnamon juice extract (FCJE) on Red SokotoBucks testicular histo-morphology and sexual ethogram.A total number of 24 apparently healthy pubertal red Sokoto buck was allotted into four treatments consists of 0, 15, 30 and 45 ml of FCJE in a completely randomized design (CRD), Duncan multiple range test were used to separate means between treatment. The animals were drenched for 8 weeks daily. Two bucks from each treatment were randomly selected and orchidectomized to obtained testes for testicular morphometry and histology determination. The result in this study suggested that bucks supplemented 45 ml FCJE has significantly ($P<0.05$) higher values of live weight, right testes weight, left testis weight, relative testis weight, right testis volume, left testis volume, and testes volume. Treatment 1 had the highest ($P<0.05$) testis density values. Non-significant ($P>0.05$) increases were observed in right testis length, left testis length, right testis width and left testis width. Considerable architectural changes were observed in the seminiferous tubules from cluster, smaller size to larger in size with increases dosage of FCJE. Similarly, wider lumens were observed in control group (T1) and T2 (15 ml FCJE). However, visible interstitial cells and wider interbular space were observed in T3 and T4 where some elongations of seminiferous tubules were prominently observed in both T3 and T4. It is therefore, concluded that supplementation of FCJE has a positive effect in changing testicular morphology and profound responses in altering testicular histological structures. However, further studies are highly suggested to validate this response and fully explore the physiological mechanisms involves for the aphrodisiac activities of fermented cinnamon juice extract observed in this study.

KEYWORDS

Fermented Cinnamon, Histo-Morphology, Bucks and Testicular

Introduction

Reproduction is central to the continued existence of animals on earth. In sexual animals, reproduction is important for both males and females for life to continue. Efficient reproduction is key to the success of any livestock enterprise (Gaddafi*et al.,* 2020). Several research efforts have been made targeted towards enhancing the reproductive performance of farm animals through scientific approaches involving some kind of genetic, nutritional and physiological manipulations or interventions (Herbert and Ukar, 2008; El-Azim and El-Kamash, 2011). The sexual behaviour of goat is an important factor for flock breeding efficiency and productivity of goat farming. Many researchers have derived different ways and means to evaluate the breeding quality of the buck. The potential fertility of breeding males can be evaluated in the field by assessment of physical examination through genital tract examination, mating ability and semen quality evaluation (Kerketta *et al.,* 2013).

Body and Testicular biometric parameters are very important for establishing reproductive patterns and consequently, the development of protocols for assisted reproduction in different species (Caldeira *et al.,* 2010). Reproductive organs are not unconditionally necessary for the individual life but they have essential role in the reproduction and genesis of species (Abreu and David-Ferreira, 1982). The knowledge of basic morphometric characteristics of the reproductive organs have been found to provide valuable information in the evaluation of breeding and fertility potential of the animals (Ogbuewu *et al.,* 2007). Togun and Egbunike reported that testes size is a good indicator of the present and future sperm production in animals. They further observed that the knowledge of basic morphometric characteristics of reproductive organs is of great value inbreeding soundness evaluation and potential fertility in breeding males. Also, Egbunike *et al.* (1976) reported that morphometric analysis on the testes of any species or breed is necessary in assessing and estimating qualitative changes in testicular component and spermatogenic functions. Similarly, Ibrahim et al. (2003) further described the mammalian testes as infallible predictors of spermatozoa production. On the other hand, the epididymis is an extremely convoluted structure, which is closely attached to the dorsal part of the lateral surface of the testes (Oyeyemi *et al.,* 2000).

Cinnamomum (family of *Lauraceae*) species, also known as "Girfa'' in hausa Language contain volatile oils, tannins, terpenoids, mucilage, oxalates and starch (Garba et al., 2022). Different chemical constituents of C. zeylanicumare known to have significant germicidal, antiulcerogenic and cytotoxic effects. In a study, the extract of cinnamomum increased the weight of testes, caudaepididymidies and seminal vesicles in the treated animal, indicating a possible stimulation of hormonal levels in the animals. Also, the sperm count and motility of the treated animals were significantly higher than the control group (Khojasteh*et al.,* 2016).

Traditional fermented foods contain a great number of probiotic microorganisms (Psani and Kotzekidou, 2006; Todorov *et al.,* 2008). Some fermented fruits extract contain live lactic acid bacteria, which they will enhance rumen activity, affect the gastrointestinal infections, help with lactose metabolism, decrease serum cholesterol, stimulate immune system, possess anti-mutagenic properties, anti-carcinogenic properties, anti-diarrheal properties, alleviate the inflammatory bowel disease symptoms, suppress the infection caused by Helicobacter pylori (Shah, 2007). Cinnamomum plant has diverse defence strategies in the fight against a huge variety of damaging biotic circumstance. It contains polyphenol compounds that are largely participate in many functions, such as colour, flavor, odour, bitterness, and astringency, and exhibit antioxidant properties that will help in scavenging, trapping, quenching and destroying the blood free radicals. Cinnamomum has long been consumed as a spice for flavoring foods as well as in traditional remedies. Recently, studies have

shown that cinnamomum bark has diverse bioactivities, including antioxidant, antimicrobial, anticancer, anti-inflammatory as well as pharmacological properties in the treatment of type II diabetes (Garba et al., 2022). Therefore, the aim of this study is to determine the effect of fermented cinnamon juice extract (FCJE) on Red Sokoto Bucks testicular histo-morphology and sexual ethogram.

Materials and Method

Experimental site

The experiment was conducted at Small Ruminant Animal Unit of Prof. Lawal Abdu Saulawa Livestock Teaching and Research Farm, Department of Animal Science, Federal University Dutsin-Ma, Katsina State, Nigeria.

Experimental Animal and Design

A total of twenty four (24) apparently healthy pubertal Red Sokoto bucks were allotted into four treatment (6 bucks per treatment) comprises four dosage level of fermented cinnomum juice extract consisting three replicate and each replicate consist two bucks in a completely randomized design (CRD). The animals were drenched orally with 0 mLs, 15 mls, 30 mls and 45 mls FCJE daily for a period of 3 weeks followed by reproductive behaviour trails by individually exposing buck with estrous doe for a period of 30 minutes.

Preparation of Fermented Juice Extract

Dry Cinnamom stem were obtained from Katsina central market. The stem was identified, sorted to remove inert materials and grinded using mortar and pestle. The powder material obtain will be grinded again using electric grinding machine to obtained fine particles. One (1 kg) of grinded powder was put in a bow and 1 Litre of molasses and 5 litres of water was added and mix together thoroughly using paddle. The mixture was poured into a plastic pail and tightly covered to assist anaerobic fermentation thereby preventing some air to get inside the plastic and was allow for fermentation for a period of one week (7 days). Fermented juice was obtained by straining mix through fine cheese cloth. The fermented extract was stored in plastic bottle under normal room temperature.

Sexual Ethogram

The video camera was mounted in the mating pen to identified copulatory behaviours. The sexual behaviour of Red Sokoto Buck was determined according to procedure outlined by Gaddafi *et al.*, (2020) with the presence of the partner, where a doe in estrous was identified, and brought near the mating pen for a limited time. Since the sampling rule is a behavioural sampling, therefore, estrous doe was brought to the breeding pen and occurrence of the following mating behaviours were recorded: vocalization, self-enurination, flehmen, anogenital sniffing, foreleg kicks, tongue flicking, chin resting, snorts, mounting without ejaculation and mounting and ejaculation. The choice for video camera was to enable the animal to exhibit mating behaviours naturally without observer distractions.

Table 1: Definition of Buck Reproductive Behaviours

Behaviours	Definition
Vocalization	Characterized by frequent louder bleating.
Self-enuranation	Downward turning of the head and shoulders towards the hindquarters and the emission of urine from the erect penis onto the face, beard and front legs.

Anogenital sniffs	Buck sniffs and licks the anal, vulva and other external genitelia.
Flehmen	When buck raise his head, curl his lip and wrinkled his nose.
Snorts	When buck forcibly produce exhalation sound/huffing.
Foreleg kicks	When buck kicked a doe`s back and shoulders with foreleg.
Nuzzling	When buck rub his nose to move the doe in cozy position.
Chin resting	Buck rises his forelegs and hanging his chest on the doe`s back.
Mounting without ejaculation	When buck chin resting properly, extrusion of penis without inserting into doe`s vagina.
Mounting with ejaculation	When resting, penis into vagina and ejaculation take place.
Non courtship behaviours	Walking, laying, feeding e.t.c.

Source: (Gaddafi et al., 2020)

Testicular Morphology

After experimental period (8 weeks), the buck scrotal circumference was measured prior to ochidectomy of the bucks using open castration technique. Open castration was carried out to measure the actual size and weight of the testes. The buck was physically restrained, lidocine was infused intradermaly, thereafter, 3cm long pre-scrotal incision was made. The underlying fascia was dissected bluntly and the left testis was forced out through the incision by the pressure over the scrotum. The tunica vaginalis was be cut though to expose the testis and isolate the spermatic cord. Three artery forceps was used to clamp the spermatic cord at three successive points leaving a small gap between each forceps. The spermatic cord was then ligated at the gaps between the forceps (double ligation) using chromic catgut size 1. Following the ligation the spermatic cord were transected at the outer most forceps (the closest to the testis). A double ligature was then place at the base of the gubernaculums testes after which it was also transected and the testis was removed. The second testes (right) was grasp and pushed towards the incision point, where it was further milked out through the incision. The tunica vaginalis were incised and the same procedure conducted for the left testis was repeated on the right. 1 ml of penicillin-streptomycin was infused into the scrotal sac. The testicles were examined grossly for abnormalities. Testicular weight and length was determined. After morphological data determination the testicular tissues was further preserved and fix in 10% formaline solution and transported histology laboratory from histological preparation of testicular tissue.

Result

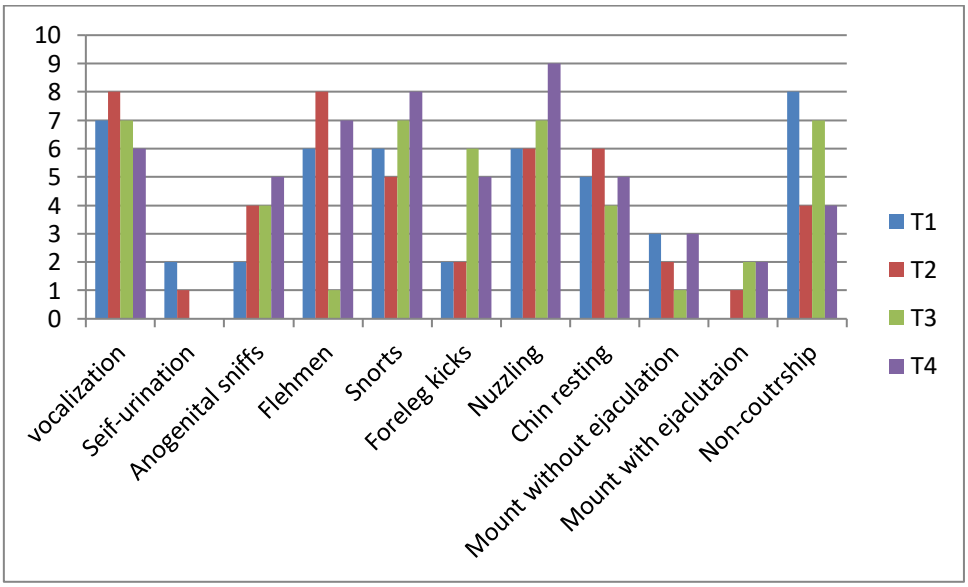

Figure 1:

Figure 2: Present s the duration (Min: Sec) of Red Sokoto bucks supplemented Fermented cinnamon juice extract. From the figure, treatment 2 shows the highest (5:21min) duration taken in exhibiting vocalization behaviour, this vocalization may serve as a stimulator for estrus. Vielma et al. (2005) states that buck vocalization may stimulate estrous in local anovulatory female goats during the anestrous period. While treatment 4 (3:59 min) was the lowest duration. Highest self-urination (3:5 min) was recorded in treatment 1 and self-enuration is more likely to occur when the buck van view, but not breed an estrous. Price et al. (1986) reported that self-enuration may occur when buck is separated from a female by a fence, or when the male is in the post-ejaculatory refractor period. While no self-urination behaviour was observed in both treatment 2 and 4.

Bucks supplemented 45 mls of FCJE spend long durations exhibiting Anogenital sniffing, flehmen reaction, nuzzling, chin resting and mount with ejaculation. Highest duration in snorts, foreleg kicks and non-courtship were observed in bucks supplemented 30 mls of fermented cinnamon juice extract.

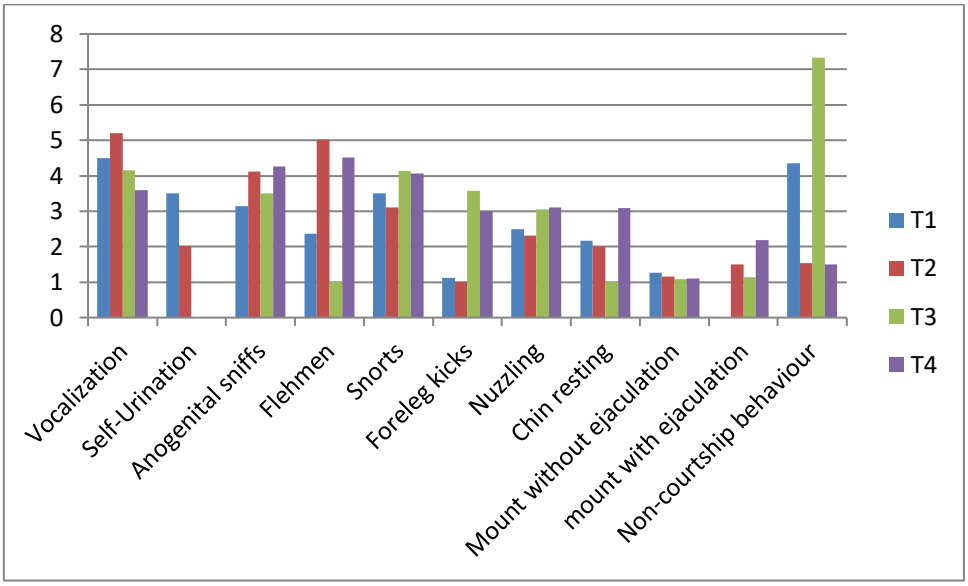

Figure 2: Duration (Min:Sec) of Reproductive behaviours of Red Sokoto bucks supplemented FCJE

Nuzzling the behaviour that assist buck to arranged does in a cozy corner this support the statement made by Gaddafi et al. (2020) that buck displayed nuzzling behaviour and spent more time to move and arrange the does in a comfortable and cozy position for mating and these may be attributed to mating inexperience nature of the does.

Table 1: Testicular morphometry of Red Sokoto buck supplemented fermented cinnamon juice extract

Parameters	T1	T2	T3	T4	SEM	LOS
Live weight (kg)	13.070^{c}	13.306^{b}	13.950^{b}	15.250^{a}	0.121	*
Right Testis Weight (g)	36.373^{c}	39.497^{bc}	44.447^{b}	56.103^{a}	1.018	*
Left Testis Weight (g)	40.403^{c}	43.970^{c}	49.217^{b}	59.880^{a}	0.781	*
Relative Testes Weight (g)	0.580^{c}	0.623^{bc}	0.670^{b}	0.760^{a}	0.011	*
Right Testis Volume (ml)	17.683^{c}	20.280^{b}	28.733^{b}	39.900^{a}	1.529	*
Left Testis Volume (ml)	14.900^{c}	27.633^{b}	34.033^{b}	44.190^{a}	1.707	*
Testes Volume (ml)	32.583^{d}	47.913^{c}	62.767^{b}	84.090^{a}	1.555	*
Testes Density	2.353^{a}	1.790^{b}	1.490^{b}	1.383^{b}	0.076	*
Right Testis Length (cm)	9.367^{a}	9.500^{a}	9.733^{a}	9.700^{a}	0.059	NS
Left Testis Length (cm)	9.467^{a}	9.500^{a}	9.567^{a}	9.600^{a}	0.036	NS
Right Testis Width (cm)	5.000^{a}	5.100^{a}	5.167^{a}	5.150^{a}	0.036	NS
Left Testis Width (cm)	5.133^{a}	5.100^{a}	5.133^{a}	5.167^{a}	0.024	NS

The result of testicular morphometry of Red Sokoto bucks supplemented fermented cinnamon juice extract was presented in table 1. The result revealed that treatment 4 (45 ml FCJE) had the highest Live weight (kg) followed by treatment T3, T2 and T1 was the lowest. The result also clearly indicates that there are significantly ($P<0.05$) linear increases with increase dosage of FCJE of Right testes weight (g) Left testis weight, relative testes weight, right testis volume (ml), left testis volume, and testes volume. Highest ($P<0.05$) Testes density was observed in control group (0 ml FCJE) followed by T2, T3 and T4 was the lowest. In this study the testicular morphometry variables of right testis length, left testis length, right testis width and left testis width revealed non-significant ($P>0.05$) difference.

From the testicular histology carried out in this study the testicular microphotographs of Red Sokoto bucks supplemented FCJE were presented from plate A to D. Considerable architectural changes were observed in the seminiferous tubules from cluster, smaller size to larger in size with increases dosage of FCJE. Similarly, wider lumen was observed in control group (T1) and T2 (15 mLs FCJE). However, visible interstitial cells and wider interbular space were observed in T3 and T4 where some elongations of seminiferous tubules were prominently observed in both T3 and T4.

Testicular Histology

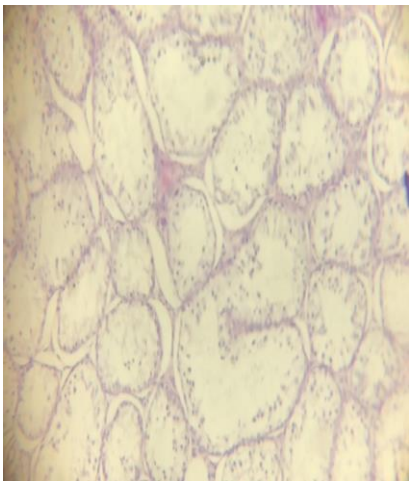

Plate A: Photomicrograph of testicular tissue of Red Sokoto Buck supplemented with 0 mL CFJE. The seminiferous tubules were cluster and majority are smaller in size with wider lumen. (Haematoxylin and Eosine staining x 10 magnification).

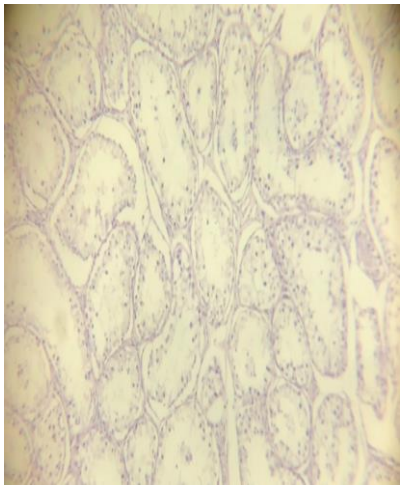

Plate B: Photomicrograph of testicular tissue of Red Sokoto Buck supplemented with 15 mLs CFJE. Smaller size seminiferous tubules with elongations. (Haematoxylin and Eosine staining x 10 magnification).

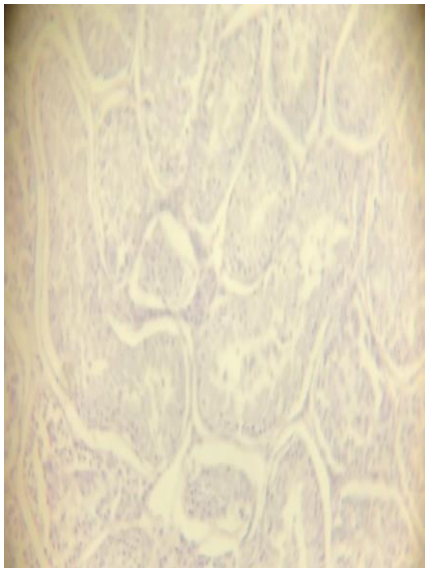

Plate C: Photomicrograph of testicular tissue of Red Sokoto Buck supplemented with 30 mLs CFJE. Visible interstitial cells, seminiferous tubules become larger with minimal lumen, pronounced enlongation of the tubules. (Haematoxylin and Eosine staining x 10 magnification).

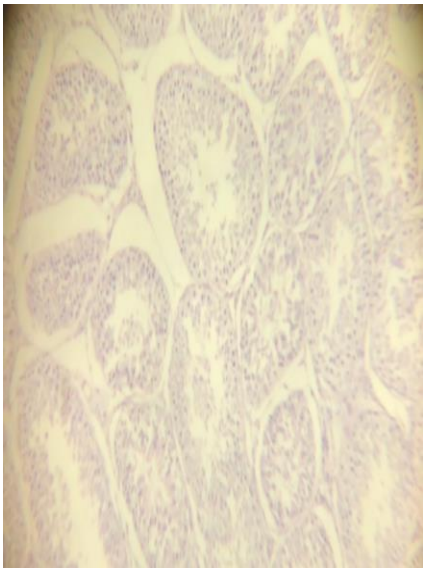

Plate D: Photomicrograph of testicular tissue of Red Sokoto Buck supplemented with 45 mLs CFJE. Wider intertubular space, dense and larger seminiferous tubules ready for division. (Haematoxylin and Eosine staining x 10 magnification).

Conclusion

Supplementation of FCJE has a positive effect in changing testicular morphology and profound responses in altering testicular histological structures. However, further studies are highly suggested to validate this response and fully explore the physiological mechanisms involves for the aphrodisiac activities of fermented cinnamon juice extract observed in this study.

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