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Research Article

Additive Effect of Maca (*Lepidium Meyenii*) Powder on Semen Characteristic and Testicular Histo-Morphology of Red Sokoto Bucks in Sudan Savanna Zone Nigeria

Garba MG, Gaddafi S* and Yahaya MA

Department of Animal Science Department, Federal University Dutsin-Ma, Katsina State, Nigeria

***Corresponding Author:** Gaddafi S, Department of Animal Science Department, Federal University Dutsin-Ma, Katsina State, Nigeria.

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Abstract

Reproduction is central to the continued existence of animal on earth. This study was designed to determine the additive effect of Maca powder on Testicular histo-morphology, semen kinetic and spermatozoa morphological defect of Red Sokoto bucks. A total of twenty-four (24) apparently healthy pubertal Red Sokoto bucks were allotted into four treatments (6 bucks per treatment) comprises four additive level of maca powdered consisting three replicate and each replicate consist two bucks in a completely randomized design (CRD). The animal were giving four different diet consist of 0g, 2g, 4g and 6g of maca powdered per kg diet. The experiment last for a period of 60days. The result of testicular morphometric in this study revealed a significantly ($P < 0.05$) higher values of right testis weight, left testis weight, relative testis weight, right testis volume, left testis volume and testes volume. Sperm cell kinetic in this study indicates significant ($P < 0.05$) difference in percentage motility, progressive motility, linearity, curvilinear velocity, straight line velocity and acrosome integrity. Considerable changes were also observed in seminiferous tubules of Red Sokoto bucks testicular tissue. It is therefore concluded that adding maca powder up to 6g/kg diet has a profound effect on testicular morphometry, sperm cell kinetic and increased percentage of normal sperm cell without deformity. Considerable changes of seminiferous tubules and insterstial cells are the architectural variables observed in this study.

Keywords: Bucks; Histo-Morphology; Maca; Semen

Introduction

Climate change refers to a change in climate which is attributed directly or indirectly to human activities and natural variability that alters the composition of the global atmosphere over a long period of time [1]. The variations in climate parameters affect different sectors of the economy, such as agriculture, livestock production, heath, water, energy, e.t.c. According to Intergovernmental Panel on Climate Change, Africa is one of the most vulnerable continents to climate change and climate variability [1]. In mammals, global warming causes significant increases in body temperature above the physiological homoeothermic point (hyperthermia) with consequent organic suffering (heat stress) that leads to impaired physiological and reproductive activities [2]. Heat stress has become one of the most important factors affecting the reproductive performance of mammals. In male animal, excessive heat affects spermatogenesis in the testis, reduces the quality and quantity of

sperm, and even causes infertility. The testes contain different cell types, heat stress has different effects on these cells such as apoptosis, DNA damage, blood-testis barrier (BTB) disruption, and hormone secretion disorders [3].

Hormones and other industrially synthetic medical therapy have long been employed to reduce the adverse effect of heat stress in livestock reproductive performance. However, unwanted side effects or fear of adverse risks of hormone therapy to enhance animal reproduction makes livestock farmers so reluctant to continue and/or commence hormone therapy [4]. Complementary and alternative therapies [5,6] are currently available and claim to provide a wide array of benefits for reproductive enhancement, among such alternative means are the use of maca plant (*Lepium meyenii*) which is widely used for its putative fertility-enhancing and aphrodisiac properties [7,8].

Maca is a vegetable root native to Peru. While its scientific name is *Lepidium meyenii* and also known in Hausa language as "*Sauyar Maca*" or "*Albasar tamoji*" while Yaroba Langauge it known as ''*Isu baka*''. Maca (*Lepidium meyenii*) has been used traditionally for centuries as a medicinal plant with variety of biological function such as tonic, fertility enhancer [9,10], involves in treating ailments such as rheumatism, respiratory disorders, anaemia, promoting mental clarity [11], treating chronic fatique syndrome in both human and animals, it also used as an alternative to anabolic steroids by bodybuilders due to its richness in sterols among others. Maca is also used as an immunostimulant, for anaemia, tuberculosis, menstrual disorders, menopause symptoms, stomach cancer, sterility and other reproductive and sexual disorders as well as to enhance memory [12]. This study was designed to determine the additive effect of Maca powder on Testicular histo-morphology, semen kinetic and spermatozoa morphological defect of Red Sokoto bucks.

Materials and Methods

Experimental site

The study was carried out in the ruminant unit, Prof. Lawal Abdu Saulawa, Livestock Teaching and Research Farm, Department of Animal Science, Federal University Dutsin-ma. The site lies between latitude 12°27'18' North and 7°29'29' East and 605 meters above sea level with an average rainfall of 700mm within the Sudan Savannah zone [13].

Experimental animals and management

A total of twenty-four (24) pubertal Red Sokoto bucks were procured from Dutsin-Ma Wednesday market. The animals were given a prophylactic treatment (Broad spectrum Antibiotic and Albendazole as dewormer against possible infection and parasites infestation) prior to the commencement of the experiment during 14 days period of acclimatization.

Experimental designed

A total of twenty-four (24) apparently healthy pubertal Red Sokoto bucks were allotted into four treatments (6 bucks per treatment) comprises four additive level of maca powdered consisting three replicate and each replicate consist two bucks in a completely randomized design (CRD). The animal were giving four different diet consist of 0g, 2g, 4g and 6g of maca powdered per kg diet. The experiment last for a period of 60days

Semen collection

Semen samples used in this study were collected from the buck weekly for a period of four weeks consecutively using electro-ejaculator techniques as described by Oyeyemi., *et al.* [14] with little modification. Prior to the semen collection preliminary training were given to the bucks. The animals were gently restrained, the bucks were placed on its side and the penis was extended from the sheath by stretching the sigmoid flexure. The penis was than grasped with the sterile gauze and the gland penis was diverted into a 50ml disposable tube that was insulated by the hand of the collection technician. The animals were gently massaged the accessory glands by exerting a downward pressure on the bottom of the rectum for 10 to 15 second prior to the inserting electro-ejaculator. The lubricated electro-ejaculator probe was than inserted into the rectum of the animal and turned on were the voltage increased manually for three to eight seconds and then the animal is allowed to rest for 15-20 seconds and repeated till ejaculation. After ejaculation, the semen was covered to maintain its temperature and taken to the laboratory for processing.

Semen morphological traits

These are traits on a deformed sperm cell making it abnormal in its structure as opposed to normal sperm cell. The sperm cells abnormalities to be evaluated include:

- **Acrosome swelling:** This is a swelling on the acrosome which is located at the head region of the sperm cell.
- **Detached head:** This is the head of a sperm cell which has been separated from other parts of a complete sperm cell.
- **Bent tail:** The bent tail is a tail type found on a sperm cell with its tail bent to direction.
- **Coiled tail:** A coiled tail is a tail types found on a sperm cell with its tail coiled round.
- **Full tail:** A full tail is that tail which has been detached completely from the body and head of a sperm cell. This morphological defect amongst other types has been evaluated by Tabatabaei., *et al.* (2009) [15]. The determination of these defects will be done on smeared slides prepared from raw semen and stained by Giemsa stain according to Watson (1975) [16]. The defects sperm cells will be counted using a hand tally counter a microscope.

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 intradermally, thereafter, three (3) cm 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 for histological preparation of testicular tissue. The testicles were fixation, dehydration, clearing, embedding and sectioning, and staining will be done appropriately according to procedure. After that the slide will be viewed at x 10 magnification.

Statistical analysis

Data obtained in this study for testicular morphology, semen kinetic and semen morphology were analysed using General Linear Model of Statistical Analysis System (SAS) [17] Software, were treatment means were separated using Duncan Multiple Range Test of the same software.

Result and Discussion

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 [18]. Togun and Egbunike they future observed that the knowledge of basic morphometric characteristics of reproductive organs is of great value inbreeding soundness evaluation and potential fertility in breeding males [19]. The additive effect of maca powdered on testicular morphometry of Red Sokoto bucks were presented in table 1. The result revealed a significantly higher ($P < 0.05$) values of Live weight (kg). There are linear ($P < 0.05$) increases in live weight of Red Sokoto bucks with increases inclusion level of maca powdered. Similar trend ($P < 0.05$) of linear increases in gonadal morphometry was observed in right testis weight left testis weight, relative testes weight, right testis volume, left testis volume and testes volume. The testes size and weight increases in this study would be a good future and attributes for better spermatogenesis and expression of good libido. Togun and Egbunike reported that testes size is a good indicator of the present and future sperm production in animal [19].

Sperm cell kinetic of red Sokoto buck fed maca powdered

Sperm motility is an important feature for spermatozoa to be capable of reaching and fertilizing the oocyte. Sperm motility analysis is an essential parameter for evaluation of sperm quality due to the close correlation it has with other parameters such as

Parameters	T1	T2	T3	T4	SEM	LOS
Live weight (kg)	13.080^{c}	13.3186^{b}	13.965^{b}	15.320^{a}	0.122	*
Right Testis Weight (g)	36.380^{c}	39.500^{bc}	44.447^{b}	56.213^{a}	1.028	*
Left Testis Weight (g)	40.413^{c}	43.980^{c}	49.317^{b}	59.890^{a}	0.786	*
Relative Testes Weight (g)	0.580^{c}	0.623^{bc}	0.670^{b}	0.760^{a}	0.011	*
Right Testis Length (cm)	9.380^{a}	9.520^{a}	9.740^{a}	9.720^{a}	0.060	NS
Left Testis Length (cm)	9.597^{a}	9.600^{a}	9.57^{a}	9.610^{a}	0.036	NS
Right Testis Volume (ml)	17.683^{c}	20.280^{b}	28.733^{b}	39.900^{a}	1.529^{c}	*
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	*

Table 1: Testicular morphometry of Red Sokoto buck fed additive varying level of Maca during hot season of Sudan Savanna zone, Nigeria.

morphology and membrane integrity and thus fertilization [20]. The sperm cell kinetic in this study revealed a profound ($P < 0.05$) effect of maca on Red Sokoto buck sperm cell percentage motility (%), progressive motility (%), Linearity (%), curvilinear velocity (µm/s), straight line velocity (µm/s), liveability (%) and acrosomal integrity (%) they all shows increased trend with increased dosage of maca powder in this study. This study were inconformity with Clement., *et al.* [21] who's stated that in pre-pubertal breeding bulls fed a mix of all three dried maca phenotypes at 233 mg/kg body weight, an increase in sperm motility was noted with an increase in ejaculation volume. Similarly, Gonzales., *et al.* [22] reported that black maca aqueous extract in rats, showed a significant improvement in sperm motility after 42 days of treatment. while this study contradict chung`s., *et al.* [23] study whose reported that rats treated with yellow maca aqueous extract has no effect on sperm motility. This significant ($P < 0.05$) increases in sperm cell kinetic may be attributed to the protective role of maca on spermatogenesis [24], ability to improve gonadal-androgen synthesis pathway [25], semen antioxidant properties and others.

Parameters	T1	T2	T3	T4	SEM	LOS
Percentage motility (%)	39.76^{c}	51.96^{b}	62.40^{a}	64.65^{a}	1.698	*
Non-Progressive motility (%)	4.25^{a}	2.35^{bc}	2.70^{b}	1.56^{c}	0.268	*
Progressive motility (%)	34.96^{c}	49.90^{b}	59.80^{a}	61.45^{a}	2.424	*
Linearity (%)	63.58^{b}	68.60^{ab}	71.15^{a}	74.97^{a}	2.424	*
Curvilinear velocity (µm/s)	3.64^{b}	3.87^{b}	4.19^{ab}	4.60^{a}	0.141	*
Straight line velocity (µm/s)	2.21^{c}	2.47^{a}	2.35^{b}	2.50^{a}	0.031	*
Straightness (%)	70.20^{a}	71.60^{a}	67.95^{a}	71.80^{a}	2.286	NS
Liveability (%)	75.15^{a}	80.62^{a}	82.25^{a}	90.02^{a}	3.006	NS
Acrosome Integrity (%)	81.10^{b}	91.30^{a}	91.53^{a}	94.58^{a}	0.960	*

Table 2: Spermatozoa kinetic of Red Sokoto Buck fed Maca Powdered during hot season of Sudan Savanna zone Nigeria.

Sperm cell morphological defect of red Sokoto buck fed maca powdered during hot season of Sudan savanna zone Nigeria

Mammalian semen samples contain spermatozoa with different kinds of malformation; therefore it is essential to evaluate semen morphological defect. Defective spermatogenesis and some epididymal pathologies are commonly associated with an increased percentage of spermatozoa with abnormal shapes. Abnormal spermatozoa generally have a lower fertilizing potential, depending on the types of anomalies, and may have abnormal DNA [26]. Sperm cell morphological characteristics of Red Sokoto bucks fed additive lev-

els of maca powdered in table 3. Mammalian semen samples contain spermatozoa with different kinds of malformations [26]. There are significant ($P < 0.05$) differences in normal sperm cell with increased level of maca additives, T4 had the highest normal sperm cell percentage without morphological defect. This result clear supports the aphrodisiac properties of maca through improvement of normal sperm cell percentages. This study revealed that T1 had the highest ($P < 0.05$) bent tail (20.50%) and detached head (24.80%) sperm cell deformity. High ($P < 0.05$) of proximal cytoplasmic droplets and coiled tail deformity was observed in T2. The deformity in the membrane-bound vesicle on the midpiece at the head-neck junction (Cytoplasmic droplets) in T2 were not clearly understood, however, this condition is produced from a defective spermatogenic process, which characterized by large amount of irregular stained cytoplasm, one third or more of the sperm head size as stated by Cooper, (2005) [27]. The reason for Low cytoplasmic droplets in this study is because cytoplasmic droplets are osmotically sensitive since they are not well preserved by routine air-drying procedures as stated by Chantler and Abraham-Peskir, (2004) [28]. There were no significant ($P > 0.05$) differences in coiled tail and micro-cephalic sperm cell deformity in this study, although there are numerical variation exist between treatment.

Parameters (%)	T1	T2	T3	T4	SEM	LOS
Normal	50.10^{b}	68.40^{a}	82.00^{a}	88.30^{a}	3.69	*
Bent tail	20.50^{b}	10.10^{a}	6.30^{b}	4.10^{c}	0.91	*
Detached head	24.80^{a}	13.40^{b}	4.00^{c}	1.20^{d}	0.31	*
Proximal cytoplasmic droplets	0.10^{b}	0.60^{a}	0.00^{b}	0.00^{b}	0.01	*
Coiled tail	4.00^{a}	6.50^{a}	5.50^{a}	3.40^{a}	1.20	NS
Micro-cephalic	0.50^{a}	1.00^{a}	2.20^{a}	3.00^{a}	0.02	NS

Table 3: Morphological Characteristics of sperm cell of Red Sokoto Bucks fed varying additive levels of Maca during hot season of Sudan Savanna zone Nigeria.
SEM: Standard Error Means, LOS: Level of Significance

Testicular Histology of Red Sokoto bucks fed varying additive levels of Maca during hot season of Sudan Savanna zone Nigeria

The testicular histology of Red Sokoto buck fed maca powdered was presented in figure 1 to 4. There are considerable positive changes in seminiferous tubules density, size, morphological shapes and appearance from figure 1 to 4. About 80% of the testicular microscopic structures we obtained in this study were covered by seminiferous tubules which consists convoluted structures such as spermatogenic cells and supporting sertoli cells and between the tubules are the interstial or leydigs cells which produce the male sex hormone. The density and enlargement of the tubular regions in this study is clearly indicates the normal testicular exocrine and endocrine physiological activities. These are sperm production and secretion of the male hormone (androgen).

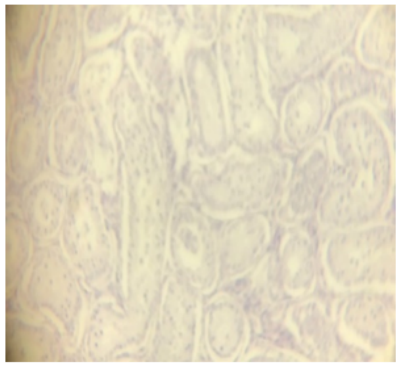

Figure 1: Seminiferous tubules are many, smaller in size and cluster. (Haematoxylin and Eosine staining x 10 magnification).

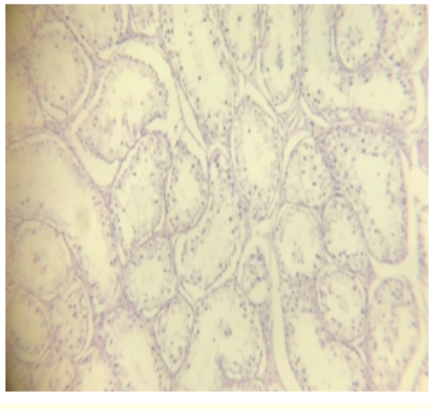

Figure 2: Cluster seminiferous tubules with a larger lumen. (Haematoxylin and Eosine staining x 10 magnification).

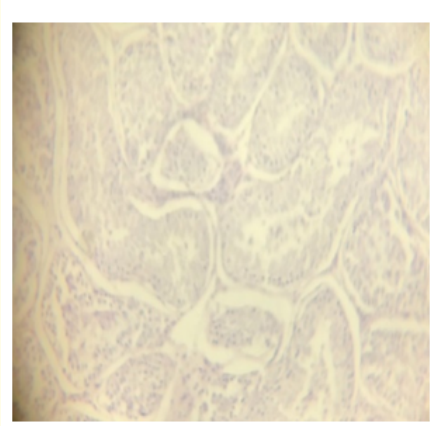

Figure 3: Elongations and larger size seminiferous tubules and visible interstitial cells. (Haematoxylin and Eosine staining x 10 magnification).

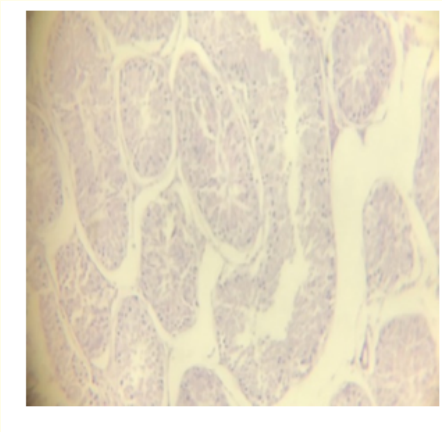

Figure 4: wider lumen and intertubular spaces, elongation of seminiferous tubules. (Haematoxylin and Eosine staining x 10 magnification).

Conclusion

It could be concluded that adding maca powder up to 6g/kg diet has a profound effect on testicular morphometry, sperm cell kinetic and increased percentage of normal sperm cell without deformity. Considerable changes of seminiferous tubules and insterstial cells are the architectural variables observed in this study.

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