Nigerian J. Anim. Sci. 2026, 28 (1): 39-47

Biopotency of Maca (*Lepidium meyenii* walp) powder additive on reproductive hormones, semen quality and kinetic characteristics of Yankasa rams during dry hot season

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Abstract

The study evaluated the biopotency of lepidium meyenii (Maca) powder supplementation on reproductive hormones, semen quality, and sperm kinetic characteristics of Yankasa rams during the hot-dry season of Sudan Savannah zone Nigeria. Twenty pubertal Yankasa rams of similar age and weight were randomly assigned to four dietary treatments containing 0, 5, 10 and 15g/kg maca powder in a completely randomized design. The experiment lasted four months, during which semen and blood samples were collected for analysis of reproductive hormones (testosterone, follicle-stimulating hormones (FSH) and Luteinizing hormone (LH)), semen characteristics and sperm kinetics. Results revealed significant ($P < 0.05$) increases in testosterone, FSH and LH concentrations with increasing levels of maca supplementation. Semen quality parameters such as sperm motility, and percentage of normal cells improved ($P < 0.05$) significantly, while sperm abnormalities and dead sperm cells decreased with higher maca inclusion. Furthermore, sperm kinetic parameters including progressive motility, linearity, and curvilinear velocity were significantly ($P < 0.05$) enhanced in supplemented groups. However, semen volume, pH, and some velocity parameters showed no significant ($P > 0.05$) differences. The findings suggest that maca supplementation, particularly at 15 g/kg, effectively enhances reproductive performance of Yankasa rams under heat stress conditions, likely due to its antioxidant and endocrine-modulating properties. It is therefore recommended that maca powder could be included in the diets of livestock from 5g up to 15g/kg diet as it is safe and improves animal reproductive performance through improved semen quality characteristics, semen kinetics and reproductive hormones. Therefore, farmers and livestock nutritionists can utilize it in livestock production as natural feed additives and aphrodisiac and reproductive enhancer without deleterious effect.

Key words: Maca, Yankasa rams, Hormones, Semen, Kinetic

Description of the Problem

Seasonal heat stress is a major constraint to small ruminant productivity in tropical and subtropical regions, particularly during the hot-dry season (1). Elevated ambient temperatures, low relative humidity, and intense solar radiation negatively affect physiological homeostasis, endocrine function, and reproductive performance of breeding males. In rams, heat stress has been associated with impaired spermatogenesis, altered reproductive hormone secretion, reduced semen quality, and compromised sperm motility and kinetics, ultimately leading to decreased fertility and flock reproductive efficiency (2). Indigenous breeds such as Yankasa rams, although relatively adapted to harsh climatic conditions, are not immune to the deleterious effects of prolonged thermal stress during the hot-dry season.

Reproductive hormones, especially testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), play crucial role in the regulation of spermatogenesis, libido, and semen quality. Heat stress disrupts the hypothalamic-pituitary-gonadal axis, resulting in hormonal imbalances that adversely affect testicular function (3). Additionally, exposure to high environmental

temperatures increase oxidative stress within the testes, leading to lipid peroxidation of sperm membranes and subsequent deterioration of semen quality characteristics, including sperm concentration, motility, viability and morphology. Advanced semen kinetic parameters, which provide detailed information on sperm movement patterns and fertilizing potential, are particularly sensitive indicators of thermal stress.

Phytogenic feed additives intervention has gained considerable attention as a sustainable strategy to mitigate heat-induced reproductive dysfunction in livestock. Among these, *Lepidium meyenii* Walp., commonly known as Maca, has been traditionally used as fertility-enhancing agent (4). Maca powder is rich in bioactive compounds such as *Macamides*, *macaenes*, flavonoids, alkaloids, sterols and essential minerals, which have been reported to possess antioxidant, adaptogenic, and endocrine-modulating properties. Several scientific evidences have demonstrated the potential of maca supplementation to enhance libido, improve semen quality. Modulate reproductive hormone profiles without exerting adverse hormonal overstimulation (5).

Despite increasing interest in the reproductive benefits of maca, limited information is available on its effects in ruminant species, particularly under conditions of environmental heat stress. Therefore, this study was designed to evaluate the effect of dietary supplementation of maca powder on reproductive hormone profiles, semen quality characteristics, and sperm kinetic parameters of Yankasa rams raised during hot-dry season.

Materials and Methods

Experimental site

The experiment was carried out at Prof. Lawal Abdu Saulawa Livestock Teaching and Research, Small Ruminant Unit of Livestock Teaching and Research Farm, Department of Animal Science, Federal University Dutsin-Ma, Katsina State. The site lies between latitude 12º27`18`N and 7º29`29`E and 605 meters above sea level with an annual average rainfall of 700mm (6).

Experimental animals and design

A total of twenty (20) pubertal Yankasa rams of the same weight and age was divided into four treatment groups of 0, 5, 10, 15 g/ Kg powdered maca with five (5) rams each per treatment in a completely randomized design (CRD). The rams were allowed to acclimatized for two weeks during which the prophylactic treatment of broad-spectrum antibiotics against infection and deworming using albendazole against endo-parasites. Feed and water were giving *ad-libitum*.

Experimental feed preparation

All the feed materials that were used in the experimental diets' preparation was purchased from selling and processing centres in Dutsin-Ma. Maize and cotton seed cake was ground and packed in sacks for experimental diets compounding. Whereas groundnut hay was chopped before mixing, other feed ingredients such as wheat offal, maize bran, bone meal, and table salt were purchased from the different centers in Dutsin-Ma town.

Diet was formulated to meet the dietary requirements for breeder rams according to dietary recommendation of NRC (7) for tropical rams.

Preparation of Maca Powder

Fresh maca was procured from herbal vendor in Dutsin-Ma Market, the maca was washed by tap water, and the fibrous roots was separated from the top. the roots were sliced to 2-cm-thick pieces and sun dried for 72 hours at Animal Science Laboratory, Department of Animal Science, Federal University Dutsin-Ma to the moisture content of 6-9%. The maca slices were

ground into powders, using laboratory mortar and pastel. Thereafter the ground maca were sieved through 1-mm wire-mesh, and stored at -20ºC before use.

Data collection

Semen collection

Prior to semen collection, the rams were given preliminary training for semen collection. This was done a week before commencement of semen evaluation. Thereafter, three (3) rams from each treatment were chosen randomly for semen collection and semen ejaculates were collected into a calibrated test tube and labelled accordingly. Electro-ejaculation semen collection was done in the morning (between 7:00 a.m. – 10:00 a.m.) once every three weeks for a period of four months. Semen samples collected was taken to the laboratory for evaluation in a warm water bath.

Semen characteristics and morphological defect determination

The semen colour, volume and pH were taken immediately after collection and recorded. Semen smear was prepared on a glass slide to determine the motility of the semen using a field microscope at magnification of x100 then stained with eosin nigrosine. It was air dried to determine the semen live and dead ratio along with the sperm morphological defects. , Another smear was made on a different glass slide to evaluate semen concentration. Evaluation of the semen quality characteristics was done in the fertility laboratory of the Artificial Insemination Unit National Animal Production Research Institute, Shika-Zaria.

Semen colour: Semen colour was scored by visual assessment based on the appearance into 1, 2 and 3 for (creamy, milky and watery respectively) which should be devoid of pus or blood as described by (8).

Sperm Volume: Ejaculate volume was measured to the nearest 0.01 ml in 5 ml tube a method described by (9) after the used of artificial vagina ending up in ejaculation of semen and the collection in a calibrated tube.

Semen pH: Initial semen pH was obtained by means of comparative pH paper as described by (10). The pH paper is calibrated from 1 to 14 with different colours. One inch of the paper was inserted into each sample of the semen then it was removed and checked for the colour match on the pH paper chart.

Sperm motility: Sperm motility was scored according to an arbitrary scheme of classification which ranges from 1 – 5 grades (immotile spermatozoa, stationary bunting, oscillatory movement, vigorous progressive movement and very vigorous forward motion respectively) according to the method described by (11). The wave pattern of semen was observed through a light microscope.

Sperm concentration: Sperm concentration ($x10^9$) was measured by Thomas-Zeis haemocytometer for counting the sperms per cubic millimeter (12) and multiplying by a dilution factor.

Live/dead sperm ratio: The stained slides with pigments of eosin and nigrosine solution technique were used to calculate the total percentages of live sperm in the sample (9). The dead sperm cells take up the stain while the live once remains unstained.

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 flagella 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 (13). The determination of these defects was done on smeared slides prepared from raw semen and stained by Giemsa stain according to (14). The defects sperm cells were counted using a hand tally counter a microscope.

Results and Discussion

Effect of Maca Supplementation on Reproductive Hormones of Yankasa Rams

The result of the effect of maca supplementation on plasma concentration of reproductive hormones of Yankasa rams were presented in Table 1. The result clearly showed that there were linear increases in plasma testosterone with increasing levels of maca in the experimental diet. Rams in T4 had the highest testosterone level of 7.855 ng/ml, followed by 5.385, 4.105 and 3.800 ng/ml for T3, T2 and T1, respectively. Testosterone plays a crucial role in the reproductive and overall physiological functioning of rams such as development of male reproductive organs as it is responsible for growth and development of the testes, penis and accessory sex glands during fetal development and puberty. Testosterone is involved in spermatogenesis through stimulating and maintaining the process of sperm production in the seminiferous tubules of the testes where works in conjunction with follicle-stimulating hormone (15).

Testosterone promotes sexual desire and mating behaviour in rams, making them more active and responsive to estrous ewes. Testosterone also influences secondary sexual characteristics such as muscle mass and thicker necks (16).

The result revealed that there were significant ($P < 0.05$) differences in follicle stimulating hormone (FSH), where T4 had 3.715 ng/ml, followed by T3 (2.935 ng/ml), T2 (1.00 ng/ml) and T1 (0.670 ng/ml). The FSH is a gonadotropin hormone secreted by anterior pituitary gland and works in conjunction with luteinizing hormone and testosterone to regulate male fertility. It stimulates spermatogenesis by acting on sertoli cells in the seminiferous tubules of the testes and promotes the production and maturation of sperm cells, a process essential for fertility (17).

The FSH enhances sertoli cells activity through the production of androgen-binding protein (ABP) which helps concentrate testosterone in the testes for optimal spermatogenesis. During puberty, FSH contributes to the development of the seminiferous tubules and onset of sperm production. FSH regulation is by feedback mechanism because its secretions are regulated by inhibin a hormone produced by sertoli cells. When sperm production is sufficient, inhibin levels rise and signal the pituitary to reduce FSH secretion (18).

The result further revealed significant ($P < 0.05$) differences in luteinizing hormones (LH). The T3 had the highest LH values of 4.385 ng/ml, T4 (3.925 ng/ml), T2 (3.560 ng/ml) and T1 (2.850 ng/ml). LH is secreted by the anterior pituitary gland in response to gonadotropin-releasing hormone (GnRH) from the hypothalamus. The LH acts on the leydig cells (also called interstitial cells) located in the testes to stimulate production, secretion of

testosterone and indirectly supports spermatogenesis (19).

Table 1: Effect of maca supplementation on reproductive hormones of Yankasa rams

Parameters (ng/ml)	T1	T2	T3	T4	SEM	LOS
Testosterone	3.800^{c}	4.105^{c}	5.385^{b}	7.855^{a}	0.401	*
FSH	0.670^{c}	1.000^{c}	2.935^{b}	3.715^{a}	0.206	*
LH	2.850^{b}	3.560^{ab}	4.385^{a}	3.925^{ab}	0.389	*

^{abc} = means with different superscripts across the row differ significantly, *= significant at 5% level of significance (0.05), FSH = Follicle stimulating hormone, LH= luteinizing hormone, SEM = Standard error of mean, LOS = Level of significance.

Semen Characteristics of Yankasa Rams Supplemented with Maca Powder

The result of the effect of maca supplementation on Yankasa rams' semen characteristics were presented in Table 2. Semen colour is an important parameter in semen analysis, providing insights into male reproductive health and potential underlying pathologies (20). It was reported that normal, freshly ejaculated semen has a creamy to milky white colour. The creamy colour observed in maca supplemented diet group could be due to riboflavin content which is secreted from ampulla or seminal vesicles. No abnormal semen colour was recorded in the study and this signifies the rams were absent from genital infection, injuries or inflammation. Because infection with *Pseudomonas aerogenosa* causes yellowish green semen, dark red or pink semen that occurs due to urethra or penis injury while curdy and chunk clots semen occur due to genitalia inflammation (20). The result revealed that there were no significant ($P > 0.05$) differences in semen volume and pH. Despite non-significant ($P > 0.05$) differences the semen volume and pH fall within the normal semen parameters of mature ram as reported by (21) semen volume ranges from 0.5 – 1.5ml while pH ranges from 5.9 – 7.3 in mature ram.

Sperm motility is crucial for successful fertilization, as it determines the ability of sperm to reach and penetrate the ovum. Significantly ($P < 0.05$) higher sperm cell motility was recorded in rams fed diet T4 with 76.330 %, followed by T3 (73.670 %), T2 (73.000 %) while T1 sperm cells had lesser motility (65.000 %). The study clearly indicates that maca plays a significant role in enhancing sperm motility percentage and this is in conformity with earlier scientific findings in rams that found out that maca-fed animals had higher percentages of motile and progressively motile sperm compared to control group (22). The result also corroborates with other findings in canine (dogs) as reported by (23) who reported significant improvement in sperm count and progressive motility.

The result revealed that there were significant ($P < 0.05$) differences in sperm cell concentration. The result has demonstrated linear increases in sperm cell concentrations with increasing supplementation levels of maca where T4 significantly ($P < 0.05$) had higher concentration values ($281.00x10^{6}$/ml) followed by $280.00x10^{6}$/ml, $251.00x10^{6}$/ml and $185.00x10^{6}$/ml for T3, T2 and T1, respectively. These findings are in accondance with that of Lavana (22) who stated that rams fed maca exhibit significantly higher sperm concentrations, possibly due to enhanced Leydig cell activity. Another study in bulls, indicated that maca supplementation increases sperm concentration in bull, likely due to its effect on testicular function and hormonal regulation.

Sperm cells deformity is the poor-quality characteristics of ejaculate. The study had

demonstrated that T1 had ($P < 0.05$) higher spermatozoa deformity (27.67 %) while groups supplemented with maca diets have demonstrated decreasing sperm cells defect with increasing maca level. The percentage normal cells were significantly ($P < 0.05$) different, where T4 had the higher (84 %) normal cell percentage followed by 79.67 %, 78 % and 72.33 % for T3, T2 and T1, respectively. Higher livability of 91.67 % was recorded in T4 followed by T3 (86%), T2 (84.33%) and83% for T1. Sperm livability is the measure that helps to determine the ability of sperm cells to survive and remain functional. This depends on precise environmental conditions; intact structural components and energetic capacity (24). The results further demonstrate that with increased maca supplementation there were decreasing percentage of dead sperm cells in which T4 had the lowest value (8.33 %) followed by T3 (14 %), T2 (15.67 %) while T1 had the highest dead cell percentage (17 %). There were no significant ($P > 0.05$) differences in reaction time.

Table 2: Semen Characteristics of Yankasa Rams Supplemented with Maca Powder

Parameters	T1	T2	T3	T4	SEM	LOS
Semen colour	Milky	Creamy	Creamy	creamy	-	-
Sperm volume (mL)	0.883	0.893	0.967	0.950	0.213	NS
Semen pH	6.567	6.567	6.583	6.500	0.288	NS
Sperm motility (%)	65.000^{b}	73.000^{a}	73.670^{a}	76.330^{a}	3.280	*
Sperm concentration ($x10^{6}$)	185.00^{c}	251.00^{b}	280.00^{ab}	281.00^{a}	24.82	*
Defect (%)	27.67^{a}	22.00^{ab}	20.33^{ab}	16.00^{b}	3.21	*
Normal cell (%)	72.33^{b}	78.00^{ab}	79.67^{ab}	84.00^{a}	3.21	*
Liveability (%)	83.00^{b}	84.33^{ab}	86.00^{ab}	91.67^{a}	3.28	*
Dead (%)	17.00^{a}	15.67^{ab}	14.00^{ab}	8.33^{b}	3.28	*
Reaction time	2.67	3.00	3.00	2.33	0.52	NS

^{abc} = means with different superscripts across the row differ significantly, *= significant at 5% level of significancy (0.05), SEM = Standard error of mean, LOS = Level of significance , NS = Not significant

Effect of Maca Supplementation on Sperm Kinetic of Yankasa Rams

The result of the effect of maca supplementation on sperm kinetic characteristics of Yankasa rams were presented in Table 3. The result revealed that there were significant ($P < 0.05$) differences in percentage motility where T4 had the highest sperm cell motility (76.33 %) followed by T3 (73.67 %), and then T2 (73.00 %) while T1 (65.00 %) had the lowest percentage motility. Non-progressive motility values were significantly ($P < 0.05$) higher in T1 (21.00 %) followed by T2 (14.33 %) while T4 had the lowest non-progressive motility (3.67 %). The percentage semen progressive motility values were significantly ($P<0.05$) higher in rams supplemented with maca, where T4 had 41.67 % progressive motile sperm cells followed by T3 (38.33 %), T2 (32.67 %) while lower value was recorded in T1 (29.33 %). The study suggests that rams in T4 and T3 may likely have sperm cells that will have higher cervical mucus penetration ability compared to other groups. Suarez and Pacey (25) stated that progressive motility is essential for sperm to traverse the cervical mucus while abnormal motility leads to poor mucus penetration and reduced fertility.

Mortimer (26) suggested that sperm cells with higher linearity greatly have straighter movement. Therefore, rams fed maca diets at T4 had significantly ($P < 0.05$) higher linearity (LIN) followed by rams in T2 while T1 had the lower LIN. This clearly indicates that maca will greatly assist sperm

cells to swim against fluid currents in the female tract implying that high utero-tubal swimming ability will be more in T4 sperm cells. The result showed that straight line velocity (VSL), average path velocity (VAP) and straightness were not significant ($P > 0.05$) across the treatment groups. The results were however showed significant ($P<0.05$) differences in curvinearity velocity (VCL). The T4 had higher VCL (4.83 µm/s) relative to others and this clearly indicates hyperactivation. Chang and Suarez (27) stated that hyperactivated motility is high in VCL and amplitude lateral mucosal head displacement (ALH) which helps in escaping mucosal trap and high ability of oocytes zona pellucida penetration during capacitation as cited by Kirman-Brown and Smith, (28).

Table 3: Sperm Kinetic Characteristics of Yankasa rams Supplemented with Maca Powder

Parameters	T1	T2	T3	T4	SEM	LOS
Motility (%)	65.00^{b}	73.00^{a}	73.67^{a}	76.33^{a}	3.28	*
Non-progressive motility	21.00^{a}	14.33^{b}	6.33^{c}	3.67^{c}	2.83	*
Progressive motility (%)	29.33^{b}	32.67^{b}	38.33^{a}	41.67^{a}	2.17	*
Linearity	51.73^{b}	67.02^{ab}	62.40^{ab}	84.89^{a}	11.82	*
Straight line velocity (µm/s)	2.45	2.77	2.23	2.13	0.32	NS
Curvinearity velocity (µm/s)	2.53^{b}	3.63^{ab}	3.87^{ab}	4.83^{a}	0.56	*
VAP	3.60	4.03	4.07	4.00	0.48	NS
Straightness	67.67	66.00	69.33	72.20	4.76	NS

, ^{abc} = means with different superscripts across the row differ significantly, *= significant at 5% level of significance (0.05),, VAP = Average path velocity, SEM = Standard error of mean, LOS = Level of significanceNS = Not significant

Conclusion and Application

In conclusion, dietary supplementation of maca (*lepidium meyenii*) powder significantly improved reproductive hormone profiles, semen quality, and sperm kinetic characteristics of Yankasa rams during the hot-dry season. The superior performance recorded at 15 g/kg supplementation level suggests that maca can serve as an effective natural phytogenic additive for mitigating heat stress-induced reproductive challenges in rams.

It is highly recommended that maca powder could be included in the diets of livestock from 5g up to 15g/kg diet as it is safe and improves animal reproductive performance through improved semen quality characteristics, semen kinetics and reproductive hormones. Therefore, farmers and livestock nutritionists can utilize it in livestock production as natural feed additives and aphrodisiac enhancer without deleterious effect. Further studies are therefore encouraged to fully explore the physiological and nutritional mechanisms involved in maca actions on different animal models especially during the heat stress condition.

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