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Effect of melatonin administration on conception rate and biochemical properties of the epididymis in West African Dwarf (WAD) ewes and rams, respectively in dry season

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***Target Audience**: Small ruminant farmers, Animal Physiologists and Researchers*

Abstract

The effect of melatonin administration on conception rate and biochemical properties of the epididymis in West African Dwarf (WAD) ewes and ram respectively were investigated in an experiment with twenty-four sheep (16 ewes and 8 rams) aged, 12 - 18 months, with average weight of 16.5 ± 2kg. Animals were randomly allotted to four treatment groups, each group consisting of six animals and varied doses of melatonin were administered for 30 days. Melatonin was administered at 0mg (T1, control), 5mg (T2), 10mg (T3) and 15mg(T4) to each group, which contained 4 ewes and 2 rams for 30 consecutive days. Twenty-five percent (25%) and fifty percent (50%) conception rate were observed in ewes that received 10 mg and 15 mg of melatonin, respectively. One fetus was obtained from a single ewe in those administered 10mg melatonin, while two fetuses from two ewes , were obtained from those on 15 mg melatonin. Lactate dehydrogenase, was significantly (P<0.05) higher in rams administered 15mg (917.50iu/L) than those on 0mg, 5mg and 10mg melatonin respectively. Lactate dehydrogenase of rams on 5mg, and 10mg (435.50iu/L and 655.50iu/L, respectively) melatonin treatment were significantly (P<0.05) higher than those in control group (375.50iu/L). Total cholesterol was significantly (P<0.05) higher in rams receiving 0mg, 5mg and 10mg melatonin than those that received 15mg melatonin treatment. Glucose was significantly (P<0.05) higher on animals that received 10mg (17.00mg/dL) melatonin compared to those on 0mg (12.00mg/dL), 5mg(12.50mg/dL) and 15mg(11.65mg/dL). Zinc was significantly (P<0.05) higher in animals administered 10mg and 15mg melatonin, however both were significantly different from animals on 0mg, and 5mg melatonin. The paired epididymis, sperm reserves of rams on 15mg (21.50 X 10^6 cell/mL) melatonin treatment was significantly (P<0.05) higher than those on 0mg (11.20±1.36), 5mg (6.47±1.36), and 10mg (0.90±1.36). No Significant difference was observed in Total Protein, Sodium, Potassium, Chloride, Magnesium and calcium in all the treatment groups. In conclusion exogenous melatonin influenced the conception rate of ewes and improved the quality of epididymal sperm cells of the rams in the dry season.

Keywords: Melatonin; epididymal biochemical properties; sperm reserves; West African Dwarf sheep; Dry season

Description of Problem

Small ruminant especially sheep is faced with many challenges which has affected its breeding program. Seasonal variations have posed reproductive challenges on breeding of sheep throughout the year (1). The Ewes have a distinct breeding season, their oestrus cycles generally starts when day length is decreasing and ends when day length is increasing. Although semen production continues

throughout the year in rams, sperm quality is lower in the non-breeding season (2). Studies have shown that reproductive activities of our indigenous breed follow the change of dry and rainy season and are influenced by temperature and humidity (3). Non- Breeding season is a season characterised by high intensity and increased length of sunlight. In the temperate region, this period is typical of 'summer' but in Nigeria, it is typical of 'Hot season' (November- March). In this period the reverse in the breeding season occurs with higher sunlight intensity, low melatonin production and low level of GnRH, LH and FSH production, thereby making it almost impossible for these animals to breed (4). With these challenges, it is imperative to find possible ways to mitigate this shortfall so as to breed our indigenous West African Dwarf Sheep throughout the year and by so doing encourage farmers to go into sheep production hence increasing the availability of derived fresh animal product (cheese, meat and milk) throughout the year.

Materials and Methods

Experimental location

The experiment was carried out at sheep unit of The Federal University of Technology Research Farm, Owerri Imo state on latitude 5˚29'N and longitude 7˚02'E between the months of January to April. The region is in the South Eastern agro-ecological zone of the rain forest zone of Nigeria with a mean annual rainfall of 2200mm (5).

Experimental animal, management and diet

Twenty-four sheep (16 ewes and 8 rams) aged 12 - 18 months, with average weight of 16.5 ± 2kg were housed within the sheep pen and were dewormed and vaccinated against PPR prior to the commencement of the experiment. The animals were fed a mixture of groundnut husk/sugarcane hay and supplemented with concentrate diet of 16.47% CP as shown in Table 1. They were fed at 3% body weight daily and water was given regularly.

Study duration

This study lasted for 12 weeks

Experimental design

Animals were randomly allotted to four treatment groups, each treatment consisted of six animals and varied doses of melatonin were administered for 30 days as shown in Table 2. Oestrus was synchronized in the ewes after melatonin was administered for 30 days and followed by the immediate introduction of rams. Mating trial was conducted for fertility assessment in ewes and rams. The mating trial was allowed for a 5-day period across treatments, thereafter the rams were then separated from the ewes.

Fertility Assessment

Parameters assessed include:

Conception rate of WAD ewes administered melatonin

Conception rate is the number of animals that become pregnant as a proportion of the total number of animal serviced. The conception rates of the ewes were calculated as:

$$\text{Conception rate CR (\%)} = \frac{\text{Number of pregnant ewe X 100}}{\text{Total Number of animals mated}}$$

Litter size which is defined as the number of offspring produced at one birth by an animal was also calculated as the number of foetus per pregnant ewe. At eight weeks post- mating, the ewes were assessed by method of rectal palpation to confirm conception after which they were sacrificed. The lower abdomen was immediately opened up to affirm conception and the number of available foetus.

Table 1: Gross composition of diet for experimental animals

Ingredients	Quantity (%)
Maize	45.00
Wheat offal	45.00
Soyabean	5.00
Bone meal	3.00
Salt	2.00
Total	100.00
Calculated Values	
Crude protein	16.47
DE (Kcal.kg)	2292.80
Dry matter	84.15
Ether extract	3.65
Crude fibre	5.02

Table 2: Melatonin administration and mating ratio

	Varied doses of melatonin per treatment			
	T1 (0mg)	T2 (5mg)	T3 (10mg)	T4 (15mg)
Mating ratio (rams : ewes)	2:4	2:4	2:4	2:4

*Total number of sheep per treatment is six.

Epididymal biochemical properties and sperm reserve

After the rams were separated from the ewes, the rams were castrated and the epididymis carefully removed. Epididymal biochemical properties were determined by modified homogenate technique (6). The epididymis was carefully removed, and portions from the caput, corpus and cauda epididymis were each homogenized by maceration with a pair of surgical scissors for about 5 minutes in a beaker containing 10 ml physiological saline solution. The homogenate was then filtered through a double layer cheese cloth and filtrate diluted to ratio 1:20 with deionized water. A homogenized portion of the pooled segments (caput, corpus and cauda) of the total epididymis (left and right epididymis) was used to determine the biochemical indices of the epididymis. The biochemicals assessed were glucose, total protein, lactate dehydrogenase, total cholesterol, and the electrolytes which are: sodium, potassium, chloride, magnesium, calcium and zinc. Glucose was analysed by glucose oxidase method. The total protein was determined by the Biuret method, the lactate dehydrogenase by Seralyzer Lactate dehydrogenase method as described (7), total cholesterol was determined by cholesterol oxidase method. The electrolytes were assessed by the ISE method (computerized Ion selective electrode).

Sperm cell concentration ($x10^6$ cell/ml) was determined through the use of an improved Neubauer chamber haemocytometer at 40X magnification on a simple light microscope .

The concentration of the sperm cells per milliliter of the epididymal homogenate was calculated as:

Sperm concentration per ml = N x D x 32,000 x 5
N= no of sperm cells counted in 5 squares diagonally
D= Dilution factor

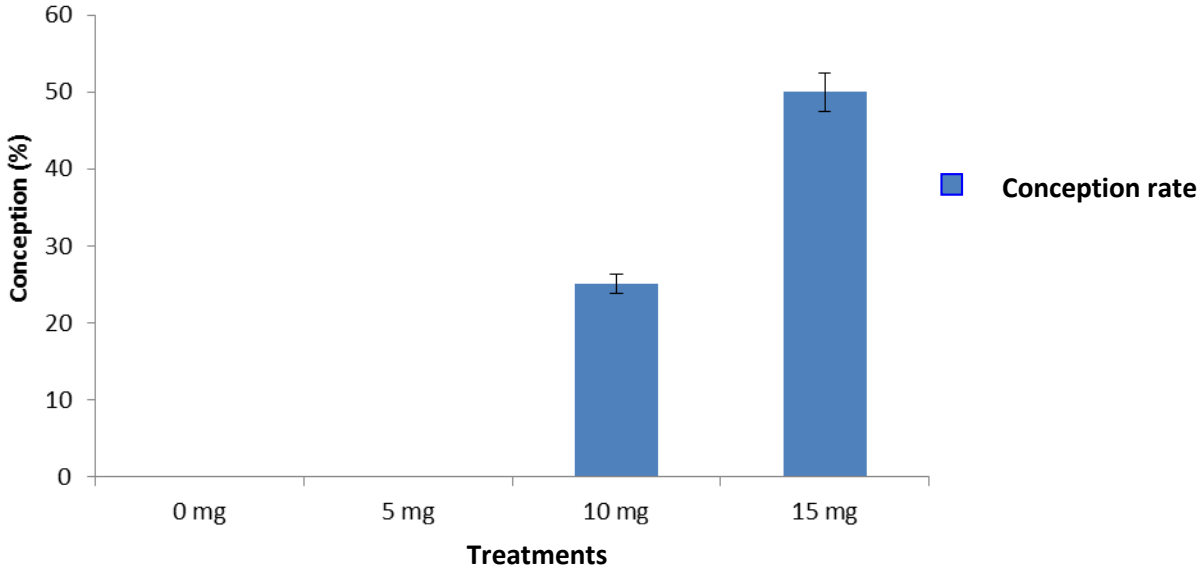
Statistical Analysis

Data were analysed using the one way ANOVA (8) procedure and Duncan multiple range test option of the same statistical software was used to separate the treatment means.

Results

Conception rate of WAD ewes administered melatonin

The conception rate of ewes administered melatonin is shown in the Figure below. Twenty five percent (25%) and fifty percent (50%) conception rate was observed in ewes receiving 10 mg and 15 mg of melatonin, respectively. One fetus was obtained from a single ewe in those administered 10mg melatonin , while two fetuses were obtained from two ewes from those on 15 mg melatonin.



Effect of melatonin on Conception rate of ewes

The biochemical properties of the epididymis of WAD rams administered melatonin are presented in the Table 3. Lactatedehydrogenase, was significantly ($P<0.05$) higher in rams administered 15mg (917.50iu/L) compared to those on 0mg, 5mg and 10mg melatonin administration. Lactatedehydrogenase of rams on 5mg, and 10mg (435.50iu/L and 655.50iu/L, respectively) melatonin treatment were significantly ($P<0.05$) higher than those on control group (375.50iu/L). Total cholesterol was significantly ($P<0.05$) higher in rams given 0mg, 5mg and 10mg melatonin than those on 15mg melatonin treatment, being the least (1.66mg/dL). Glucose was significantly ($P<0.05$) higher in animals that received 10mg (17.00mg/dL) melatonin compared to those on 0mg (12.00mg/dL), 5mg (12.50mg/dL) and 15mg(11.65mg/dL) melatonin administration

which were not significantly different from each other. Zinc was significantly ($P<0.05$) higher with animals that received 10mg and 15mg melatonin respectively, however both were significantly different from animals on 0mg, and 5mg melatonin administration. The highest value of zinc (94.78mg/dL) was recorded in the rams that received 15mg melatonin. No significant difference was observed in Total Protein, Sodium, Potassium, Chloride, Magnesium and calcium in all the treatment groups.

Table 3: Effect of melatonin on biochemical properties of the epididymis

PARAMETERS	0mg	5mg	10mg	15mg	SEM
Protein (g/dL)	0.81^{a}	0.49^{ab}	0.50^{ab}	1.00^{a}	0.35
Lactate dehydrogenase(iu/L)	375.50^{c}	435.50^{b}	655.50^{ab}	917.50^{a}	97.25
Total Cholesterol (mg/dL)	13.49^{a}	11.08^{a}	12.61^{a}	1.66^{b}	6.26
Glucose (mg/dL)	12.00^{b}	12.50^{b}	17.00^{a}	11.65^{b}	5.86
Sodium (Mmol/L)	179.60^{a}	167.75^{a}	184.40^{a}	169.82^{a}	9.87
Potassium (Mmol/L)	11.78^{a}	6.86^{ab}	7.49^{ab}	13.09^{a}	3.55
Chloride (Mmol/L)	175.50^{a}	170.20^{a}	175.80^{a}	165.50^{a}	4.19
Magnesium (mg/dL)	1.70^{ab}	1.65^{ab}	2.10^{a}	2.30^{a}	0.32
Calcium (mg/dL)	2.43^{ab}	2.42^{ab}	2.58^{a}	3.40^{a}	0.49
Zinc (mg/L)	61.75^{b}	72.81^{b}	90.41^{a}	94.78^{a}	11.88

Means along the same row with different superscripts are significantly ($P<0.05$) different. SEM: Standard error of mean.

Effect of Melatonin on epididymal sperm reserve of WAD rams

Total epididymal sperm reserve of WAD rams administered varied doses of melatonin is presented in Table 4. In the right epididymis, sperm reserves from rams administered 10mg melatonin was significantly lower (0.25×10^6 cell/mL), than those on 0mg (1.60×10^6 cell/mL), 5mg (0.73×10^6 cell/mL) and 15mg (10.00×10^6 cell/mL) melatonin administration. In the left epididymis, sperm reserves from rams on 0mg melatonin administration was not significantly different from those on 15mg melatonin administration, however both were significantly ($P<0.05$) different from those administered 5mg and 10mg melatonin. In the paired epididymis, sperm reserves of rams on 15mg (21.50×10^6 cell/mL) melatonin administration was significantly ($P<0.05$) higher than those on 0mg (11.20×10^6 cell/mL), 5mg (6.47×10^6 cell/mL), and 10mg (0.90×10^6 cell/mL) melatonin administration respectively, while rams on 10mg melatonin administration was significantly lower than those on 0mg, 5mg, and 15mg melatonin administration.

Table 4: Effect of melatonin on total epididymal sperm reserve of WAD rams

PARAMETERS (x10^6cell/mL)	0mg	5mg	10mg	15mg	SEM
Right Epididymis Sperm reserves	1.60^{b}	0.73^{b}	0.25^{c}	10.00^{a}	0.46
Left Epididymis Sperm reserves	9.60^{a}	5.75^{b}	0.65^{c}	11.50^{a}	1.50
Paired Epididymis Sperm reserves	11.20^{b}	6.47^{ab}	0.90^{c}	21.50^{a}	1.36

Means along the same row with different superscripts are significantly different ($P<0.05$). SEM: Standard error of mean

Discussion

Studies with temperate breeds of sheep have revealed that exogenous melatonin can be used to influence conception in ewes (9). Conception rate of the ewes were influenced by exogenous melatonin, conception was achieved in ewes administered 10mg and 15mg melatonin and none in the 0mg and 5mg melatonin. This implies that melatonin was able to improve the quality of sperm cells during the non-breeding season. This report supports the findings of (10) who observed significant results of spermiograms in WAD goat as influenced by exogenous melatonin. This result could also imply that onset of ovarian activities was influenced by melatonin as reported by (11).

Any disruption of the epididymal micro-environment through congenital abnormalities, intrinsic alterations in pH, protein composition and concentrations, temperature and biochemical properties may lead to male post-testicular infertility (12). According to (13), melatonin maintains mineral and electrolyte balance by acting as free radical scavenger and preventing electron loss, thereby preventing cellular oxidation. Therefore, the relevance of testicular biochemicals is due to their synergism in achieving a balance in fertility and body functions, for example, (14) explained that increased levels of melatonin above normal is associated with bone loss and fracture as a result of Ca^{2+} loss which happens when luteinizing hormone being in excess stimulates excess release of testosterone.

From the results, the protein concentration was maintained across the treatments. This implies that the epididymis under the influence of melatonin via androgenic activities was not disrupted in its function in the modification and maturation of sperm cells as noted by (15).

Magnesium, although not statistically different across the treatment groups increased on animals administered 10mg and 15mg melatonin. This increase in magnesium went along with the increase in zinc concentrations in animals administered 10mg and 15mg melatonin, this could imply that at these doses, endogenous melatonin production was activated in the pineal gland. This supports the findings of (16) who said that zinc and magnesium are key activators of endogenous melatonin synthesis from the pineal gland, and are needed to be in the right proportion and in constant circulation. (17) also reported that balanced magnesium status is required to obtain efficiency of the suprachiasmatic nuclei

and of the pineal gland which are necessary pathway of melatonin production from light via pineal gland and as such, reduction or depletion of magnesium comes with decreased production of melatonin. This result shows that exogenous melatonin supplementation during the non-breeding season can help boost endogenous melatonin production thereby improving fertility during this period.

Sodium and potassium concentrations were maintained across treatments. Neurotransmitters are sustained in the cells by sodium and high levels of potassium concentrations. This result implies that melatonin influenced the sustenance of neurotransmitters, this conformed to the findings of (13) who reported that melatonin maintains mineral and electrolyte balance by acting as free radical scavenger and preventing electron loss, thereby preventing cellular oxidation.

Calcium and chloride were also maintained across the treatment groups which reveals that melatonin maintains mineral and electrolyte balance (13).

Cholesterol concentrations in animals administered 5 mg and 10 mg melatonin were not different significantly from the control animals, but a significant decrease in concentration was recorded in animals that received 15mg melatonin. Following the make-up of total cholesterol where low density lipoprotein (LDL) makes up 2/3 of the total cholesterol, thus high levels of total cholesterol depicts a negative influence. Therefore, low result could imply that fertility was positively influenced as explained by (18), that cholesterol in the testes must be precisely and continuously regulated to allow cell survival, if the function of cholesterol regulators (hormone sensitive lipase) are challenged by lipid pathologies (LDL- low density lipoprotein), the testicular functioning will be modified which possess significant repercussion in male fertility, female fertility and embryonic death.

The glucose concentration in animals treated with 5mg and 15mg melatonin were not significantly different from the control but were lower in animals treated with 10mg melatonin. It was observed that animals administered 10mg melatonin had the least percentage of abnormal sperm cells and also the least sperm reserves and yet 25% conception was recorded in this treatment. The high concentration of glucose in this treatment could have been beneficial in sperm capacitation and thus promoting fertilization. This result supports the findings of (19) who reported that glucose is beneficial for optimum sperm capacitation and fertility. Glucose at 15mg was not significantly higher than those at 10mg but was not different from 0mg and 5mg. This result showcases the maintenance work of melatonin, in that at increasing dosage, melatonin strives to balance out biochemical discrepancies for optimum body performance.

The control group had the lowest concentration of Lactate dehydrogenase, while animals on 15mg melatonin administrations had the highest concentration. These results established the importance of lactate dehydrogenase in fertility and support the findings of (20) who reported that Lactate dehydrogenase is involved in the energy metabolism of spermatozoa in sperm capacitation and fertility.

Conclusion and Applications

1. The increase in epididymal sperm cell, conception rate and lactate dehydrogenase with reduced cholesterol following melatonin administration showed that melatonin can be of great benefit in reproduction in WAD sheep.

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