



## Effects of Taurine and Season on Semen Characteristics and Sperm Morphology of West African Dwarf Rams

P.O., Atte., M., Abdulrashid, O.M., Daudu, O.M., D. Zahraddeen.

Department of Animal Science, Faculty of Agriculture. Ahmadu Bello University, Zaria

### Abstract

*The study was conducted to determine the effect of taurine and season on semen characteristics and morphology of thirty-two West Africa Dwarf rams with an average weight of 12kg in a Completely Randomized Design involving four dietary treatments containing 0, 0.5, 1 and 1.5% levels of inclusion of taurine respectively. Semen was collected during the wet and dry season. Each ejaculate was evaluated for its various characteristics and morphology. The colours of ejaculates for all treatments were creamy, pH ranged between 6.61 – 6.77. The semen volume ranged from 0.54ml (0%) - 1.76ml (1.5%). Sperm motility and concentration ranged between (62.35ml (0%) – 86.23ml (1.5%) and  $2.30-5.17 \times 10^6$  respectively. There was no significant seasonal variation in reaction time, semen colour, pH, volume and concentration however, maximum sperm motility was observed during the dry (79.67%) and minimum in wet season (74.00%). The percentage of normal cells ranged from 79.20 (0%) – 91.54% (1.5%). The abnormalities of the cells were highest for rams fed the 0% and least for those fed 1.5% taurine inclusion. The range of the abnormalities is; mid – piece droplet (2.30-3.38%), coiled tail (1.05–4.78%), bent tail (1.97-4.21%), detached tail (2.14 - 4.20%) and free tail (1 - 4.33%) respectively. The results showed that season had no significant effect on the variables. The study showed that taurine improves sperm quality, morphology and also helps in ameliorating the effect of seasonal stress. It was therefore recommended that taurine should be incorporated at 1% in the diet of ram for optimal production.*

**Keywords:** Taurine, Season, Semen Characteristics and sperm Morphology

### Introduction

The West African Dwarf (WAD) sheep is the predominant breed found in the area extending from the Southern Guinea Savannah to the very humid Atlantic Ocean Coast in the South (Otchereet *et al.*, 1990). This breed is characterized by exceptional reproductive abilities such as high prolificacy, sexual precocity and good fertility (Hassan *et al.*, 2015). The West African dwarf sheep are particularly useful in the improvement of food security of the populations and thus escaping poverty (FAO, 2017). Their adaptability traits include hardiness and resistance to some local diseases like trypanosomiasis (Hassan *et al.*, 2015). Season is an important factor that have

influenced the variation in semen quality and fertility (Granleese, 2015). Seasonal changes in testicular function vary among species and can range from complete arrest of spermatogenesis during part of the year to poor sperm production (Chemineau *et al* 2008). Sperm morphology refers to the form or shape of sperm, which is the male reproductive cell, it is a very strong indicator of a person's bodily and thus testicular health, which is strongly related to bodily, physiological and environmental stresses, far more than any other organ and sometime a contributing factor that can have an important impact on male fertility (Mataveli, 2008).

Taurine, or 2-aminoethanesulfonic acid, is an organic acid widely distributed in animal tissues. It is a derivative of cysteine, an amino acid which contains a sulf- hydryl group (Hariprasath *et al.*, 2012). Taurine has been identified as an important free  $\beta$ -amino acid in the male reproductive system (Aly and Khafagy, 2014). This amino acid has been found in the male reproductive system especially in peritubularmyoid cells (Li *et al.*, 2013), Leydig cells, vascular endothelial cells, and some other interstitial cells of testis (Yang, 2010). Taurine has various roles in the male reproductive system and sperm cells. It is known for its ability to stabilize cell membranes and regulate cellular osmosis (Ahmed, 2015), and can traverse the sperm plasma membrane (Kumar and Atreja, 2012) but it also acts as a sperm motility factor and as a capacitating agent (Aly and Khafagy, 2014). Taurine is able to inhibit lipid peroxidation (LPO) due to its antioxidant properties (Ahmed, 2015) and protect the sperm cells against the accumulation of reactive oxygen species (ROS) (Chhillar *et al.*, 2012). Taurine is also known as the most abundant free amino acid of the sperm cells and seminal fluid (Yang, 2010; Li *et al.*, 2013). Taurine is also important during the regulation of the male reproductive system and spermatogenesis (Ahmed, 2015). Taurine have been found in seminal plasma and

spermatozoa of numerous species and are known to have beneficial effects on spermatozoa characteristics in mammals. Taurine is a cyto - protective agent with broad study prospects, which can protect the integrity of cell membranes and biological systems from oxygen free radical damage, based on these functions of taurine, it therefore enhances the antioxidant capacity of sperm, inhibit LPO and then protect the structure of the sperm (Jong *et al.*, 2011). Hence, the aim of the present study was to evaluate the effect of taurine supplementation during the wet and dry season on semen characteristics and sperm morphology for better breeding efficiency.

**Materials and Methods**

A total of sixteen West Africa Dwarf (WAD) rams with an average body weight of 12kg were used for this study. On arrival they were given prophylactic treatment and quarantined for two weeks during which they were treated against ectoparasite using Diasentol and dewormed with Albendazole (2.5% oral suspension) per kg body weight. Four diets were formulated with graded levels of supplemental taurine at 0, 0.5, 1.0 and 1.5 respectively (Table1). Sheep were divided into four treatments groups with four replicates and were fed at 3% of their body weight in a completely randomized design that lasted for ninety days.

**Table I: Experimental Diets**

Ingredients	Levels of inclusion (%)
Maize offal	78.0
Soya bean cake	6.5
Groundnut cake	8.6
Oil	5.0
Limestone	1.5
Salt	0.4
Metabolizable Energy (Kcal/kg)	2800

Semen Collection: Semen was collected from rams adequately restrained in standing

position. The samples were collected with the aid of a battery operated Bailey electro

ejaculator (MOD2, Western Instrument Company, Denver, Colorado USA). The Bailey ejaculator is powered by a 6-volt battery. The probe was lubricated with petroleum jelly for easy insertion into the rectum and pushed forward slowly and a series of short electrical stimulation was done intermittently for approximately 2–5 seconds, until erection and ejaculation were achieved. Ejaculates were placed in a water bath at 37°C before microscopic evaluations. Semen was collected three times from August-October to cover the wet season and December - February to cover the dry season.

**Semen Evaluation:** Semen samples were evaluated according to the method described by Zemjanis (1977). The gross semen characteristics that were examined include pH, colour, volume, motility and concentration. The pH was obtained using pH strips and the colour change in the paper was compared with the standard strip. The colour of the semen was assessed visually in the collection tube immediately after collection. The semen was collected and read from the graduated collecting tubes immediately. The sperm motility was determined as described by Oyeyemi *et al.* (2009) using Celestron Penta View microscope (LCD-44348 by RoHS, China). A drop of raw undiluted semen on a pre-warmed slide was covered by a slip and viewed under a field microscope at  $\times 10$  magnification. The Sperm concentration was determined by using the haemocytometer method. A semen sample was sucked into the red blood cell diluting pipette up to 0.1- mark and the volume made up to 1 mark with 10 % formal saline and mixed thoroughly. Live - dead ratio of the sperm cells was determined as described by Estes *et al.* (2006). A thin smear of the semen sample was made on clean, grease free glass slides and stained with Eosin - Nigrosin stain. The dead sperm cells were identified as those that absorbed the stain. At least 400 sperm cells were counted using a light microscope at  $\times 40$

magnification. Sperm abnormalities were determined as described by Estes *et al.* (2006). A thin smear of the semen sample was made on clean grease free glass slide and fixed with buffered formal saline and the preparation was examined and abnormal sperm cells counted in a regular sequence using light

### Statistical Analysis

Data obtained were subjected to analysis of variance (SAS, 2000) and where significant differences occurred means were separated using Duncan Multiple range test of the same package

### Results and Discussion

#### Semen Characteristics of WAD Sheep Fed Graded Levels of Dietary Taurine

The results for the semen characteristic of WAD sheep fed graded dietary levels of taurine are shown in Table 2. The colours of the ejaculates for all treatments were all milky. Mataveli (2008) reported that the normal healthy colour of semen varies between milky – creamy colour for good quality sperm. This was an indication that the taurine diet fed to the rams resulted in a good semen appearance. The semen pH ranged from 6.61 – 6.77, this implies that the pH is slightly alkaline. This agreed with the report of Dhurvey *et al.* (2012) who reported that the pH of ram should be slightly alkaline. The values obtained in this present study indicated that supplementation with taurine did not adversely affect the buffering capacity of the seminal plasma of the sperm cells. There was no significance difference between animals with respect to semen colour and pH; this could be attributed to similar age of the animals under study and same environmental condition.

The semen volume of ram fed 1.0% (1.68 ml) and 1.5% (1.76 ml) dietary treatment are similar but are significantly ( $P < 0.05$ ) higher than those sheep fed 0.5% (1.28 ml) dietary

treatment while those fed 0% (0.54 ml) dietary treatment had the lowest semen volume. This implies that the semen volume increases as levels of taurine increases. This result is contrary to the findings of Jiancheng *et al.* (2010) who reported that taurine had no effect on semen volume. However, the volume obtained in this study is higher than the range of 0.60 – 1.6 ml reported by Moghaddam *et al.* (2012) and within the range of 1.62 – 2.13 ml reported by Al-Anazi *et al.* (2017). This implies that antioxidant activity of taurine reduces the production of free radicals leading to improvement in semen volume.

The sperm motility in this present study ranged between 62.35 (0%) – 86.23 (1.5%) this is within the mean range of 60 – 90% reported by Moghaddam *et al.* (2012). There was an improvement in semen motility of the rams fed dietary taurine diet. This result is similar to the study conducted by Yang (2010) reported an increase in motility of sperm as the levels of taurine increased. This may be as a result of

taurine playing an important role in the maintenance and stimulation of sperm motility and stimulation of capacitation and acrosome reactions (Tadros *et al.*, 2005).

Semen concentration of rams fed 1.0 and 1.5% dietary treatment are similar however, they are significantly ( $P < 0.05$ ) higher than those fed 0.5% dietary taurine while those fed the control diet had the least concentration. The semen concentration in this present study ranged within  $2.30 - 5.17 \times 10^6$ . This is higher than the value of  $2.6 - 4.44 \times 10^6$  ml reported by Al-Anazi *et al.* (2017) and fell within the normal range of  $2.0 \times 10^6$ /ml to  $6.0 \times 10^9$ /ml reported by Ahemen *et al.* (2011). Higher concentration of semen observed in this study may be as a result of taurine supplementation. This agreed with the findings of Castellini *et al.* (2003), who reported that improvement of semen antioxidant stability can be achieved through diet fortification, especially antioxidants, of which taurine which is a powerful antioxidant molecule.

**Table 2: Semen Characteristics of WAD Sheep Fed Graded Levels of Dietary Taurine**

Parameters	Levels of taurine (%)				SEM	P- value
	0	0.5	1.0	1.5		
Semen Colour	Milky	Milky	Milky	Milky	-	-
Reaction Time (s)	83.11	82.50	82.50	81.67	1.72	0.11
Semen pH	6.60	6.61	6.77	6.76	0.24	0.18
Semen Volume (ml)	0.54 <sup>c</sup>	1.28 <sup>b</sup>	1.68 <sup>a</sup>	1.76 <sup>a</sup>	0.32	0.04
Sperm Motility (%)	62.35 <sup>c</sup>	74.60 <sup>b</sup>	78.83 <sup>a</sup>	86.23 <sup>a</sup>	5.44	0.03
Sperm Concentration (x10m <sup>6</sup> ml)	2.26 <sup>c</sup>	3.50 <sup>b</sup>	4.17 <sup>a</sup>	5.17 <sup>a</sup>	1.19	0.01

<sup>abc</sup>: means with different superscript in the same row are significantly ( $P < 0.05$ ) different



### Effect of Season on Semen Characteristics of WAD Sheep Fed Graded Levels of Dietary Taurine

The results of effect of season on semen characteristic of WAD sheep fed graded dietary levels of taurine are shown in Table 3. There was no significant seasonal variation ( $P>0.05$ ) in all the parameters under investigation with the exception of sperm motility. This present study supports the finding of Maina *et al.* (2006), who reported that season had no significant effect on semen volume and concentration. Maximum sperm motility was observed during the dry season (79.67%) while the minimum occurred in wet season (74.00%). This result is in consonance with the result obtained from the findings of

Moghaddam *et al.* (2012) and Maina *et al.* (2006). Both researchers reported that sperm motility was highest during the dry season. Li *et al.* (2013) reported that taurine performs important roles in the maintenance and stimulation of sperm motility and stimulation of capacitation and acrosome reactions in vivo and in vitro. Also Hwang and Wang (2001) found that taurine could inhibit lipid peroxidation in rabbit spermatozoa and protect the loss of motility. You *et al.* (2010) reported that taurine acts as both a cell protecting agent by modulating the cell membranes fluidity and health, as well as exerting anti-oxidant like effect thereby alleviating effect of stress. All these potentials put together may as well eliminate the effect of seasonal variation in semen properties reported in this present study.

**Table 3: Effect of Season on Semen Characteristics of WAD Sheep Fed Graded Levels of Dietary Taurine**

Parameters	Season		SEM	P- value
	Wet	Dry		
Semen Colour	Milky	Milky	-	-
Reaction Time (s)	82.00	82.60	0.05	0.17
Semen pH	6.64	6.67	0.16	0.63
Semen Volume (ml)	1.10	1.21	0.08	0.04
Sperm Motility (%)	74.00 <sup>b</sup>	79.67 <sup>a</sup>	2.74	0.01
Sperm Concentration ( $\times 10^6$ )	4.52	4.95	0.78	0.04

<sup>ab</sup>: means with different superscript in the same row are significantly ( $P<0.05$ ) different

### Sperm Morphology of WAD Sheep Fed Graded Levels of Dietary Taurine

Sperm morphology of WAD sheep fed graded levels of dietary taurine is presented in Table 4. The study showed that the percent of normal sperms in the semen of WAD ram is significantly ( $P<0.05$ ) influenced by taurine. The mean percentage of normal sperms

recorded significantly increased as the level of taurine supplementation increases. This result was similar to the findings of Jiancheng *et al.* (2010) who observed that taurine can improve the semen quality in male animals. The enhancement effects of taurine on male reproduction may be attributed to the stimulation of T secretion, promotion of testis

homeostasis and antioxidation. The mean percentages of normal sperm recorded was significantly ( $P < 0.05$ ) highest for sheep fed 1.5% dietary taurine (91.54%) which is statistically ( $P > 0.05$ ) similar to those fed 1.0% (90.71%) dietary taurine while sheep fed the 0% dietary taurine had the least (79.20%) normal cells. There was significant ( $P < 0.05$ ) effect of dietary taurine on the incidence of sperm abnormality percentage in the semen of WAD rams. The mean percentage of semen abnormalities tends to follow the same pattern. The abnormalities of the mid – piece droplet, coiled tail, bent tail, detached tail and free tail were highest (3.28%, 4.78%, 4.21%, 4.20% and 4.33%) respectively for sheep fed 0% dietary taurine. However, the mid – piece, coiled tail and bent tail were statistically similar for those animals fed 0.5 and 1.0% dietary taurine while those fed 1.5% dietary

taurine had the least (2.30%, 1.05%, 1.97%, 2.14% and 1.00%) respectively. Abnormal sperms have fundamental consequences on quality of the spermatozoa and fertility. A high ratio of spermatozoa abnormalities indicates damage to the spermtogenic epithelium or pathologic conditions of the excretory duct system (Bitto *et al.* 2000). Baseline values of sperm morphological abnormalities for WAD rams recorded in this study were in agreement with values reported by previous workers for Nigerian breeds (Bitto *et al.* 2000). In this present study the mean percentage of semen abnormalities tends to reduce as percentage of taurine increases in the diet. This result is similar to the findings of Hwang and Wang (2001) and Jiancheng *et al.* (2010). They found out that taurine could inhibit lipid peroxidation in rabbit spermatozoa and protect loss of its qualities therefore reducing abnormalities.

**Table 4: Sperm Morphology of WAD Sheep Fed Graded Levels of Dietary Taurine**

Parameters (%)	Levels of taurine (%)				SEM	P-value
	0	0.5	1.0	1.5		
Normal cells	79.20 <sup>c</sup>	87.90 <sup>b</sup>	90.71 <sup>a</sup>	91.54 <sup>a</sup>	3.08	0.01
Mid – Piece Droplet	3.28 <sup>a</sup>	2.27 <sup>b</sup>	2.31 <sup>b</sup>	2.30 <sup>b</sup>	0.37	0.03
Coiled Tail	4.78 <sup>a</sup>	2.30 <sup>b</sup>	1.10 <sup>c</sup>	1.05 <sup>c</sup>	1.96	0.04
Bent Tail	4.21 <sup>a</sup>	2.33 <sup>b</sup>	2.30 <sup>b</sup>	1.97 <sup>b</sup>	0.56	0.01
Detached Tail	4.20 <sup>a</sup>	2.50 <sup>b</sup>	2.47 <sup>b</sup>	2.14 <sup>b</sup>	0.55	0.01
Free Tail	4.33 <sup>a</sup>	2.70 <sup>b</sup>	1.11 <sup>c</sup>	1.00 <sup>c</sup>	1.23	0.02

<sup>abc</sup>: means with different superscript in the same row are significantly ( $P < 0.05$ ) different

**Effect of Season on Sperm Morphology of WAD Sheep Fed Graded Levels of Dietary Taurine**

Table 5 presents the effect of season on sperm morphology of WAD sheep fed graded levels of dietary taurine. The results showed that season had no significant ( $P > 0.05$ ) effect on the observed variables however, there were slight variations amidst the observed values. The mean (91.24%) of normal cells collected

during the wet season was similar to those collected during the dry season (90.85%). There was no significant ( $P > 0.05$ ) effect imposed by season on all semen abnormalities considered in this study. Morphology of the cells during the wet season was similar to those collected during the dry season. This result disagreed with the results of several authors; Karagiannidis *et al.* (2000), Maina *et al.* (2006) and Jiancheng *et al.* (2010). They

reported a significant seasonal variation in semen quality (percentage of motile spermatozoa, percentage of abnormal spermatozoa and rate of progressive motility). Semen has been reported by several workers to be influenced by seasonal weather conditions. The deviation observed from this study may be

as a result of antioxidant nature of taurine. According to Li *et al.* (2013), taurine perform important roles in the maintenance and stimulation of sperm motility, stimulation of capacitation and acrosome reactions *in vivo* and *in vitro* and maintenance of sperm quality and characters.

**Table 5: Effect of Season on Sperm Morphology of WAD Sheep Fed Graded Levels of Dietary Taurine**

Parameters (%)	Season		SEM	P- value
	Wet	Dry		
Normal cells	91.42	90.85	2.57	0.06
Mid – Piece Droplet	1.69	1.77	0.08	2.89
Coiled Tail	2.08	1.98	0.10	1.62
Bent Tail	3.44	3.82	1.38	0.08
Detached Tail	0.91	1.26	1.35	1.02
Free Tail	0.46	0.32	0.14	0.07

### Conclusion and Recommendation

The study showed that taurine improves sperm quality (volume, motility and concentration) as well as its morphology. The oxidizing nature of taurine helps in maintaining the quality of the semen in both seasons by ameliorating the effect of seasonal stress. It was therefore recommended that taurine should be incorporated in the diet of ram at 1% for optimal production.

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