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GROWTH AND SEMEN CHARACTERISTICS OF WEST AFRICAN DWARF RAMS FED A*MMONIUM SULPHATE* SUPPLEMENTED DIETS

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ABSTRACT

Ammonium sulphate (AS) is widely used as a feed additive for animals. It is generally used to provide supplemental sulphur to the diet, particularly in diets containing non-protein nitrogen sources. This study assessed the growth and reproductive performance of Yankasa rams fed diets supplemented with different levels of ammonium sulphate. Ammonium sulphate was added at 0, 0.5, 1.0, and 1.5g/kg of the concentrate diet as T0, T0.5, T1.0 and T1.5 respectively. Each treatment had four replicates while semen was collected once from all replicates in the treatments. Rams were fed experimental diets for 70 days. The parameters determined were: feed intake, body weight gain, feed conversion ratio, mass activity, motility, livability, sperm volume, sperm concentration, scrotal circumference and length. There were significant differences in the feed intake, body weight gain and the feed conversion ratio of the West African dwarf rams fed the supplemented diets. The rams fed diet T1.0 had the highest feed intake and body weight gain of 403.75g/day and 195.83g/day respectively above other treatments. The FCR in T1.0 was the lowest at 2.11 below 6.68 (T0) There was significant difference in the sperm concentration ($x10^6$spz/ml) with T1.5 (160.25) having the highest while T0 (136.00) had the lowest. The total sperm ejaculate follow the same trend as sperm concentration with the lowest value recorded in T0 (98.95) while T1.5 (151.65) had the highest value. There were no significant differences in the values recorded for the scrotal circumference and length, however, scrotal weight, total testicular weight, scrotal length and scrotal circumference values in T1.5 was numerically higher than other treatments. It can be concluded that inclusion of ammonium sulphate in the feed of rams at 1.0g/kg enhanced the efficiency of feed utilization and improved the semen quality positively.

Keywords: scrotal weight, ejaculate, livability, weight gain, supplementation

INTRODUCTION

Ammonium sulphate, a source of non -protein nitrogen is more stable than urea in release of volatile ammonia (NH_3), (Arora *et al.*, 1973) and it's a source of sulphur to rumen microbes, can be "Used as a dual source of nitrogen and sulphur in the ration of ruminants (Arora *et al.*, 1974). The sulphur in ammonium sulphate is required by rumen microbes to manufacture several amino acids that help in the conversion and breakdown of urea into nitrogen. Ammonium sulphate (AS) is widely used as a feed additive in feedlot rations. It is generally used to provide supplemental sulphur to the diet, particularly in diets containing non-protein nitrogen sources. Therefore, the most important thing to be aware of regarding sulphate-containing compounds is their optimum levels in the diet as low ruminal sulphur concentration can depress microbial growth (Bal and Ozturk, 2006).

It is well established that ruminants fed roughages containing low-nitrogen sources respond to a source of protein (Orskov, 1982; Preston and Leng, 1987). Supplementing low quality forage-based diets with nitrogen sources elevates ruminal ammonia N concentration to provide rumen bacteria with their requirements to achieve maximum rates of fermentation (Fike *et al.*, 1995). A major limitation to animal production in Nigeria is poor reproductive performance (RIMS, 1992). Other factors are skyrocketing prices and scarcity of conventional animal feed rich in protein. Enjalbert (2006) attributed many reproductive health disorders in animals to diet inadequacy. It is well documented that protein deficient feeds reduces meat quality and sexual activity in bulls (Brown, 1994; Rekwot *et al*., 1997), which may likely be the same for rams.

Similarly, seminal attributes are affected by many factors, including the breed, body weight, age, management, climatic conditions, nutrition, method of semen collection and degree of sexual stimulation (Zamiri and Heidari, 2006). This study was conducted to evaluate the effect of ammonium sulphate supplementation on the growth and semen characteristics of West African dwarf rams.

MATERIALS AND METHOD

Experimental Site

The experiment was carried out at the Sheep unit of the Teaching and Research Farm, University of Ibadan, Oyo state, South West Nigeria. The location is between latitude 7.270 N and longitude 3.450 E and 200 – 300 m above sea level. Found in the tropical rainforest zone, the daily temperature and humidity are within the ranges of 25^0C - 32^0C and 55% - 75 % respectively depending on the season. The total annual rainfall ranges between 2032 mm – 3048 mm with a bimodal rainfall pattern that begins in late March and ends in late October.

Experimental Design, Animals and Management

In a complete randomized design (CRD), sixteen (16) healthy West African dwarf (WAD) rams were tagged and allotted into four treatments with four replicates per treatment according to their weight. The rams were allowed two weeks of acclimatization during which weighted amount of experimental diets were given. Animals were housed in different pens which were properly disinfected and the concrete floor covered with wood shavings. Water was supplied *ad-libtum* throughout the period of the study. Necessary management practices for sheep production were carried out according to Baiden *et al.* (2007).

Experimental Diets

The ration formulated comprised of brewer's dried grain, cassava peels, palm kernel cake, salt, urea, di-calcium sulphate and premix (growers). Ammonium sulphate was then added into the rations at varying inclusion levels of 0, 0.5, 1.0 and 1.5% for T0, T0.5, T1.0 and T1.5. Table 1 shows the gross composition of the experimental diets.

Proximate Composition Analysis

Proximate composition of the feed was determined according to AOAC (2005) and detergent fibre fractions by the method of Van Soest *et al.* (1991).

Live Weights and Feed Intake

Live weight was taken weekly following an overnight fast except for water. Feed intake was determined daily as the difference between the amounts of feed offered and refusals. Feed was offered to the animals at 5% of their body weight. The initial body weight of the animals varied from 19.25kg (T1.5) to 19.75kg (T0) and (T1.0). The final body weight ranged from 23.00kg (T0 and T1.5) to 31.00kg (T1.0).

Table 1: Gross composition of experimental diets

	Ammonium sulphate level in the diets (%)			
Ingredients%	**T0**	**T0.5**	**T1.0**	**T1.5**
BDG	55	55	55	55
Cassava peels	26	26	26	26
PKC	12	12	12	12
Salt	2	2	2	2
Urea	1	1	1	1
DCP	3	3	3	3
Premix(grower)	1	1	1	1
Ammonium sulphate	0	0.5	1.0	1.5

BDG: Brewers Dry Grain. PKC: Palm Kernel Cake. DCP: Di-calcium phosphate

Body Weight Measurements

Body weights of the rams were measured in kilograms by following the procedure as described by Akpa *et al*. (1998). The weight of the observer was taken first, and then the body weight of each animal was taken by carrying the animal individually and standing on a weighing scale. The difference between this weight and that of the observer provided the weight of the animal. Weighing was done at the beginning of the study and subsequently on weekly basis.

Scrotal Measurements/ Testicular Measurement

Scrotal circumference was measured weekly in centimeters using a flexible measuring tape at the widest scrotal diameter by applying pressure with a hand above the head of the epididymis, thereby gently forcing the testes into the scrotum, then the flexible measuring tape was placed at the widest scrotal diameter to take the reading. The measurements were carried out as follow:

Testicular Length (TL)

This was measured in centimeter with a flexible measuring tape as the distance along the caudal surface of the scrotum, from its point of attachment to the tip of the scrotum as described by Bratte *et al*. (1999) and Akpa *et al*. (2012).

Testicular Circumference (TC)

This is the maximum dimension around the pendulous scrotum after pushing the testes firmly into the scrotum (Akpa *et al*., 2006). It was measured in centimeters (cm).

Testicular Width (TW)

This was taken as the division of Testicular Circumference by two.

Testicular Weight (TWT)

This was determined using Bailey *et al.* (1996) formulae as given below;

TWT = 0.5533 x TL x TW

Where; TWT = Testicular weight TL = Testicular length TW = Testicular width

Semen Collection

Semen was collected once from the rams using electro-ejaculation (EE) method. The rams were adequately restrained; hair around the penis was trimmed and the area washed with 6 % sodium chloride solution to avoid contamination of the semen being collected. The probe of the electro-ejaculator was lubricated using petroleum jelly and inserted into the rectum and switched on, with the current being alternated from 0 to 5 volts and returned to zero every 5 to 10 seconds, with the subsequent stimulation being higher such that at about the fifth a maximum of 10-15 volts was reached, this resulted in erection and subsequently ejaculation. Ejaculated semen was collected in a calibrated tube.

Semen Evaluation

Semen samples collected were evaluated as described by Zemjanis (1970) with modifications. After collection by electro-ejaculator, the volume of each ejaculate was measured in a graduated tube. The proportion of spermatozoa with an intact apical ridge was evaluated. After fixation in a buffered 2% glutaraldehyde solution and examined under Differential Interference Contrast microscope at magnification of 400. Total number of spermatozoa per ejaculate was calculated as the product between sperm concentration and volume of the ejaculate. Percentage of abnormal spermatozoa (considering all normal forms in sperm head, intermediate piece and tail) were estimated.

Sperm Volume

The volume of the ejaculate was measured with a graduated cylinder. Thereby, loss of volume associated with transfer from the collection tube to either another tube or a pipette was avoided (Jorgensen *et al.*, 1997).

Sperm Concentration

Sperm concentration was determined using a haemocytometer.

Sperm Motility

Sperm motility was assessed by the method described by Zemjanis (1977). The Microscopic examination for wave pattern (gross sperm motility)was determined by placing a drop of undiluted semen with a few drops of 2.9% sodium citrate on a pre-warmed slide then cover-slipped and viewed using a field microscope at X40 magnification.

Livability Percentage: Livability of the sperm cells was determined as described by Esteso *et al.* (2006). A thin smear of the semen sample was made on clean grease free glass slide and stained with eosin-nigrosin stain. Light microscopy at X40 magnification was used for the determination.

Morphology

On a clean, warm glass slide, a drop of semen was placed as well as two drops of Wells and Awa stain. The semen and stain were thoroughly mixed together with a smear made on another clean and warm slide. The smear was air-dried and observed using the light microscope starting with low power to high magnification. The presence of abnormal cells out of at least 500 sperm cells from several fields on the slide was counted and their total percentage estimated (Wells and Awa, 1977).

Statistical analysis

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

RESULTS

The chemical composition of the diets fortified with varying amount of ammonium sulphate is presented in Table 2. CP values obtained for the diets were 40.00%, 36.75%, 38.43% and 40.25% for T1 to T4 respectively. Similarly, the CF obtained ranged from 28.51% to 32.25% for T1 to T4 respectively. In addition, the values obtained for ether extract were 4.00% in T0, 6.40% in T0.5, 6.02% in T1.0 to 6.50% in T1.5. Whereas, the values obtained for ash were 17.00%, 11.00%, 12.00% and 11.00% for T0 to T1.5 respectively.

Table 2: Chemical Analysis of the Experimental Diets

NUTRIENT (%)	T0	T0.5	T1.0	T1.5
Crude Protein	36.75	38.43	40.00	40.25
Crude Fibre	28.51	31.25	31.99	32.25
Ether Extract	4.00	6.40	6.02	6.50
Ash	17.00	11.00	12.00	11.00
NFE	10.49	14.40	11.56	10.00
NDF	57.80	57.56	57.05	57.57
ADF	33.56	33.98	33.93	33.75
ADL	11.26	11.77	11.68	11.57

NFE: Nitrogen free extract, NDF: Neutral detergent fire ADF: Acid detergent fibre, ADL: Acid detergent Lignin, PM: *Panicum maximum*. T0 = 0 % Ammonium sulphate; T0.5 = 0.5 % Ammonium sulphate; T1.0 = 1.0 % Ammonium sulphate; T1.5 = 1.5 % Ammonium sulphate

GROWTH PERFORMANCE OF WEST AFRICAN DWARF RAMS FED AMMONIUM SULPHATE SUPPLEMENTED DIETS

Shown in Table 3 is the performance characteristic of West African dwarf rams fed varied levels of ammonium sulphate. The T1.0 was ranked highest with 195.83g/day while T0 (54.17kg) was the lowest in terms of weight gain. Feed intake T1.5 has the lowest feed intake of 307.50g/day (T1.5) while T1.0 ranked highest with value of 403.75g/day. There were ($P<0.05$) difference in feed conversion ratio among the treatments although, T0.5 and T1.5 do not differ significantly ($P>0.05$). Weight gain showed significant differences among the treatments although, T1.0 was the highest and T0 and T0.5 was the lowest. Body weight showed significant difference for T1.0 but T0, T0.5 and T1.5 were not significantly different. Feed intake showed significant ($P<0.05$) difference across the treatment while no significant ($P>0.05$) difference among T0, T0.5 and T1.5. Feed conversion ratio showed significant ($P<0.05$) difference between T0 and T1.0 although T0 and T1.5 are not significantly ($P>0.05$) different from T0.5 and T1.5.

Table 3: The performance characteristics of West African dwarf rams fed varied levels of ammonium sulphate

	Ammonium sulphate level in diet (%)				SEM
Parameters	**0**	**0.5**	**1.0**	**1.5**	
Initial body weight(kg)	19.75	19.75	19.25	19.50	2.25
Final body weight(kg)	23.00^{b}	27.75^{ab}	31.00^{a}	23.00^{b}	2.09
Body Weight gain(g/day)	54.17^{b}	91.67^{b}	195.83^{a}	58.34^{b}	13.07
Feed intake(g/day)	360.00^{ab}	388.00^{ab}	403.75^{a}	307.50^{b}	29.04
Feed conversion ratio(FCR)	6.68^{a}	5.70^{ab}	2.11^{b}	5.39^{ab}	1.16

a, b: means in the same row with different superscripts are significantly($p<0.05$) different.
SEM: Standard Error of Mean. T0= 0 % Ammonium sulphate. T0.5 = 0.5 % Ammonium sulphate, 1.0= 1.0 % Ammonium sulphate. T1.5= 1.5 % Ammonium sulphate

Presented in Table 4 is the semen characteristics of West African dwarf rams fed ammonium sulphate supplemented diets. There were no difference in semen characteristics except for sperm concentration and total sperm ejaculate which were significantly ($p<0.05$) higher on T1.0 than T0 and T0.5.

Table 4: Semen characteristics of West African dwarf rams fed ammonium sulphate supplemented diets

PARAMETERS	T1	T2	T3	T4	SEM
Colour	Creamy	creamy	creamy	creamy	
Volume (ml)	0.73	0.83	0.95	0.83	0.07
Mass Activity (1-5)	3.00	3.00	3.33	3.00	0.12
Motility (%)	84.75	89.00	97.00	92.25	5.56
Livability (%)	85.00	88.25	90.75	88.25	3.45
Morphology (%)	1.60	2.30	0.90	1.68	0.44
Sperm Conc. (x 10^{6}spz/ml)	136.00^{b}	139.25^{b}	160.25^{a}	152.75^{ab}	6.47
Total Sperm Ejaculate	98.95^{b}	114.40^{b}	151.65^{a}	124.65^{ab}	9.04

ab: means in the same row with different superscripts are significantly($p<0.05$) different.
SEM: Standard Error of Mean; T0= 0 % Ammonium sulphate. T0.5 = 0.5 % Ammonium sulphate, T1.0= 1.0 % Ammonium sulphate. T1.5= 1.5 % Ammonium sulphate

Figure 1 shows the scrotal biometry of West African Dwarf rams fed varying levels of ammonium sulphate fortified diets. Testicular weight was higher in rams fed T0.5 while scrotal circumference was higher on T1.0. Scrotal length and weight did not differ for all the treatment groups.

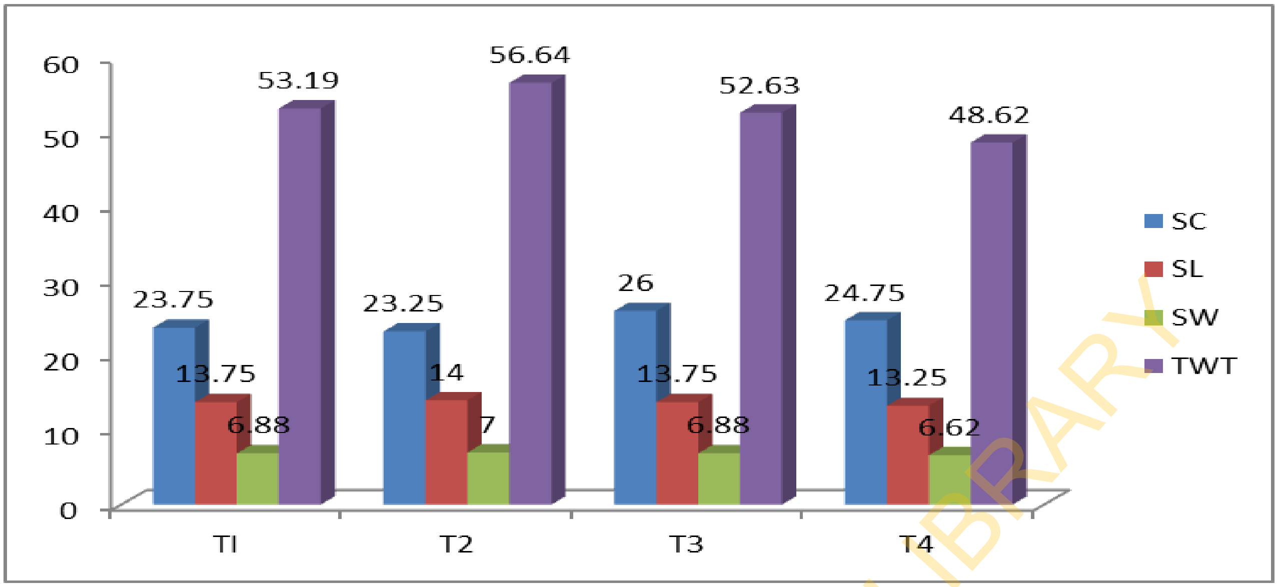

Figure 1: Scrotal biometry of West African dwarf rams fed varied levels of ammonium sulphate supplemented diet. SC: Scrotal circumference. SL: Scrotal length. SW: scrotal width. TWT: Testicular weight

DISCUSSION

Growth performance of West African dwarf rams fed ammonium sulphate fortified diets

The animals were balanced for weight prior to their placement into the treatments. Significant (P<0.05) differences were observed in the final body weights and body weight gain of animals across the treatment. The observed final body weights and body weight gain may be due to higher levels of incorporation of ammonium sulphate, it is known to depress feed intake and this has been used to limit feed intake of steers being introduced to grain based diets (May and Barker, 1989). The feed intake of rams in T3 was highest with value of 403.75g. Feed intake obtained in this study ranged from 307.50 - 403.75g/day which negate the report of Adegbola *et al.* (1985) who reported a higher value of 627-697g/day for West African dwarf sheep fed grass supplemented with concentrate. Bowman and Asplund; (1988) also noted an increased feed intake and performance in sheep with the addition of a legume to a grass diet.

The feed conversion ratio showed significant (P<0.05) difference across the treatments for animals fed ammonium sulphate supplemented diets.). Animals in T1.0 had the least feed conversion ratio. Animals placed in T3 have the ability to effectively convert feed into meat than other three treatments. Therefore, T1.0 with feed conversion ratio of 2.11 (1.0% ammonium sulphate) will fit best for West African dwarf rams.

Scrotal biometry and characteristics of West African dwarf rams fed varied levels of ammonium sulphate fortified diets

This study showed the effect of ammonium sulphate supplemented diets on scrotal biometry of West African dwarf rams. The study showed no significant (P>0.05) difference in animals fed ammonium sulphate supplemented diets in T2, T3 and T4 unlike the animals on T1 (control). The 23.75 - 26.00 cm range of scrotal circumference obtained in this study was contrary to the 15.98-19.43cm reported by Naoman and Taha (2010) for changes in testicular circumference and semen analysis following hemi-castration in adult Iraqi bucks. Scrotal circumference is an important reproductive signal for breeding soundness in animals. The obtained result in this study was higher than those reported by Ososanya *et al.* (2013) who fed pubertal West Africa dwarf rams with pineapple waste silage as replacement for dried cassava peel.

The increased scrotal size in this experiment may be due to high level of nutrition (Masters and Fels, 1984). This report is also in agreement with Fernandez *et al.* (2004) and Hotzel *et al.* (2003) who observed that testicular growth can be affected when animals were fed beyond their maintenance level. In addition, the scrotal circumference is related to testicular weight, sperm production, and semen quality, age at puberty, body weight and age in young bulls (Swanepoel and Heyns, 1990). Therefore, animals with small testicles have reduced sperm production and poor semen quality. Lindsay *et al.* (1984) also found no significant effect of improved pasture or high dietary protein on testicular dimensions. On one hand, Thompson *et al.* (1992) also reported that scrotal circumference was not an accurate determinant of sperm morphology or motility.

The study was further extended to evaluate the effect of ammonium sulphate on semen characteristics of West African dwarf rams. Semen parameters are instruments for the prediction of animal reproductive performance. The colour of the semen of rabbit bucks fed ammonium sulphate in the diet showed no significant (P>0.05) difference from the control. The creamy colour observed in this study negates the milk-white colour earlier observed in the adult WAD buck (Bitto *et al*., 1988; Bitto and Egbunike, 2012). The colour obtained in this study was in conformation with the semen characteristics of pubertal West Africa dwarf rams fed pineapple waste silage as replacement for dried cassava peel by Ososanya *et al.* (2013). The colour of the semen in this study was also similar to the report of Naoman and Taha (2010) and in agreement to the reports of Oyeyemi *et al.* (2011); for changes in testicular circumference and semen analysis following hemi-castration in adult Iraqi bucks

The mass activity of animals in T0.5 to T1.5 which were fed with ammonium sulphate supplemented diets exerted no significance with animals in the T0 which were not fed ammonium sulphate supplemented diets but this was in agreement with the result obtained by (Ososanya *et al.*, 2013). The semen volume is one of the important factors in semen evaluation and reproductive performance in the males (Ax *et al.*, 2000). The results of the present study depicted that semen volume was not significant in the treatment groups. However, animals in T0.5, T1.0 and T1.5 had higher values than those in T0 but contrary to the report of Kheradmand *et al.* (2006) that there was a tendency for semen volume to increase in the treatment groups, in comparison with the control group. The ejaculate volume was not in agreement with values of puberty in Boer goats (Corteel, 1977; Noran *et al.* 1998). However, the values obtained in this study are close to values of Hassan *et al.* (1983) and Summermatter and Flukiger (1985) who reported a range of 0.2-1.2 ml for the ejaculate volume of bucks. Furthermore, the value obtained in this study for sperm volume fell in the range of 0.62-0.92 ml obtained by Mwafaq *et al.* (2013) for semen characteristics of Meriz bucks in response to different doses of prostaglandin $F_{2.}$

The sperm concentration of the T1.0 was highly significant among other treatment fed with ammonium sulphate. Obtained result in the study were respectively lower than the corresponding values reported by Noran *et al*. (1998) for adult Katjang and Katjang x German Fawn goats. This variation may be a result of species difference in seminal characteristics. Although, the value obtained in this study fell in range of the sperm concentration (150-204) obtained by Mwafaq *et al.* (2013) for Meriz bucks in response to different doses of prostaglandin $PGF_{2.}$

The sperm motility of T0.5 to T1.5 obtained in the present study showed higher values with no significant (P.0.05) compared to the report on 13 month old Murciano-Granadina male goats in all four seasons of the year (Roca *et al*., 1992). These differences might be due to breed, age and environmental factors. However, sperm motility is an indicator of semen fertilizing ability (Blottner *et al*., 2001). The total sperm/ejaculation values observed in this study were in the range of the total sperm /ejaculate (92- 194) obtained by Mwafaq *et al.* (2013) on semen characteristics of Meriz bucks in response to different doses of prostaglandin $F_{2.}$ The total sperm of animals fed ammonium sulphated supplemented diets showed significant difference to the control group (T0) except for T0.5 which revealed no significance from animals in T0.

The livability of semen in T0.5 to T1.5 fed ammonium sulphate supplemented diets showed no significant effect from the control group. The livability of T0 which had no ammonium sulphate inclusions in their diet had the lowest value. The report on pubertal and adult bucks (Bitto and Egbunike, 2012) was higher than values of livability obtained for this study; these differences may result due to species, nutritional status, environmental differences and the differences in the methods used for semen collection.

No significant differences existed in morphology between animals in T0.5, T1.0 and T1.5 that were fed ammonium sulphate supplemented diets and the animals in T0. The report of this study were lower than the report which states that the percentage of morphological abnormalities in the semen of below average and poorly fertile bucks may be 10-15%, and above 15 respectively (Easton and Simmons, 1952; Patel, 1967; Huat, 1973; Ott, 1978). In addition, this study agrees with the report that the semen of most males contains a small amount of morphologically abnormal sperm (Hafez, 2004). In respect to this, sperm morphology is useful in semen samples presumption and fertility (Buendía *et al*., 2002). Therefore, an investigation into the percentage of morphological defects is an important indicator of semen fertilizing ability (Blottner *et al*., 2001).

CONCLUSION

Based on this study, the best performance was observed in animals placed on 1.0% ammonium sulphate (T1.0) with feed conversion ratio of 2.11. Feed conversion ratio is a measure of animal efficiency in converting feed mass into increased body mass. Animals with low feed conversion ratio are known as efficient users. Therefore inclusion of 1% ammonium sulphate provided the best feed covesion ratio.

Ammonium sulphate has no deleterious effect on the growth performance and semen characteristics up to 1% inclusion but at 1.5% ammonium sulphate inclusion feed intake, body weight gain reduced. In addition, at 1.5% inclusion of ammonium sulphate, feed conversion ratio was high indicating low feed to meat conversion efficiency. Similarly the semen volume, mass activity, motility, livability, sperm concentration, total sperm ejaculate and scrotal circumference were highest in animals fed 1% ammonium sulphate supplemented diet. Consequently, inclusion of 1% ammonium sulhate promoted better feed conversion ratio and improved semen volume, sperm livability and total ejaculate.

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