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Growth performance and blood profile of gestating wad ewes fed dietary supplementation of ammonium sulphate

Ososanya[1],T. O., Inyang,[2] U. A. and Shehu,[3] S. A.
[1,3] Department of Animal science, University of Ibadan, Nigeria
[2] Department of Animal science, University of Uyo, Nigeria
Corresponding Email: tososanya85@gmail.com

Abstract

The concern raised as a result of antibiotic usage in the livestock industry has necessitated the need for alternative feed additives such as ammonium sulphate. Hence, sixteen West African Dwarf (WAD) ewes averaging 24 kg in weight and aged eighteen months were used to determine the growth performance and blood chemistry of ammonium sulphate supplemented diets. The animals were divided into four groups of experimental diets consisting: D1 (0 %) (Control diet), D2 (0.25 %), D3 (0.50 %) and D4 (0.75 %) of ammonium sulphate (AMS) inclusion levels and were randomly allotted in a completely randomized design. They were also synchronized with prostaglandin $F_{2\alpha}$ and naturally mated with four (4) rams of proven fertility when they came on heat. Parameters determined were feed intake, dry matter intake (DMI), gestating weights and gains, and blood haematology and serum biochemistry such as white blood cells (WBC), red blood cell (RBC), packed cell volume (PCV), haemoglobin (HGB), albumin (A), globulin (G), total protein (TP), cholesterol (CHOL), blood urea nitrogen, creatinine (C), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). The results showed that supplementing AMS in ewe diets generally enhanced ($p < 0.05$) final weight (Kg), gestational weight gain (Kg) and average daily weight gain (g). The values ranged from 32.00 – 45.25 Kg; 8.25 – 22.25 Kg; 207.79 – 267.86 g/d respectively. The feed conversion ratio improved with increasing levels of AMS with animals on D4 recording the least (0.20). Animals on D1 recorded the highest values for PCV, HGB, and RBC while those on D3 had the least. The values were 44.25 and 41.00 %; 14.68 and 13.40 g/dl; and 13.02 and 11.86 x 10^6/mm^3 respectively. Animals on D4 recorded the highest values for WBC (5150.50 x 10^3/mm^3). Serum biochemical responses of pregnant sheep indicated significant differences ($p < 0.05$) of AMS supplementation on TP, A/G ratio, ALP and creatinine values. Animals on D1 recorded the highest values for TP, A/G ratio; ALT, BUN and C while those on D2 were least except for ALT where D3 was least in ALT. The values were 8.68 and 8.15 g/dl; 0.80 and 0.65; 289 and 235.50 I.U./L; 10.75 and 9.75 mg/dl; 1.48 and 1.08 mg/dl respectively. Animals on D2 recorded the highest values for G, ALP and CHOL (4.80 mg/dl; 25.25 I.U/L; and 63.00 mg/dl respectively). Inclusion of AMS improved conversion ratio thereby improving the performance of ewes and thus proved that it could be tolerated up to 0.75 % supplementation in diets for ewes.

Keywords: Ewes, ammonium sulphate, haematology, serum, weight gain

Introduction

Non conventional feed resources available have been exploited for feeding livestock at all levels of production to make up for the shortages in conventional feedstuffs. Small ruminants (sheep and goats) in the humid zone of West Africa subsist on natural pastures and wastes which are high in fibre and lignin and low in crude protein contents (Aregheore, 2000). Ruminant nutritionists have long been interested in modulating the competition among different microbial populations with the objective of improving the efficiency of energy and protein

utilization in the rumen. In evaluating other alternatives to ionophore antibiotics due to its reduced social acceptance (Stanton, 2013) in order to alter microbial population and modulate rumen fermentation feed additives has become a necessary option to achieve optimization of diet or feed formulations. Tham *et al.*, (2008) reported that cassava leaf meal improved growth performance and feed conversion in cattle diets based on urea supplemented with rice straw. Urea is most common source of non protein nitrogen but the major problem is its rapid breakdown to ammonia in the rumen. Hence, there exists a need for feed supplement that allows the use of a higher proportion of non protein nitrogen in ruminant feed without the risk for ammonia toxicity. Rumen microorganisms can utilize inorganic sources of sulphur like ammonium sulphate which is a potential source of nitrogen and sulphur. It is more stable than urea for the release of ammonia (Firkins *et al.*, 1986) as well as a protein supplement low in content of soluble protein and high in bypass protein (Leng, 2008). There are several potential benefits from using ammonium sulphate as alternative non protein nitrogen in the diets of ruminants which includes potentially higher microbial growth and efficiency as ATP is generated in the reduction of nitrate to ammonia and the nitrate can substitute fermentable nitrogen in a low protein diet. Information on effect of ammonium sulphate on non pregnant ewes are scanty, therefore, this study was conducted to evaluate the effect of ammonium sulphate supplementation on the performance and blood chemistry of pregnant ewes.

Materials and methods

The study location was the Sheep unit of Teaching and Research Farm, University of Ibadan, Oyo state, South West Nigeria. The location is between latitude 7.27^0 N and longitude 3.45^0 E and 200 – 300 m above sea level. Found in the tropical rainforest zone, the daily temperature and humidity are within the ranges of 25^0 C - 32^{0}C 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.

The animals were housed individually in cleaned, disinfected pens having wood shavings spread on the floor as bedding material. A total of 16 dry ewes (West African Dwarf breed) were used together with 4 rams of proven fertility. The ewes aged 18 months and the rams above 18 months with initial average weight of 24.00 kg. Each treatment comprised four replicates and one ram was introduced into each treatment to run for one week through natural mating after synchronization of ewes with prostaglandin $F_{2\alpha}$.

Animals were treated with ivermectin super administered subcutaneously against ectoparasites and endoparasites which was repeated two weeks after. Long acting oxytetracycline (antibiotic) was also administered intramuscularly against bacterial infections. Animals were weighed before the commencement of the study and served with experimental diets in feed troughs at 5 % body weight on dry matter basis of concentrate and guinea grass (*Panicum maximum*). They were fed once a day at 0800 h. Feed intake was recorded daily and it lasted for 170 days including 15 days of acclimatization to period of last lamb was delivered.. Clean water and salt licks were also served throughout the study period.

Ammonium sulphate used as supplement in diet contained Iron (Fe) 0.001 %, Arsenic (As) 0.0005 %, loss on drying at 1.50^0 C (1%), residue of ignition (SO_4) (0.05%),

lead (Pb) (0.001 %), nitrate (NO_3) 0.002%, chloride (Cl) 0.002%, pH 5% solution (20°C) (4.50 – 6.00), Assay on dried substance (98.50 %).

Feed ingredients were ground, as shown in Table 1, before mixing to make a total mix ration (TMR) with 50:50 concentrate to roughage ratio without supplementation (Control/T1) and TMR with 0.25% (T2), 0.50% (T3) and 0.75% (T4) ammonium sulphate respectively.

Oestrus was synchronized in all the dry ewes with prostaglandin $F_{2\alpha}$ and observed closely. Rams were introduced 24 h post injection of prostaglandin $F_{2\alpha}$ and allowed to run with the ewes for 7 days. All ewes were weighed before mating and weekly until parturition after which weekly weight gain of ewes were recorded accordingly. Data generated included body weight gain in gestation (Kg), average daily gain (g/d) and feed conversion efficiency.

Table 1: Gross composition (%) of concentrate

Ingredients	T1	T2	T3	T4
Ammonium sulphate	0.00	0.25	0.50	0.75
Urea	1.00	1.00	1.00	1.00
Dry cassava peel	60.00	60.00	60.00	60.00
Brewers dry grain	23.00	23.00	23.00	23.00
Palm kernel cake	10.00	10.00	10.00	10.00
Dicalcium phosphate	1.00	1.00	1.00	1.00
Oyster shell	2.00	2.00	2.00	2.00
Salt	2.00	2.00	2.00	2.00
Premix (growers)	1.00	1.00	1.00	1.00
Total	100.00	100.25	100.50	100.75
Calculated CP %	8.42	8.46	8.50	8.54

On day 175, 5ml of blood samples were collected from each experimental animal according to standard procedures for haematology and serum biochemical analyses. Blood samples were determined for packed cell volume (PCV) and haemoglobin (HGB) using microhaematocrit method and cyanmethaemoglobin method respectively (Schalm *et al.*, 1975). Erythrocyte count (RBC) and leucocyte count (WBC) were determined using improved Neubauer haemocytometer after appropriate dilution (Schalm *et al.*, 1975). The mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated. Serum urea was determined by urease method and creatinine by Folin-wu filtrate methods (Toro and Ackerman, 1975). Serum total protein was determined by Biuret method (Kohn and Allen, 1995), while albumin was determined using the bromocresol (BCG) method (Peter *et al.*, 1982). Serum cholesterol was measured using appropriate laboratory kits (Gowenlock, 1988), aspartate amino tranferase (AST), alanine amino transferase (ALT) and alkaline phosphatise (ALP) activities were determined using spectrophotometric methods (McComb *et al.*, 1988; Rej and Hodder, 1983).

Total mix ration (TMR) samples comprising 50:50 concentrate to roughage ratio were dried in a hot air oven at 105°C for 24 h and ground to pass through a 1mm screen. Dry matter (DM), organic matter (OM), crude protein (CP) and ether extract (EE) were analysed according to AOAC

(1990). The acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (NDF) were analysed by the method of Van Soest *et al.*, (1991).

Data collected were subjected to one way analysis of variance according to the procedures of Statistical Analysis System (SAS, 1999) to determine the significance of treatment effects on the various parameters measured and where differences were observed, the means separated using Duncan Multiple Range Test of the same package.

Result and discussion

Chemical composition

Table 2 reveals the chemical composition of ammonium sulphate supplemented diets fed to pregnant WAD ewes. The dry matter ranged from 82.50 % to 88.30 %. The crude protein content (%) fell within the range of 8.60 – 9.21 %. The fibre fractions (NDF, ADF and ADL) ranged between 52.93% - 55.37%, 31.18% - 31.84% and 19.67% - 20.02% respectively.

The content of crude protein (CP) in the diets was higher than 7.00 % CP recommended being minimum requirement for ruminants in the tropics (NRC, 1981) but below the range for minimum protein requirement of 10 – 12 % recommended by ARC (1985) for ruminants. The CP range was higher than the requirements for ewes for first 15 weeks of gestation (8.02 %) and low for last 6 weeks of gestation (9.80 %) for ewes weighing 20 Kg (Ranjhan, 2001).

Table 2: Proximate composition and fibre fractions of ammonium sulphate supplemented TMR fed to pregnant WAD ewes

Parameter	T1	T2	T3	T4	SEM
Dry matter	86.50	82.50	85.80	88.30	2.13
Crude protein	8.60	9.00	9.10	9.21	0.17
Crude fibre	10.20	11.90	12.00	11.60	0.24
Ether extract	9.80	9.50	10.10	10.10	0.21
Ash	12.80	12.80	12.59	12.88	0.25
Nitrogen free extract	59.00	56.20	52.40	56.75	1.32
Neutral detergent fibre	52.93	55.37	55.23	55.22	1.25
Acid detergent fibre	31.63	31.51	31.84	31.18	0.12
Acid detergent lignin	19.67	20.02	19.98	19.70	0.08

T1: 0.00% AMS; T2: 0.25% AMS; T3: 0.50% AMS; T4: 0.75% AMS

Haematology

The haematological profile of pregnant WAD ewes fed ammonium sulphate supplemented diets and *Panicum maximum* are presented in Table 3. The packed cell volume (PCV) ranged from 41.00 % in T3 to 44.25 % in T1. The haemoglobin (HGB) fell within the range of 13.40 g/dl (T3) – 14.68 g/dl (T1). Red blood cell (RBC) and neutrophil (N) also followed the same pattern as in PCV and HGB with the following value ranges: 11.86 – 13.02 x 10^6/mm^3 and 23.00 – 31.00 %, respectively.

The white blood cell (WBC) ranged from 4312.50 x 10^3/mm^3 in T2 to 5150.50 x 10^3/mm^3 in T4. Significant effects of the supplement were observed for platelets, MCH and MCHC. The MCH refers to the average haemoglobin content of a single RBC while MCHC is reliable for establishing iron deficiency in animals. The values obtained in this study were within the physiological range of animals said to be normochromic/normocytic condition (Mitruka and Rawnsley, 1977).

The PCV value obtained in this report was

higher than that reported by Mitruka and Rawnsley (1977) who reported 31.50 – 36.70 % for female Dorset-Delane adult sheep weighing 40-60 Kg. This difference could be due to differences in breed, nutrition and physiological status (Onifade *et al.*, 1999). The varying levels of ammonium sulphate had no significant effect on haematological indices such as RBC, WBC, HGB, lymphocytes, neutrophils, and MCV.

Table 3: Haematological parameters of pregnant WAD ewes fed ammonium sulphate supplemented TMR

Indices	T1	T2	T3	T4	SEM
PCV (%)	44.25	42.00	41.00	43.50	1.19
HGB (g/dl)	14.68	14.00	13.40	14.45	0.40
RBC x 10^6/mm^3	13.02	12.34	11.86	12.81	0.24
WBC x 10^3/mm^3	4767.50	4312.50	4550.50	5150.50	182.05
Platelets (%)	68675^b	99250^a	81250^{ab}	95000^{ab}	0.43
Lymphocytes (%)	67.75	68.00	72.75	66.00	1.34
Neutrophils (%)	31.00	27.25	23.00	29.00	1.51
MCV (μ^3)	33.95	34.06	34.53	33.90	0.44
MCH (μg)	33.17^{ab}	33.54^a	32.70^b	33.23^{ab}	0.10
MCHC (%)	33.18^{ab}	33.33^a	32.68^{ab}	33.22^b	0.11

^{a,b,c} means with different superscripts in the same row differ significantly ($p < 0.05$)
T1: 0.00% AMS; T2: 0.25% AMS; T3: 0.50% AMS; T4: 0.75% AMS

Serum biochemical indices

Serum biochemical responses of pregnant ewes fed ammonium sulphate supplemented diets are presented in Table 4. The total protein (TP) ranged from 8.15 g/dl in T2 to 8.68 g/dl in T1. There was significant effect of treatment on TP. However, animals on Control (T1) were higher numerically than those on ammonium sulphate supplemented diets. Albumin (A) and globulin (G) ranged from 3.33 g/dl to 3.63 g/dl and 4.38 mg/dl to 4.80 mg/dl respectively. The A:G ratio which ranged from 0.65 in T2 to 0.80 in T1 showed significant difference. The liver enzymes (AST, ALP and ALT) ranged from 76.25 IU/L – 108.00 IU/L, 10.25 IU/L – 25.25 IU/L and 235.50 IU/L – 289.25 IU/L respectively. Except for ALT, other liver enzymes showed significant effect of treatment. Blood urea nitrogen (BUN) ranged between 9.75 mg/dl and 10.75 mg/dl. Creatinine (C) and cholesterol (CHOL) ranged from 1.08 mg/dl to 1.48 mg/dl and 57.75 mg/dl to 63.00 mg/dl respectively. Animals on T3 and T4 recorded lower values for C and CHOL when compared with T1 and T2. Significant difference was observed in creatinine implying that there was wastage of muscle energy especially for animals on T2 and foetus growth effect.

The values for TP, albumin, globulin, A/G, AST, C and CHOL reported in this study were within the range documented by Mitruka and Rawnsley (1977) for normal healthy sheep while ALP and BUN were below normal levels and ALT was far above normal level (Mitruka and Rawnsley, 1977). The decrease in blood protein fractions (TP, A and G) could be attributed to the increase in foetus weight (Abdel Rahman *et al.*, 2012), low digestibility (Abdel-Khalek *et al.*, 2000) and low urea concentrations.. Eissa *et al.*, (1992) had reported that serum AST and ALT concentrations increased with pregnancy progress of ewes and cows. This increased

activity of ALT may be due to more requirements of amino acids during milk production (Vihan and Rai, 1987).

Table 4: Serum biochemical responses of pregnant ewes fed ammonium sulphate supplemented TMR

Indices	T1	T2	T3	T4	SEM
Total protein (g/dL)	8.68[a]	8.15[b]	8.50[ab]	8.55[ab]	0.07
Albumin (g/dL)	3.63	3.33	3.60	3.63	0.06
Globulin (mg/dL)	4.38	4.80	4.75	4.68	0.08
A:G	0.80[a]	0.65[b]	0.70[ab]	0.70[ab]	0.02
AST (IU/L)	103.75[a]	76.25[b]	77.50[b]	108.00[a]	13.45
ALP (IU/L)	12.75[b]	25.25[a]	10.25[b]	24.75[a]	1.37
ALT (IU/L)	289.25	283.75	235.50	269.00	14.91
BUN (mg/dL)	10.75	9.75	10.00	10.00	0.20
Creatinine (mg/dL)	1.48[a]	1.08[b]	1.20[ab]	1.35[ab]	0.05
Cholesterol (mg/dL)	61.75	63.00	57.75	59.50	1.42

[a,b,c] means with different superscripts in the same row differ significantly ($p < 0.05$)
T1: 0.00% AMS; T2: 0.25% AMS; T3: 0.50% AMS; T4: 0.75% AMS

Gestational weights of experimental ewes

The gestational weights (Kg) of the ewes over a period of 22 weeks are presented in Table 5. The weights increased successively until the last week from an initial weight of 23.75Kg, 23.25Kg, 25.50Kg and 23.00Kg to 32.00Kg, 32.00Kg, 34.50Kg, and 45.25Kg respectively for T1, T2, T3, and T4. Significant effect was observed in weeks 1, 9 and 22. The animals on ammonium sulphate supplemented diets (T3 and T4) performed better than control (T1) in most of the weeks except for weeks 2, 5 and 8. The result shows that supplementation of ewes diets with ammonium sulphate improved the gestation weekly weights considerably. Thus, it proved that it can be regarded as a source of non-protein nitrogen and sulphur for body cell and tissue development.

Performance of ewes

The performance characteristics of WAD ewes fed dietary supplementation of ammonium sulphate are shown in Table 6. Initial weights of ewes ranged from 23.00 kg in T4 to 25.50 Kg in T3. The final weights ranged from 32.00 Kg in T1 and T2 to 45.25 Kg in T4. Final weight (Kg) of animals on T4 were significantly different ($p < 0.05$) when compared with others. However, the average daily dry matter intake (Kg DM) ranged from 4.13 Kg (T2) to 4.42 Kg (T4). The gestational weight gain (Kg) for animals on T4 (22.25 Kg) was highest ($p < 0.05$) while those on T1 and T2 were the lowest (8.25 Kg respectively). The average daily weight gain (g/d) ranged from 207.79 g/d in T1 to 267.86 g/d in T4. The feed conversion ratio was lowest (0.20) in animals on T4 and higher for those on T1 (0.53).

Tisdale (1977) reported that protein synthesis from addition of the following inorganic sources decreased in the following order: $(NH_4)SO_4 > S > Na_2SO_4 > K_2SO_4 > CaSO_4 > MgSO_4$. Ammonium sulphate and elemental sulphur were therefore the most effective in promoting the synthesis of rumen microbial protein and certain volatile fatty acids. Both nitrogen and sulphur supplementation are noted to improve performance by enhancing bacterial protein synthesis in the rumen and amino acids balance (Morrison *et al.*, 1990).

Table 5: Gestational weights of experimental ewes

Week	T1	T2	T3	T4	SEM
1	23.75^{ab}	23.25^{ab}	25.50^{a}	23.00^{b}	0.98
2	26.50	26.50	26.25	26.00	0.87
3	22.75	23.00	23.75	27.50	0.99
4	26.50	26.50	28.25	27.75	1.35
5	27.75	26.00	27.00	27.00	1.29
6	27.50	28.25	29.50	29.00	0.89
7	28.00	26.50	28.25	30.00	0.79
8	29.00	26.75	28.50	32.00	0.92
9	27.50^{b}	29.50^{ab}	31.00^{ab}	33.25^{a}	0.81
10	29.75	30.75	31.50	34.25	0.84
11	30.25	29.25	31.50	34.75	0.97
12	30.50	31.00	34.00	35.50	1.09
13	30.75	31.50	34.50	35.75	1.06
14	31.50	31.75	34.50	36.25	1.39
15	32.50	31.75	35.00	38.50	1.31
16	32.13	33.00	36.00	40.50	1.44
17	32.00	33.25	36.25	40.50	1.42
18	32.00	33.25	36.25	38.25	1.42
19	31.75	31.50	35.25	38.25	1.32
20	32.75	32.25	36.00	40.25	1.45
21	32.50	33.25	35.00	40.75	1.49
22	32.00^{b}	32.00^{b}	34.50^{ab}	45.25^{a}	1.29

^{a,b} means with different superscripts in the same row differ significantly ($p < 0.05$)
T1: 0.00% AMS; T2: 0.25% AMS; T3: 0.50% AMS; T4: 0.75% AMS

Table 6: Performance characteristics of WAD ewes fed dietary supplementation of ammonium sulphate

Parameter	T1	T2	T3	T4	SEM
Initial weight (Kg)	23.75^{ab}	23.25^{ab}	25.50^{a}	23.00^{b}	0.98
Final weight (Kg)	32.00^{b}	32.00^{b}	34.50^{b}	45.25^{a}	0.31
Gestational weight gain (Kg)	8.25^{b}	8.25^{b}	9.00^{b}	22.25^{a}	0.14
Average daily weight gain (g/d)	207.79^{c}	207.79^{c}	224.03^{b}	267.86^{a}	0.25
Total feed intake (kg)	668.82^{b}	636.02^{c}	660.66^{b}	680.68^{a}	0.21
Average feed intake (Kg/d)	4.33^{b}	4.13^{c}	4.29^{b}	4.42^{a}	0.12
Feed conversion ratio	0.53^{a}	0.50^{a}	0.48^{a}	0.20^{b}	0.17

^{a,b,c} means with different superscripts in the same row differ significantly ($p < 0.05$)
T1: 0.00% AMS; T2: 0.25% AMS; T3: 0.50% AMS; T4: 0.75% AMS

Conclusion

Supplementation of pregnant ewe diet with ammonium sulphate indicated its effect on total protein, albumin/globulin ratio, AST, ALP and creatinine. In addition, ammonium sulphate supplementation gave the highest dry matter intake and gestational weight gain especially at 0.75% inclusion level. However, since its supplementation produced no deleterious effects in the studied animals, ammonium sulphate is recommended for inclusion in ruminant diets especially at 0.75%.

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