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NUTRIENT UTILIZATION, RUMINAL MICROBIAL POPULATION AND FERMENTATION CHARACTERISTICS OF WEST AFRICAN DWARF (WAD) RAMS FED AMMONIUM SULPHATE FORTIFIED DIETS

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ABSTRACT

The ruminal microbial population has enormous potential for fibre digestion. Microorganisms are responsible for breakdown of plant cell walls, fungi have better ability to attack lignocellulosic tissues for bacteria to act on it. Ammonium sulphate, a chemical compound that serves as a ready source of nitrogen and sulphur, plays important roles in enhancing rumen microbial activity. Therefore, the effects of ammonium sulphate fortification on rumen fermentation characteristics and microbial populations were evaluated. Sixteen (16) West African dwarf (WAD) rams weighing 12.8 ± 0.12 kg were assigned to diets containing 0g/100kg (control diet), 250g/100kg, 500g/100kg and 750g/100kg levels of ammonium sulphate (T1, T2, T3 and T4 respectively) in a completely randomized design, each ram fed 5% body weight of the diet and wilted guinea grass in a 60:40 ratio for 105 days. At the end of the feeding trial, the effects of the diets on rumen microbial population and fermentation characteristics of the rams were assessed. The fungi population in sheep fed ammonium sulphate fortified diets ranged from $3.37 - 4.36 \times 10^4$ cfu/ml. Acetate, propionate and butyrate in the ammonium sulphate fortified diets ranged from 46.37 - 48.71, 29.41 - 33.25 and 21.00 - 24.75 mmole/100ml respectively. The acetate and butyrate of the ammonium sulphate fortified diets decreased with increasing inclusion levels of ammonium sulphate, while the propionate decreased. Results obtained showed that ammonia nitrogen, fungi, bacteria and acetate were 1.45ppm, 4.36 cfu/ml, 7.01 cfu/ml, 48.71 mmole/100ml respectively, and they were significantly ($P < 0.05$) highest in rams fed diet fortified with 750g/100kg ammonium sulphate. Rams on control diet had significantly ($P < 0.05$) higher values of protozoa, pH, acetate and butyrate (5.96 cfu/ml, 6.82, 48.71 mmole/100ml, 24.75 mmole/100ml) than rams on T2, T3 and T4 because the control diet is not fortified with ammonium sulphate. It was concluded that rams fed 750g/100kg of ammonium sulphate had greater number of rumen microbes which resulted in higher microbial protein synthesis, hence improved performance and higher body weight gain.

Keywords: Ammonium sulphate, rumen fermentation, microbial populations, fungi, WAD sheep

INTRODUCTION

Ruminants have a highly efficient anaerobic fermenting vat located at the beginning of their digestive tract, allows them to digest fibrous feed (Eryavuz *et al.*, 2003). When microbes' breakdown and digest plant fibre, they produce volatile fatty acids which are absorbed into rumen, supplying about 60 to 80% of the ruminant's energy. Microbes in the reticulo-rumen include bacteria, protozoa, fungi, archaea and virus (Kamra, 2005). Bacteria are the most numerous of these microorganisms and play a major role in the biological degradation of dietary fiber. *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens* recognized as the major cellulolytic bacterial species found in the rumen (Koike and Kobayashi, 2001; Koike *et al.*, 2003). Microorganisms are capable of invading and colonizing plant tissues; others

follow up to ferment the spoils of the invasion. The microbial mass synthesized in the rumen provides about 20% of the nutrients absorbed by the host animal, the composition of microorganisms is important (McDonald *et al.*, 1988). Interactions among microorganisms in the rumen are complex and often to the advantage of the host. Large protozoa population in the rumen has been shown to reduce animal productivity, possibly lowering the amino acid to energy ratio in the absorbed products of digestion. It appears that protozoa reduce the biomass of bacteria and fungi in the rumen of animals on diets with high fibre and this may eventually reduce the rate of digestion of the fibrous feeds (Preston and Leng, 1987). Increased knowledge concerning the rumen cellulolytic bacterial population will allow insight into the fiber-digestion capabilities of ruminant animals. A number of dietary factors influence rumen fermentation and microbial population dynamics: Basal roughage sources, physical form of feed and fermentation end-products (Wanapat, 2008). Local feed resources, particularly, low-quality roughages and agricultural residues are of prime importance to ruminants raised in the tropics (Orskov, 1999).

Ruminal fungi make up only 5-10% of microbes and are absent in diets poor in fibre. Despite their low numbers, the fungi still occupy an important niche in the rumen because they hydrolyse some ester linkages between lignin and hemicellulose or cellulose, and break down digesta particles. It has been well established that anaerobic fungi are a significant part of the population of fibre-digesting ruminal micro-organisms (Akin *et al.*, 1983). Ruminal fungi degrade lignified plant cell walls totally, indicating that their enzymes are able to hydrolyze or solubilise the entire plant wall. They produce high levels of cellulases and hemicellulases and are particularly proficient in producing xylanases, which are active against forage cell wall carbohydrate (Paul *et al.*, 2003). These enzymes are regulated by substrate (especially soluble sugars) available to the organisms. These organisms are better able to colonize and degrade the lignin-containing tissues than bacteria. They are unique among rumen microbes in that, they penetrate the cuticle. Residues after incubation with fungi are physically weaker than those incubated with whole rumen fluid or with rumen bacteria, suggesting that fungi could alter the fibrous residue for easier mastication by the animal (Akin and Hogan, 1983). Examples of fibre degrading fungi include: *Aspergillus niger*, *Geotrichum candidum*, *Neocallimastix frontalis*, *Sphaeromonas communis* and *Oikomonas speices* (Kamra, 2005). Rumen microbes and the host ruminant animal require many macro and micro minerals for normal growth and development. Among these minerals, sulphur is a necessary component of the amino acids; cystine and methionine are building blocks of proteins (Olafadehan *et al.*, 2014). Morrison *et al.*, (1990) found that sulphur supplementation increased the concentration of all three microbial groups but the most dramatic increase was observed with the number of sporangial forms of rumen anaerobic fungi which helps in initial fibre degradation. The objective of this study was to determine the effect of ammonium sulphate fortified diets on population of rumen microbes and rumen fermentation characteristics of West African Dwarf (WAD) sheep.

MATERIALS AND METHOD

The study was conducted in the Sheep Unit of the Teaching and Research Farm, University of Ibadan, Nigeria. The location is 7° 27'N and 3° 45' E at altitude 200-300m above sea level. The climate is humid tropical with mean temperature of 25-29°C and the average annual rainfall of about 1250mm. Sixteen WAD sheep, aged 6-10 months; with average body weight of 12.8 ±0.12 kg were used. On arrival, the rams were subjected to anti-stress and prophylactic treatments consisting of intramuscular injection of vigmoral multi-vitamin (1ml/10kg BW) and oxy-tetracycline (1ml/10kg BW). They were dewormed with Ivomectin ® (1ml/50kg BW) and

bathed with amitraz solution to eliminate ecto-parasites (ticks, lice and nuisance flies). As well, Tissue Culture Rinderpest Vaccine (TCRV) was administered to the rams to prevent Peste de Petit Ruminant (PPR) disease. The experiment lasted for 105 days excluding the 21 days of adaptation period.

Brewers dry grain (60kg), palm kernel cake (23kg), dicalcium phosphate (1kg), oyster shell (2kg), salt (2kg), growers premix (1kg), urea (1kg) and dry cassava peel (60kg) were mixed and 0g, 250g, 500g and 750g levels of ammonium sulphate were added on top of the feed ingredients for T1, T2, T3 and T4 respectively. The mixture was poured into a plastic mould measuring 14cm x 10cm x 5cm. Guinea grass (*Panicum maximum*) was harvested at the pre-anthesis stage at a height of about 20cm. The harvested grass was chopped into small bits (about 2-3cm), allowed to wilt for 2-3 days (for each cutting), baled into jute bags and kept in a well-ventilated room. Dried cassava peels, also preserved in jute bags, were kept in well-ventilated room.

The 16 WAD rams, balanced for body weight (BW) at the commencement of the study were randomly allocated to the four dietary treatment groups and fed the experimental diets in two installments at 0800h and 1600h on daily feed allowance of 5% BW at a ratio of 60:40 of diet to wilted guinea grass for the 105-experimental period after 21 days of adaptation period. Fresh water was supplied *ad-libitum*.

10ml of rumen liquor was collected from all the rams using suction tube after 105 days of the commencement of the growth trial. Rumen liquor was immediately evaluated for physical characteristics (Rosenberger, 1979); pH of the fluid was obtained with the aid of hand pH meter (pH 315i, German) with glass electrode. Ammonia-Nitrogen concentration in rumen was determined using Markham's distillation apparatus (AOAC, 2005). Acetate, propionate and butyrate were determined using UV-spectrophotometer (Prasad *et al.*, 2010). Fibre fraction was analyzed by the procedures of Van Soest (1994).

Data generated from parameters investigated were subjected to Analysis of Variance (ANOVA) using Statistical Analysis System (SAS, 1999). Significant differences between treatment means were separated using Least Significant differences (LSD) of the same package.

RESULTS

Table 1 shows the chemical analysis of experimental diets containing varying levels of ammonium sulphate.

TABLE 1: Chemical analysis of Ammonium sulphate fortified diet and *Panicum maximum*

PARAMETERS (%)	T1	T2	T3	T4	SEM	<i>P. maxi.</i>
Dry Matter	94.15	94.33	94.51	94.69	0.02	38.50
Crude Protein	11.00 ^c	11.80 ^c	12.60 ^b	13.40 ^a	0.02	7.81
Crude Fibre	10.51 ^c	11.25 ^b	11.99 ^b	12.73 ^a	0.01	30.72
Ether Extract	0.42 ^c	0.72 ^b	1.02 ^a	1.32 ^a	0.01	0.70
Ash	12.04 ^b	12.30 ^{ab}	12.59 ^a	12.80 ^a	0.02	8.94
NDF	30.35 ^d	31.71 ^c	32.67 ^b	34.06 ^a	0.02	60.00
ADF	25.20	26.58	27.96	29.34	0.01	38.00
ADL	5.81	6.18	6.20	6.24	0.03	7.00

a,b,c: Means within rows with unlike superscripts are significantly different from each other (P<0.05). T1: 0g/100kg (NH₄)₂SO₄, T2: 250g/100kg (NH₄)₂SO₄, T3: 500g/100kg (NH₄)₂SO₄, T4: 750g/100kg (NH₄)₂SO₄, SEM: Standard Error Mean, NDF: Neutral Detergent Fibre, ADF: Acid Detergent Fibre, ADL: Acid Detergent Lignin, *P. maxi. Panicum maximum*

Table 2 showed the digestibility and performance of WAD rams fed ammonium sulphate fortified diet. The feed intake decreases with increasing level of ammonium sulphate. DM digestibility ranged from 34.15 to 35.68% while CP digestibility ranged from 36.84 to 38.76%. The NDF and ADF tended to increase with increased ammonium sulphate fortification.

TABLE 2: Nutrient digestibility and body weight gain of WAD rams fed Ammonium sulphate fortified diets

PARAMETER	T1	T2	T3	T4	SEM
DMD (%)	34.15	34.66	35.17	35.68	0.03
CPD (%)	36.84	37.48	38.12	38.76	0.04
NDFD (%)	42.80	43.06	43.32	43.58	0.02
ADFD (%)	31.76	32.86	33.96	35.06	0.05
IBW (Kg)	12.25	12.08	12.25	11.75	0.37
FBW (Kg)	18.25	18.75	19.75	19.75	0.74
LWG (Kg)	6.00	6.67	7.50	8.00	1.08
DWG (g/day)	66.67	74.11	83.33	88.89	1.02

a, b, c: Means within rows with unlike superscripts are significantly different from each other (P<0.05). T1: 0g/100kg (NH₄)₂SO₄, T2: 250g/100kg (NH₄)₂SO₄, T3: 500g/100kg (NH₄)₂SO₄, T4: 750g/100kg (NH₄)₂SO₄, SEM: Standard Error Mean, NDF: Neutral Detergent Fibre, ADF: Acid Detergent Fibre, ADL: Acid Detergent Lignin, IBW: Initial Body Weight, FBW: Final Body Weight, LWG: Live Weight Gain, DWG: Daily Weight Gain.

TABLE 3: Rumen microbial population (x10⁴cfu/ml), physical properties and rumen liquor characteristics of WAD rams fed Ammonium sulphate fortified diets

PARAMETER	T1	T2	T3	T4	SEM
Total Bacteria	6.26 ^d	6.51 ^c	6.76 ^b	7.01 ^a	0.09
Total Fungi	3.37 ^d	3.70 ^c	4.03 ^b	4.36 ^a	0.75
Total Protozoa	5.96	5.77	5.58	5.39	0.06
pH	6.82	6.68	6.54	6.40	0.04
Rumen Liquor Temp. (°C)	38.52	38.64	38.76	38.88	0.04
Density (Kg/L)	0.64 ^d	0.76 ^c	0.88 ^b	1.00 ^a	0.01
NH ₃ -N conc. (mg/100ml)	0.61 ^d	0.89 ^c	1.17 ^b	1.45 ^a	0.03
Acetate (mmole/100ml)	48.71 ^a	47.93 ^b	47.15 ^c	46.37 ^d	0.01
Propionate (mmole/100ml)	29.41 ^d	30.97 ^c	31.97 ^b	33.25 ^a	0.03
Butyrate (mmole/100ml)	24.75 ^a	23.50 ^b	22.25 ^c	21.00 ^d	0.01

a, b, c: Means within rows with unlike superscripts are significantly different from each other (P<0.05). T1: 0g/100kg (NH₄)₂SO₄, T2: 250g/100kg (NH₄)₂SO₄, T3: 500g/100kg (NH₄)₂SO₄, T4: 750g/100kg (NH₄)₂SO₄, SEM: Standard Error Mean, Temp.: Temperature, NH₃-N conc.: Ammonia nitrogen concentration.

DISCUSSIONS

Analyses of several ruminal variables and their interactions are presented in Table 3. Ruminal pH, $\text{NH}_3\text{-N}$, temperature and density were significantly ($P < 0.05$) affected by the fortification of ammonium sulphate, indicating that the diets had different levels of fermentation. Also, rumen pH was lowest at 750g/100kg inclusion level of $(\text{NH}_4)_2\text{SO}_4$ (T4) while the pH of control diet (T1) was highest. This could be attributed to different inclusion levels of ammonium sulphate in the experimental diets. Accordingly, this could cause a less acidic rumen pH. The value for the acetate production ranged from 46.37 mmole/100ml (T1) to 48.71 mmole/100ml (T4) and was significantly ($P < 0.05$) different from each other. This suggested that feeding of ammonium sulphate did not affect the rumen environment adversely with regard to these parameters.

Dry matter is an indicator of the amount of nutrient available to the animal in a particular feed and it ranged from 94.15 - 94.69 %. The CP of the experimental diets ranged from 13.40% (T4) to 11.00% (T1). The values were within the values of 10-12% recommended by NRC (2007). The CP content of ruminant diet is an indication of nutritional quality of the diet since CP content is a very important index of nutritional quality of a feed. Thus, ammonium sulphate was added to the treatments to provide adequate sulphur requirement for anaerobic fungi to commence the digestion of the components of dietary fibre to produce volatile fatty acids (Okoruwa *et al.*, 2014). Values obtained for total ash were considerably different with highest of 12.80% (T4) and lowest of 12.04% (T1). The implication was that mineral content in 750g/100kg inclusion level of $(\text{NH}_4)_2\text{SO}_4$ (T4) was higher compared to other treatments in this study. Ether extract varied from 0.42% in T1 to 1.32% in T4. The neutral detergent fibre differed significantly ($P < 0.05$) in rams on 500g/100kg $(\text{NH}_4)_2\text{SO}_4$ (T4) compared with control diet (T1). Generally, digestible nutrient intake is a function of apparent nutrient digestibility and dry matter intake. Relative to urea with or without S supplementation is consistent with previous studies. Olafadehan *et al.* (2014) attributed improved intakes of digestible nutrients to greater digestibility of CP of the diet. The EE digestibility obtained ranged from 30.56 to 32.57%. Digestible DM and CP intakes showed the same trend as the digestible EE and ash, they were not statistically different from each other ($P > 0.05$). Results revealed no significant ($P < 0.05$) differences in acid detergent lignin. Enhanced neutral detergent fibre (NDF) digestion of $(\text{NH}_4)_2\text{SO}_4$ supplemented diets increased dry matter intake (DMI). Increasing the $(\text{NH}_4)_2\text{SO}_4$ level from control diet to 750g/100kg increased the digestibility of NDF from 42.80 to 43.58%. The result showed that sulphur helps in the proliferation of fungi and hence they colonize the more rigid lignocellulosic tissue of fibre (Gordon and Philips, 1998).

Thomas *et al.*, (1951) demonstrated that the addition of inorganic sulphate to a sulphur deficient purified ration improved weight gain and the nitrogen and sulphur retention of sheep. The tendency for a negative effect of control diet (T1) on live weight change may be due to reduction in muscular development as a result of depletion of the sulphur-containing amino acids necessary for formation of sulphur-amino acids (Onwuka *et al.*, 1992). Promkot *et al.*, (2007) reported that goats on low sulphur, cassava-based diets had the greatest weight losses as compared to sulphur supplemented groups. It appears that supplementation of sulphur with urea had tendency to improve feed utilization. Sulphur, being a precursor for the other S-containing amino acids (NRC, 2000), improved the quality of synthesized microbial protein or amino acid. It was shown by Ferreiro *et al.*, (1977) that addition of 1g ammonium sulphate per kg of fresh sugar cane improved daily gain significantly on a ration composed otherwise of only sugar cane and urea.

Microbial yield in the rumen is very important because, it is an index of the amount of microbial protein made available to the sheep daily (Kissada *et al.*, 2010). This is because sulphur is one of the important factors for fungi growth and proliferation (Gordon and Philips, 1998) that enhances microbial protein synthesis in the rumen. Low levels of sulphur supplementation in the diet containing cassava foliage could reduce microbial biomass in the rumen (Promkot *et al.*, 2007). Rams on T4 had the highest population of fungi because sulphur supplementation increased the concentration of all three microbial groups but the most dramatic increase was observed with the number of sporangial forms of rumen anaerobic fungi as shown in Table 3 which ranged from $3.37 - 4.36 \times 10^4$ cfu/ml. Fungi have an additional advantage of better penetration of the lignocellulosic feeds over the cellulose-degrading bacteria because of the presence of different fibre-degrading enzymes (cellulases, hemicellulases, xylanases, avicelases, glucosidases e.t.c.) found to be associated with rhizomycelia which enable it to degrade fibres (Paul *et al.*, 2003). Thus, ammonium sulphate was added to the treatments to provide adequate sulphur requirement for anaerobic fungi to maximally digest the components of dietary fibre leading to the production of energy to the animal (Okoruwa *et al.*, 2014). These results are consistent with the premise that increased rumen function due to addition of sulphur to the diet is due to increased microbial activity.

Bowen (2009) reported that rumen pH is an important factor that measures the acidity and alkalinity of rumen contents. Thus, for optimum rumen microbial fermentation, the rumen pH should lie between 6.00 and 7.00. However, the rumen pH values obtained in this study ranged from 6.40 (T4) to 6.82 (T1) and these were not significantly different from each other as shown in Table 3. However, the rumen pH values observed in this study lies within the range of values (6.00 - 7.20) for maximum microbial growth as reported by (Demba *et al.*, 2011).

The rumen liquor temperature ($^{\circ}\text{C}$) levels were different amongst treatments. It ranged from 38.52°C in T1 to 38.88°C in T4. This observed variation might be as a result of difference in evolution of heat from microbial fermentation activity in the diets. This is in consonance with the finding of (Adeyosoye *et al.*, 2010) who reported that the trends at which temperature rise in the rumen following ingestion of feeds is due to the evolution of heat in the fermentation process which has been used as a measure of microbial fermentation rate in the rumen. However, the rumen temperature values obtained in this study were within the relative constant range values ($38.00-40.00^{\circ}\text{C}$) for continuous microbial fermentation as reported by (Okoruwa *et al.*, 2014).

Fermentation in the rumen produces $\text{NH}_3\text{-N}$, and in the unionized state, ammonia is readily transferred across the rumen wall and may be excreted in the urine as ammonium salts, used in transamination to form glutamine or converted to urea in the liver and excreted or recycled back to the rumen (Emerick, 1988). $\text{NH}_3\text{-N}$ concentration in T4 (1.45 ppm) was significantly ($P < 0.05$) highest followed by T3 (1.17 ppm), T2 (0.89 ppm) and then T1 (0.64 ppm) as shown in Table 2. Also, it ranged from T1 (0.64 ppm) to T4 (1.45 ppm). The variation obtained in $\text{NH}_3\text{-N}$ concentration might be due to the sulphur and nitrogen combination from ammonium sulphate inclusion levels in the treatments which influenced the nitrogen intake by the rumen microbes. Isah *et al.*, (2014) reported that rumen $\text{NH}_3\text{-N}$ concentration has a good profile with values between 2 and 5 mg/100ml as a minimum rumen fluid to maximize rumen microbial synthesis, 15 mg/100ml rumen fluid to maximized fibre digestion and 20 mg/100ml rumen fluid to maximize intake. Notwithstanding, the $\text{NH}_3\text{-N}$ concentration values obtained in this study were

similar to the finding of Mohsen *et al.*, (2013) who reported that the most suitable rumen $\text{NH}_3\text{-N}$ concentration levels for microbial activities range between 5 and 20 mg/100ml.

Acetate concentrations were actually increased in the high concentrate diet with the addition of humates (HA) according to Varadyova *et al.*, (2009) and this is similar to the current findings with the addition of 0.75% $(\text{NH}_4)_2\text{SO}_4$ resulting in a higher acetate production that is used by ruminant for energy production. Nevertheless, this finding have resulted in improved animal performance in rams consuming 750g/100kg $(\text{NH}_4)_2\text{SO}_4$ than other treatments.

CONCLUSION

The problem associated with scarcity of forages that are available for ruminants during the dry season can be solved by adopting the use of ammonium sulphate fortified diets that are cereal based in compounded ration. This would results to production of feed at least cost and high nutrient profile for livestock productivity. The nutrient digestibility, weight gain and rumen fermentation characteristics obtained for the West African Dwarf rams in this study confirmed that ammonium sulphate fortified diets can be utilized up to 750g/100kg $(\text{NH}_4)_2\text{SO}_4$ inclusion level in compounded ration.

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