Trop. Anim. Prod. Invest. 19 (2): 26 - 33 (2016)
ISSN: 1115-2540
http://animalsci.agric.ui.edu.ng/TAPI/index.php/tapijournal/index

ORIGINAL RESEARCH ARTICLE

Volatile fatty acid and microbial load of West African dwarf rams fed ammonium sulphate-fortified diets

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ABSTRACT

Growing West African Dwarf (WAD) rams, aged between 6 and 8 months with mean body weight of 12.80 ± 0.12kg were used to predict rumen microbial population and volatile fatty acids (VFAs) for 105 days. The growing WAD rams were randomly allotted to four dietary treatments with four rams per treatment group in a completely randomized design. The compared experimental diets were: Each group was assigned to experimental diet shown below and ammonium sulphate at inclusion level of 0, 2.5, 5.0, 7.5g/kg $(NH_4)_2SO_4$ were added to it as T1, T2, T3 and T4 respectively. Results showed that, rumen ammonia nitrogen concentration (1.17mg/100ml), rumen bacteria (7.17cfu/ml), rumen fungi (4.47 cfu/ml), rumen pH (6.82) and propionate acid (33.25mmol/100ml) were significantly ($P < 0.05$) highest in growing rams on T4 compared to other treatment diets. Rumen temperature (38.95^0C), rumen protozoa (5.39ml), acetic acid (21.51%) and butyric acid (12.85%) were significantly ($P < 0.05$) better in growing WAD rams on T1. Propionate formation can be considered as a competitive pathway for hydrogen use in the rumen and it ranges from 19.25-22.41 mmole/100ml and is statistically difference because sulphate reducing bacteria compete with methanogenic archaea for hydrogen when sulphate is present. Also, ammonium sulphate can be referred to as anti-methanogenic compounds because it reduces the protozoal numbers in the rumen and it ranges from 5.36 to 5.95 ml. It was concluded that, ammonium sulphate fortified diets is a potential source of readily available nitrogen and sulphur which enhances the growth of microbial population due to their high solubility and ability to be rapidly degraded to NH_3 in the rumen thereby enhancing ruminant productivity especially when fed at 7.5g/kg $(NH_4)_2SO_4$.

Keywords: Ammonium sulphate, rumen microbes, volatile fatty acids, WAD rams

INTRODUCTION

Digestion processes in the rumen of sheep are dominated by the activities of very complex and variable microbial population (Smith, 1989). However, the most important thing to be aware of regarding sulfate-containing compound is optimum levels in the diet. Low ruminal sulfur concentration can also depress microbial growth (Bal and Ozturk, 2006). Despite this, there have been a lot of investigations on using various sulfate-containing compounds for improving performance and health of ruminants but there is lack of available data indicating sulfate-containing compound sources and levels depress methanogenesis in the rumen.

Notwithstanding, the rumen that form the largest part of the reticulorumen, serves as the primary site for microbial fermentation of ingested feed components. Gerard and Frederique (2006) reported that the nature of feed given to ruminant to support productivity is one of the several abiotic factors that can alter the balance of rumen microbial population and their activities which may lead to either decrease in performance or increase the risk of health problems. Volatile fatty acids (VFA) like acetic, propionic and butyric acids are end products of carbohydrate fermentation in the rumen. The various proportions of these VFA depend on type of diet fed (Perry, 1980) and the condition prevalent in the ruminal environment. However, some of these VFAs are better utilized than the others. However, ban on growth-promoting antibiotics necessitates the need for the production of animal proteins that are safe for human consumption. Hence, use of ammonium sulphate $(NH_4)_2SO_4$ becomes a viable alternative to growth-promoting antibiotics due to their ability to dissociate easily in the rumen (Mc Donald *et al.*, 1998). Therefore, the objective of this study was to investigate the

effect of ammonium sulphate-fortified diets on volatile fatty acid and microbial load of WAD rams.

MATERIALS AND METHODS

Experimental site: The experiment was conducted at the Small Ruminant Unit of the Teaching and Research Farm, University of Ibadan, Nigeria. The location is 7^0 27'N and 3^0 45'E at altitude 200-300m above sea level. The climate is humid tropical with mean temperature of 25-29^{0}C and the average annual rainfall of about 1250mm.

Experimental diets: Brewers dry grain (23 kg), palm kernel cake (10 kg), dicalcium phosphate (1kg), oyster shell (2kg), salt (2kg), growers premix (1kg), urea (1kg) and dry cassava peel (60kg) were mixed and 0g, 2.5g, 5.0g and 7.5g levels of ammonium sulphate were added to the feed ingredients.

Experimental Animals and Management: Sixteen growing West African Dwarf (WAD) rams, aged between 6 and 8 months with average initial body weight of 12.8 ± 0.12 kg were used for the study. The rams were purchased at Oranyan market and randomly allotted to 4 dietary treatments with 4 replicates per treatment and 4 WAD rams per replicate in a completely randomized design. On arrival, the WAD rams were given prophylactic treatments against ecto and endo parasites and allowed a period of 21days for acclimatization. Thereafter, they were individually housed in demarcated pens. The pens were adequately ventilated; cleaned daily and wood shavings were changed fortnightly. The experimental diets were fed to rams once daily at about 8:00am in the morning and guinea grass in the evening. Drinking water was provided *ad-libitum* throughout the experimental period. The experiment lasted for 105 days excluding the 21 days of adaptation period.

Rumen Study: Rumen fluid sample (40ml) was taken before feeding from four rams per treatment after 105 days. The rumen fluid sample was collected by means of suction tube thrust into the rumen compartment. As soon as the sample was obtained, rumen fluid temperature and pH were determined within two minutes of collection by using thermometer and digital pH meter, respectively. The digital pH meter was stabilised in distilled water with specific pH recommendation before used for the reading. 20ml of the rumen fluid samples was used for direct microscopic counts of rumen protozoa, bacteria and fungi. While the other 20ml sample of rumen fluid was bulked for each animal before made free of coarse particle by filtration with cheese cloth. Thereafter, 5ml sample of the filtrate was used for determination of volatile fatty acid fractions. The other 15ml of the filtrate sample was used for analysis of ammonia nitrogen ($NH_3 – N$) concentration.

Laboratory Analysis: Samples of the experimental diets ammonium sulphate fortified diets and guinea grass offered to rams were analysed for proximate composition using the procedure AOAC (1990). Microscopic counts of bacteria and fungi were determined using pour-plate method (Butterworth, 1964). Total number of protozoa was counted using microscope with the aid of a counter after staining with methylblue formalin solution (Keiji *et al.,* 1991). Rumen ammonia nitrogen concentration and total volatile fatty acids production was determined by steam distillation process using Markham micro-distillation apparatus as reported by Yusuf *et al.* (2013). Individual volatile fatty acids were determined using UV-spectrophotometer (Prasad *et al.,* 2010).

Statistical Analysis: Data obtained from rumen microbial population and volatile fatty acids components were subjected to analysis of variance (ANOVA) and significant difference between means were separated using least significant difference (LSD) using statistical analysis system (SAS, 2002).

RESULTS AND DISCUSSION

The proximate compositions (% dry matter) of the experimental feedstuffs (ammonium sulphate-fortified diets and guinea grass) are shown in Table 1. The result indicated that, the dry matter (DM) contents of the experimental feedstuffs and concentrate supplement that ranged from 94.05% to 94.91% were relatively high, suggesting the feeds can be stored for a longer period of time without spoilage. The crude protein value of ammonium sulphate-fortified diets ranges from 11.01% to 14.91% were above the 10% crude protein level recommended by Bengaly *et al.* (2007) for maximum growth in ruminant animals. Thus, 7.81% crude protein of guinea grass basal diets was added to the feedstuffs to provide adequate nitrogen requirement for rumen microbes to maximally digest the components of dietary fibre leading to the production of volatile

fatty acids (Okoruwa and Igene, 2014). Ash contents were considerably different in values, being highest in ammonium sulphate- fortified diets (12.04-12.80%) and lowest in

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

PARAMETERS (%)	T1	T2	T3	T4	SEM	*P. maxi.*
Dry Matter	94.90	94.73	94.05	94.91	0.25	38.50
Crude Protein	11.01[c]	11.95[c]	12.28[b]	14.91[a]	0.37	7.81
Ether Extract	0.62[c]	0.70[b]	0.98[b]	1.63[a]	0.01	0.70
Ash	12.04	12.30	12.59	12.80	0.02	8.94
NDF	30.35	31.71	32.67	34.06	0.01	60.00
ADF	25.20	26.58	28.80	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/kg $(NH_4)_2SO_4$, T2: 2.5g/kg $(NH_4)_2SO_4$, T3: 5.0/kg $(NH_4)_2SO_4$, T4: 7.5g/kg $(NH_4)_2SO_4$, SEM: Standard Error Mean, NDF: Neutral Detergent Fibre, ADF: Acid Detergent Fibre, ADL: Acid Detergent Lignin, *P. maxi: Panicum maximum*

guinea grass (8.94%), respectively. This implies that the mineral content present in ammonium sulphate fortified diets is highest compared to guinea grass. Ether extract of guinea grass fell within the same range with ammonium sulphate-fortfied diets which ranges between (0.62-1.63%), indicating fats and oil present in the experimental diets. Neutral detergent fibre values that ranged from 30.35% to 34.06% were considerably high and reflect high energy content of the feeds.

Presented in Table 2, is the rumen microbial population in growing rams fed experimental diets. The rumen fluid temperature (^{0}C) level was significantly (P<0.05) higher in rams on T1 (40.10^{0}C) and T4 (40.00^{0}C) compared with diet T2 (39.40^{0}C). This observed difference might be a reflection of the difference in evolution of heat from microbial fermentation activity in the diets. This is in agreement with the finding of Guobin *et al.* (2017) who reported that the trend 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 for bacteria in the rumen. Notwithstanding, the rumen temperature values obtained in this study were almost within the relative constant range values (38.00 to 40.00^{0}C) for continues microbial fermentation as reported by Guobin *et al.* (2017). The rumen ammonia nitrogen (NH_3 –N) concentration was significantly (P < 0.05) highest in T4 (1.17 mg/100ml), followed by T3 (0.89 mg/100ml) before T2 (0.76 mg/100ml). This variation obtained in NH3 – N concentration might be due to varying level of ammonium sulphate inclusions which influenced the nitrogen uptake by the rumen microbes. The rumen NH_3 – N concentration values (0.33 – 1.17 mg/100ml) obtained in this study fell in the range of values reported by Isah *et al.,* (2014). These authors reported that rumen NH_3 –N concentration had a good profile, with values between a minimum of 2mg/100ml and a maximum of 30mg/100ml suggested for maximum microbial growth in the rumen. Similarly, Lindela and Lewis (1995) also reported that ruminal NH_3 –N concentration has a good profile with values between 2 and 5mg/100ml as a minimum rumen fluid for maximize rumen microbial synthesis, 15mg/100ml rumen fluid to maximum fibre digestion and 20mg/100ml rumen fluid to maximize intake. However, the reported optimum rumen ammonia concentration ranging 5 – 20mg/100ml for the most suitable microbial activities by Leng and Nolan (1984) is also in consonance with the values reported in this study but contradict the suggested ruminal NH_3 –N concentration above 20mg/100ml that is required for sufficient voluntary intake of low quality roughage as reported by Yusuf *et al.* (2013).
Microbes yield in the rumen is very important because is an index or a function of the amount of microbial protein made available to the ruminant daily. Anaerobia fungi are reported to be the first

to reduce the tensile strength of feed particles and increase the particles breakdown in rumination, thus they are important initiators of fermentative breakdown of insoluble plant cell wall materials (Okoruwa *et al.,* 2013).

TABLE 2: Rumen microbial population (x10^4cfu/ml) of WAD rams fed Ammonium sulphate-fortified diets

PARAMETER	T1	T2	T3	T4	SEM
Rumen Liquor Temp. (^{0}C)	40.10	39.40	39.80	40.00	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)	0.33^d	0.68^c	0.89^b	1.17^a	0.08
Bacteria (cfu/ml)	6.67^d	6.76^c	7.01^b	7.17^a	0.09
Fungi (cfu/ml)	3.70^d	3.70^c	4.37^b	4.47^a	0.75
Protozoa (/ml)	5.95	5.80	5.62	5.39	0.06

$^{a, b, c}$: Means within rows with unlike superscripts are significantly different from each other (P<0.05). T1: 0g/kg $(NH_4)_2SO_4$, T2: 2.5g/kg $(NH_4)_2SO_4$, T3: 5.0g/kg $(NH_4)_2SO_4$, T4: 7.5g/kg $(NH_4)_2SO_4$, SEM: Standard Error Mean, Temp.: Temperature, NH_3-N conc.: Ammonia nitrogen concentration.

However, rumen fungi population that ranged from 3.37 to 4.47cfu/ml was significantly (P < 0.05) highest in T4 and lowest in T1. This variation could primarily influenced by the rate of attaching plant particles of the feed to size reduction. This agrees with the findings of Morrison *et al.* (1990) that enhanced fungal activity can cause a significant decrease in the resistance of plant particles to size reduction and then, the weakening and /or fragmentation of plant particles would also perhaps increase the surface area suitable for bacteria colonization and attack. Major changes in rumen protozoa population counts were observed in the study due to different levels of test ingredients inclusion in the diets. Marked significant (P < 0.05) higher difference was observed in rumen protozoa counts for rams on T1 (5.95 x 10^3ml) compared to rams onT4 (5.39 x 10^3ml). This increment in rumen protozoa counts with higher rumen pH in T1, undoubtedly result in an increased outflow of volatile fatty acids in the rumen as testify in Table 3. This further confirms the previous findings of Getachew and Makkar (2002) that volatile fatty acids are the major fermentation end products of increased outflow of protozoa population counts in the rumen. In addition, ruminal pH could be stabilize by stimulating ciliate entodiniomorphid protozoa which are known to engulf starch granules very rapidly and thus compete effectively with amylolytic bacteria for their substrate. Hence, starch is fermented by protozoa as a slower rate by amyloytic bacteria and the main end product is volatile fatty acid not lactate. However, rumen bacteria are the principal agents for fermenting plant cell wall carbohydrates, being the largest population of microbes in the rumen than protozoa and fungi. The rumen bacteria count values that ranged from 6.67 to 7.17 x 10^9ml was significantly (P < 0.05) highest in T4 and lowest in T1. The highest number of rumen bacteria counts observed in T4, might be responsible for high digestion of more protein and fibre by attaching the plant particle to provide more NH_3 – N concentration and total volatile fatty acids which enhance microbial activities. Bacteria count of rumen fluid is dependent in rumen NH_3 – N concentration and pH of rumen fluid and both depend on the type of diet (Yusuf *et al.,* 2013). This implies that, increasing lactate utilizing bacteria species (*Megashaera alsdenii* and *Selenomonas ruminantium*) population outnumber the lactating producing bacteria species (*Streptococcus bovis*) in T4, leading to less accumulation of lactate in the rumen that would have led to metabolic acidosis in the rams (Gozho *et al.,* 2005).

Chaucheyras e*t al.* (2008) also reported that the major – fibre degrading bacterial species which are *Fibrobacter succinogenes, Ruminoccus albus and Ruminoccus flavfaciens* are particularly increase in population under high rumen pH but sensitive to low rumen pH. However, the low

bacteria counting in T1 might indicate ammonia is limiting for bacteria growth and present of high protozoa count that encourage defaunation (Okoruwa *et al.,* 2013).

Presented in Table 3, is the rumen volatile fatty acids (VFAs) parameters of growing WAD rams fed ammonium sulphate fortified diets with guinea grass. The rumen pH values that ranged from 6.27 to 6.82 were significantly ($P < 0.05$) higher in T4. This difference could be attributed to the different inclusion levels of the test ingredient in the diets, specifically, increased in ammonium sulphate inclusion levels in the experimental diets would have reduced the rumen pH values. Ruminal pH is an important factor that measure the acidity and alkalinity of rumen content in ruminants and for optimum rumen microbial fermentation, the rumen pH should ranged between 6.00 and 7.00 (Bowen, 2009). Gozho *et al.* (2005) also reported that when the ruminal pH is low, microbial diversity is reduced as protozoa numbers may sharply declined and the bacterial population is altered and largely reduced. However, the rumen fluid pH values observed in T4 (6.82) compared favourably with the range values (6.00 to 7.00) for maximum microbial growth as reported by Lindela and Lewis (1995). Total volatile fatty acids was significantly ($P < 0.05$) higher in T4 (81.11mmol/litre) than T1 (75.37mmol/litre). This variation in the result might be connected with the inclusion levels of the test ingredients in the experimental diets, which increase the fermentation of the feeds offered which result in the production and accumulation of more total volatile fatty acids at low pH. It has been reported (Lindela and Lewis, 1995) that high production of volatile fatty acid in the rumen is linked with ruminal lysis of microbes and fermentation of microbial cell.

Yusuf *et al.* (2013) also reported that, if the volatile fatty acids production rate exceeds the clearance rate, volatile fatty acids will accumulate in the rumen; this may lower rumen pH and cause the metabolic disturbance known as rumen acidosis. However, there was no case of rumen acidosis in this study, meaning that the rumen pH was still within the normal range for the rams. Nagaraja and Lechtenberg (2007) reported that ruminal pH value that drop below 5.2 to 5.5 should be considered as the threshold for rumen acidosis in ruminants fed high concentrate diet. Volatile fatty acids are classified as one of the universal end – product of anaerobic microbial fermentation of carbohydrates in the rumen that contribute about 70% for the calories requirement of ruminants and the proportion of major partials of volatile fatty acid concentration in the rumen depends largely on the type of feed consumed by the animals (Dung *et al.,* 2011). The significant ($P<0.05$) higher value of acetic acid observed in rams on T1 (47.37mmol/100ml) compared to T4 (41.71mmol/100ml) might be due to the fact that they depend solely on experimental diets without ammonium sulphate fortification and guinea grass. Widiawati and Thalib (2009) reported that feeds resulting in increase of acetate production will promote an increase of methane and carbon dioxide production, which will represent a net loss of feed energy as well as inefficiency in feed utilization but opposite, was the case here, because as the inclusion levels of ammonium sulphate increases the acetic value decreases. The proportion of propionic acid was significantly ($P < 0.05$) highest in T4 (22.41mmol/100ml), followed by T3 (21.69 mmol/100ml), T2 (20.97 mmol/100ml) and T1 (19.25 mmol/100ml). Butyric acid proportion did not follow the same pattern of variation as observed in propionic acid. The butyric acid values ranged between 11.00 to 14.75% was significantly ($P < 0.05$) highest in T1 (14.75 mmol/100ml) and T2 (13.50 mmol/100ml) and T3 (12.25 mmol/100ml) but lowest in T4 (11.00 mmol/100ml). However, the highest proportion of propionic acids obtained in T4 revealed the better rumen fermentation of feeds by the rumen microbial activity to yield energy, while the low propionic acid of T1 could constrain rams productivity as propionic acid has been reported to be increased by concentrate diet and classified as the major precursors of glycogenic fatty acid in ruminants (Vasta *et al.,* 2009). The inverse relationship showed between acetic and propionic acids in this study further buttress the fibre and energy contents in the experimental diets. Notwithstanding, the significant ($P<0.05$) reduction in acetic and butyric in T4 (41.71mmol/100ml) and (11.00mmol/100ml) compared to T1 (47.37mmol/100ml and (14.75mmol/100ml) could probably be an indicative of increased bacteria activities. Moreover, it is interesting to note that the overall microbial counts in the rumen fluid for growing WAD rams on T4 could

be an indicative of normal rumen environment (normal pH, availability of NH_3 –N and volatile fatty acids) for microbial growth.

TABLE 3: Rumen Volatile Fatty Acids (VFAs) Parameters of Growing WAD rams fed Ammonium sulphate-fortified diets

PARAMETER	T1	T2	T3	T4	SEM
pH	6.27[d]	6.42[c]	6.68[b]	6.82[a]	0.06
Total VFAs (mmol/litre)	75.37[d]	77.62[c]	79.87[b]	81.11[a]	0.06
Acetate (mmol/100ml)	47.37[a]	45.15[b]	43.93[c]	41.71[d]	0.01
Propionate (mmol/100ml)	19.25[d]	20.97[c]	21.69[b]	22.41[a]	0.03
Butyrate (mmol/100ml)	14.75[a]	13.50[b]	12.25[c]	11.00[d]	0.01

[a, b, c]: Means within rows with unlike superscripts are significantly different from each other ($P<0.05$). T1: 0g/kg $(NH_4)_2SO_4$, T2: 2.5g/kg $(NH_4)_2SO_4$, T3: 5.0g/kg $(NH_4)_2SO_4$, T4: 7.5g/kg $(NH_4)_2SO_4$, SEM: Standard Error Mean, Temp.: Temperature, NH_3-N conc.: Ammonia nitrogen concentration.

This would be invariably led to increase in the production of microbial protein.

CONCLUSION

Ammonium sulphate $(NH_4)_2SO_4$ is a potential source of readily available nitrogen and sulphur which becomes a viable alternative to growth-promoting antibiotics due to their high solubility and ability to rapidly degraded to NH_3 in the rumen which enhances ruminant productivity. However, base on the result obtained in this finding, it was therefore concluded that feeding of ammonium sulphate fortified diets with guinea grass offered a balance of essential nutrient requirement for growing WAD rams. Thus, ammonium sulphate fortified diets at 7.5g/kg can be recommended as an appropriate feeding strategy to improve growing WAD rams' performance without any negative effects on rumen microbial population and volatile fatty acids in growing WAD rams.

CONFLICT OF INTEREST

There is no conflict of interest in the conduct and publication of this research work.

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