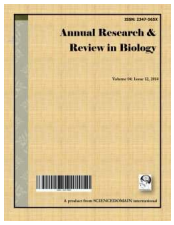

Annual Research & Review in Biology
10(4): 1-7, 2016, Article no.ARRB.25775
ISSN: 2347-565X, NLM ID: 101632869

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Evaluation of Dietary Supplementation of Ammonium Sulphate on *in vitro* Gas Production and Rumen Fermentation Characteristics of WAD Ewes

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Authors' contributions

This work was carried out in collaboration between all authors. Author TOO designed the study and wrote the protocol. However, author SAS anchored the field study, gathered the initial data and performed preliminary data analysis. Authors TOO and UAI managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2016/25775
Editor(s):
(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Andell Edwards, University of Trinidad and Tobago, Trinidad and Tobago.
(2) Ozdal Gokdal, Adnan Menderes University, Turkey.
(3) R. E. Barde, College of Agriculture Lafia, Nigeria.
Complete Peer review History: http://sciencedomain.org/review-history/14885

Original Research Article

Received 19th March 2016
Accepted 20th April 2016
Published 2nd June 2016

ABSTRACT

Ammonium sulphate, a chemical compound was utilized to evaluate its potential through *in vitro* gas production technique and rumen fermentation characteristics as a rumen stimulator and mitigates methane gas. Four experimental diets were formulated with ammonium sulphate (AS) included at varying levels: T1 (control/0% AS), T2 (control diet + 0.25% AS), T3 (control diet + 0.50% AS) and T4 (control diet + 0.75% AS). Rumen fluid was collected from the ewes, sixteen in number and weighing 24 kg averagely, using suction tube method to evaluate *in vitro* gas production, determine microbial population and rumen fermentation characteristics. Gas production was determined over a 96 hour period. Other data collected included organic matter digestibility (OMD%), short Chain Fatty Acids (SCFA µml), Metabolisable Energy (ME MJ/KJ DM), Degradability (D%), Volatile Fatty Acids (VFA), rumen pH, temperature and ammonia nitrogen (AN). Results showed no significant differences ($p>0.05$) for *in vitro* gas production profiles (i.e. total gas, OMD, ME, SCFA). However, as inclusion increased it stimulated the parameters

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measured to be numerically higher than control. Methane gas was highest for control diet (4 ml/200 mg DM) while T2 recorded the least (2 ml/200 mg DM). Degradability increased as the inclusion level of AS increased. Control diet was the lowest (32%) while T4 had the highest (45%). The rumen fermentation characteristics showed significant ($p<0.05$) differences for all the parameters measured except rumen temperature. Animals on T4 diet recorded the highest ($p<0.05$) pH value (6.85) while those on T2 diet had the least (6.53). Animals on Control diet recorded the highest values ($p<0.05$) for AN and acetic acid (100.40 mg/l and 8.98 mol/100L) while the least values were observed in animals on T2 diet (AN) and those on T4 (acetic acid). Increase inclusion of AS caused a decrease in acetic acid. Rumen bacteria was highest ($p<0.05$) in animals on T4 (1.40 x 10^6cfu/mm^3) and lowest in those on T3 (0.65 x 10^6cfu/mm^3). For fungi and protozoa, animals on T4 and T2 diets were higher ($p<0.05$) respectively than those on Control diet (1.15 and 1.09 x 10^6cfu/mm^3 compared to 1.10 and 0.85 x 10^6cfu/mm^3 respectively). Supplementation of AS has been validated by this study, with a 0.75% inclusion level resulting in lowest methane gas, and higher ME, SCFA, pH, bacteria and fungi and may be recommended for ruminant feeding.

Keywords: Ewes; ammonium sulphate; in vitro; rumen fermentation; rumen microbes.

1. INTRODUCTION

Apart from volatile fatty acids and microbial proteins, methane gas and carbon (IV) oxide (CO_2) are also released into the atmosphere as a result of rumen microbial fermentation. Enteric fermentation from livestock is a large source of methane, which has a global warming potential 23 times than that of CO_2 [1]. Ruminant livestock estimate for enteric methane is estimated to be 17-37% [2]. Hence, a need to mitigate methane is of utmost importance due to its effect on the environment and man. Several feed additives have been utilized to suppress this gas such as probiotics, antibiotics, plant extract and essential oils. Methanogenesis can be reversed by using sulphate as the terminal electron acceptor [3]. This submission had earlier been reported that methane gas is decreased immediately following the addition of sulphate [4,5]. It had been suggested the more energetically favourable nitrate and sulphate as having potential for high affinity electron acceptors competing with CO_2 for hydrogen and thus maintaining the availability of oxidized cofactors generated in fermentative degradation of carbohydrates, which at the same time lowers methane production [6]. The product of nitrate and sulphate reduction is the ammonium ion and sulphate ion respectively. Hence, this study was carried out to evaluate the effect of ammonium sulphate on *in vitro* gas production, degradation of diets and rumen fermentation characteristics of WAD sheep.

2. MATERIALS AND METHODS

2.1 Experimental Site

The study was carried out at the sheep unit of Teaching and Research Farm and in the laboratories of the Department of Animal Science, University of Ibadan, Oyo State, Nigeria.

2.2 Collection and Preparation of Experimental Diets

The feed ingredients as presented in Table 1 were sourced from the locality dried and ground before mixing to make a total mix ration (TMR) with 50:50 concentrate to roughage (*Panicum maximum*) ratio without supplementation (T1) and TMR with 0.25% (T2), 0.5% (T3) and 0.75% (T4) ammonium sulphate respectively. Ammonium sulphate was gotten from a chemical store. Sixteen dry ewes aged 18 months with initial average weight of 24.00±0.13 kg were randomly allocated so as to ensure uniformity in the four treatments.

2.3 Rumen Fermentative Characteristics and *In vitro* Gas Production Procedure

About 100ml of rumen liquour was collected before morning feeding from each ewe with the aid of suction tube as described by [7]. One portion was used for determination of temperature and pH immediately after collection using a portable thermometer and digital pH meter respectively. The liquor was further processed by straining and the filtrate was analyzed for ammonia-nitrogen using micro kjedahl method [8], volatile fatty acid was determined on HPLC system (Shimadzu IOAD, Japan) according to procedure described by [9]. Carbon dioxide (CO_2), Hydrogen sulphide (H_2S) and methane (CH_4) were analyzed for. Total viable bacteria, protozoa and fungi were enumerated by the method of [10,11].

For the *in vitro* gas production, incubation procedure was as reported by [12] and carried out at 39±1℃ and the volume of gas production was measured at three hourly intervals up to 96 hours. Rumen liquor was obtained with the help of a stomach tube, transferred into pre heated thermos flask, strained through a sieve cloth and flushed with CO_2. The buffer containing 9.8 g $NaHCO_3$ + 2.77 g Na_2HPO_4 + 0.57 g KCl + NaCl + 0.12 g $MgSO_4.7H_2O$ + 0.16 g $CaCl_2.2H_2O$ was used and kept in the incubator for warming prior to being mixed with rumen fluid (1:4) as inoculums, all under continuous flushing with streams of CO_2. About 200 mg of the substrate (sealed within porous bags) was measured and introduced into the syringe after removing the plunger. At post incubation period, 4 ml of NaOH (10m) was introduced to estimate methane production as reported by [13]. Data for gas production were fitted to an exponential equation as proposed by [14]: $GP = a + b (1 – \exp^{-ct})$. Where: GP = Gas production (ML) at time t; a + b = Gas Potential Production; C = Rate of gas production (ML/h). The organic matter digestibility (OMD%), short chain fatty acids (SCFA mmol/200 mg), metabolisable energy (ME; MJ/Kg DM) and degradability were calculated using formula – OMD% = 14.88 + (0.889*GV) + (0.45*CP) + (0.651*XA); SCFA= -0.00425 + (0.0222*GV ml); ME= 2.20 + 0.136*GV + 0.057*CP + 0.0029*CP2 and Degradability= (Initial weight of substrate – Final weight after incubation)/Initial weight*100%.

2.4 Chemical and Statistical Analysis

Dried and ground samples of the experimental diets were used for chemical analysis. Crude protein, crude fibre, ether extract and ash were determined according to methods of [8]. Fibre fractions were determined using the methods of [15]. The values are as presented in Table 2. Data collected were subjected to one-way analysis of variance according to the procedure of [16] to determine the significance of treatment effects on the various parameters measured and where differences were observed, the means were separated using Duncan Multiple Range Test of the same package.

Table 1. Gross composition (%) of experimental diets

Ingredients	T1	T2	T3	T4
Ammonium sulphate	0.00	0.25	0.50	0.75
Urea	1.00	1.00	1.00	1.00
Dried cassava peels	60.00	60.00	60.00	60.00
Brewer dried grains	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

T1 – Control; T2 – Control + 0.25 ammonium sulphate; T3 – Control + 0.50% ammonium sulphate and T4 – Control + 0.75% ammonium sulphate

Table 2. Chemical composition (%) of ammonium sulphate supplemented diets and *Panicum max*

Treatment	T1	T2	T3	T4	*P. maximum*
DM	86.50	82.50	85.80	88.30	38.50
CP	8.60	9.00	9.10	9.2.1	9.42
CF	10.20	14.90	17.00	11.60	35.50
EE	9.80	9.50	10.10	10.10	0.70
ASH	12.80	12.80	12.59	12.88	10.38
NFE	59.00	56.20	52.40	56.75	46.00
NDF	52.93	55.37	55.23	55.22	44.00
ADL	19.67	20.02	19.98	19.70	6.19

P. maximum – Panicum maximum; DM – Dry Matter; CP – Crude Protein; CF – Crude Fibre; EE – Either Extract; NDF – Neutral Detergent Fibre; ADL – Acid Detergent

3. RESULTS AND DISCUSSION

3.1 *In vitro* Gas Production and Fermentation Characteristics

Table 3 shows the *in vitro* fermentation characteristics of ammonium sulphate diets at 96 hr. The potential of gas production 'b' was highest in T2 (10.50 ml) while the lowest was recorded for T4 (6.38 ml). The advantage of 'b' is for predicting feed intake and can account for 88% of variance intake [17]. It is well known that gas production is basically the result of fermentation of carbohydrate to volatile fatty acids [18]. Low (a+b) recorded in T1 (7.50 ml) was probably due to absence of ammonium compound. The highest inclusion level of ammonium sulphate did not produce the highest volume of gas and this supports the finding of [19] who reported that protein fermentation does not lead to much gas.

3.2 Methane Gas Production and Degradation

Methane (ml/200 mg DM) production for 96 hr as shown in Fig. 1 ranged from 2 ml – 4 ml / 200 mg DM, with the least being from T2 and the highest from T1. Increased inclusion of ammonium sulphate in the diet resulted in increase in methane gas that was still lower than control (T1). It had been reported that methane increased with increase in ammonium sulphate application rate, caused by competition for substrates between sulphate – reducing bacteria and methanogens [20] and this agrees with our finding.

Shown in Fig. 2 was the result of dry matter degradability (%) of ammonium sulphate supplemented diets (TMR). Ammonium Sulphate (AS) supplementation was effective in stimulating degradability as Increased inclusion of AS

Table 3. *In vitro* fermentation characteristics of ammonium sulphate supplemented diets at 96 hr

Parameter	T1	T2	T3	T4	SEM
A	1.00	1.25	1.50	1.50	0.12
B	6.50	10.50	10.25	6.38	1.28
a+b	7.50	11.75	11.75	7.88	0.40
C	0.11	0.10	0.04	0.96	1.31
Y	3.75	3.75	3.25	3.50	0.46
OMD (%)	32.40	35.48	35.90	33.58	1.14
ME (MJ/kg DM)	3.53	4.16	4.27	4.84	0.18
SCFA (mmol/L)	0.12	0.22	0.22	0.33	0.03

T1 – Control; T2 – T1 + 0.25%AS; T3 – T1 + 0.50%AS; T4 – T1 + 0.75%AS

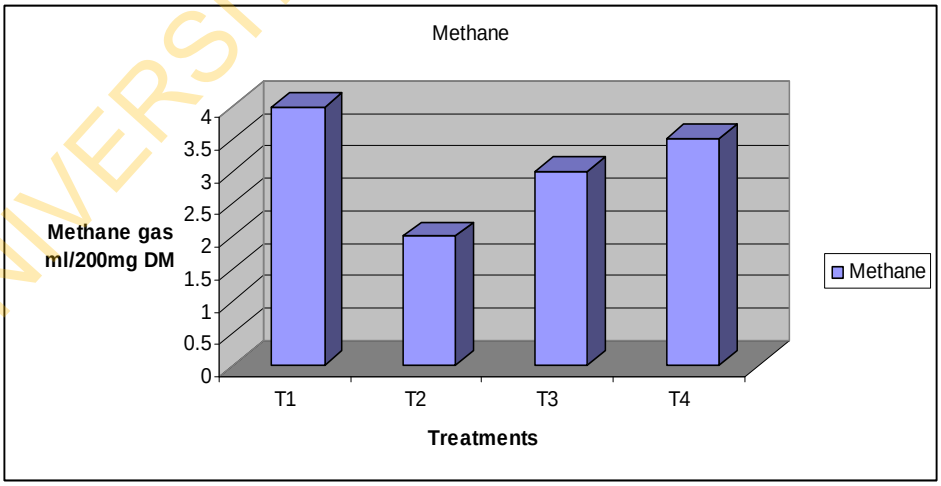

Fig. 1. Methane gas (ml/200 mg DM) at 96 hours incubation

T1 – Control; T2 – T1 + 0.25%AS; T3 – T1 + 0.50%AS; T4 – T1 + 0.75%AS

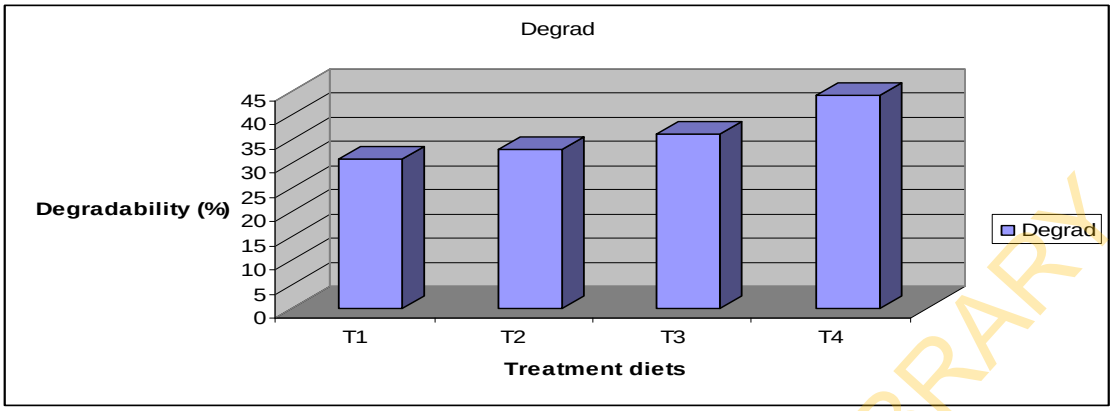

Fig. 2. Degradability (%) of ammonium sulphate supplemented diets
T1 – Control; T2 – T1 + 0.25%AS; T3 – T1 + 0.50%AS; T4 – T1 + 0.75%AS

indicated that it stimulated the rumen bacterial proliferation thereby resulting in a greater rate of degradation. The present result was in agreement with those of [21,22]. Bacteria can use carbohydrates as carbon skeletons for proteins in combination with ammonia [23] and also utilize sulphur (organic and inorganic) to synthesize sulphur containing amino acids [24] to produce microbial protein.

3.3 Rumen Fermentation and Microbial Count

Table 4 shows the rumen fermentation and microbial count of WAD Sheep fed ammonium sulphate supplemented diets. Rumen temperature did not differ significantly ($p > 0.05$) across the treatments and was close to the range for sheep in the tropics (39 – 40℃). However, significant ($p<0.05$) difference was observed in rumen liquor pH amongst supplemented diets. Ewes on T4 recorded the highest (6.85) while those on T2 had the lowest (6.53), but the values recorded were within the level for optimum ruminal fermentation. The ammonia-nitrogen was highest ($p < 0.05$) for animals on the control diet (100.40 mg/L) while those supplemented with AS were lower compared to control. The reason may be attributed to higher ammonia-nitrogen uptake by microbes in the AS supplemented animals. This observation is accordance with the submission of [25] of reduced ammonia nitrogen in sulphate supplemented diets.

The volatile fatty acids were significantly ($p < 0.05$) different across the treatment diets with animals on T2 recording the highest (14.19 mol/100ml) and the least was observed for those on T4 (13.11 mol/100 ml). Increased inclusion of AS elicited a decrease in VFA values and this was also seen in acetic acid values. The propionic values were higher for AS

Table 4. Rumen fermentation parameters and microbial count of WAD sheep (ewes) fed ammonium sulphate supplemented diets

Parameter	T1	T2	T3	T4	SEM
Temperature (℃)	38.88	38.88	38.38	38.35	0.11
pH	6.74^{ab}	6.53^{b}	6.69^{ab}	6.85^{a}	0.04
NH_3-N (mg/l)	100.40^{a}	71.00^{d}	89.00^{c}	91.00^{b}	0.34
VFA (mol/100 ml)	14.12^{b}	14.19^{a}	13.27^{ab}	13.11^{ab}	0.09
Propionic (mol/100 ml)	2.55^{ab}	2.66^{b}	2.60^{ab}	2.82^{a}	0.69
Butyric (mol/100 ml)	2.58^{ab}	2.72^{a}	2.48^{ab}	2.50^{ab}	0.67
Acetic (mol/100 l)	8.98^{a}	8.80^{b}	7.96^{ab}	7.79^{c}	0.08
Acetic acid: Propionic acid	1:4	1:3	1:3	1:3	-
Bacteria (x 10^6 cfu/ml)	1.15^{c}	1.30^{b}	0.65^{d}	1.40^{a}	0.00
Fungi (x 10^3 cfu/ml)	0.85^{c}	1.09^{a}	0.81^{d}	0.90^{b}	0.00
Protozoa (x 10^3 cfu/ml)	0.85^{c}	1.09^{a}	0.81^{d}	0.90^{b}	0.00

a,b,c,d means with different superscript within the same row are significantly (p<0.05) different

supplemented diets over control indicating a shift in molar proportion of acids and the ability to reduce methane gas.

Ewes fed diet T4 had the highest ($p<0.05$) bacterial population ($1.4x10^6$ cfu/ml) while those on T1 recorded the least ($1.15x10^6$ cfu/ml). The animals on AS supplemented diets showed an increase in bacterial population as AS inclusion increased over those on control (T1) diet except for those on T3. This is suggestive of AS stimulating the proliferation of bacterial population.

4. CONCLUSION

This study has validated the potential of ammonium sulphate as a feed additive in ruminant nutrition. Supplementation of ammonium sulphate led to increase in rumen fermentation, efficiency of rumen microbes and stimulating them which occasionally higher *in vitro* gas production, and fermentation characteristics. The animals were able to tolerate 0.75% inclusion level of ammonium sulphate, hence it is recommended for supplementation in ruminant feeding.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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