EFFECT OF INTRARUMINAL INFUSSION OF SATURATED AND UNSATURATED FATTY ACIDS ON ORGANIC MATTER DEGRADABILITY, TOTAL VOLATILE FATTY ACID AND METHANE PRODUCTIONS IN WEST AFRICAN DWARF SHEEP

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ABSTRACT

This study describes the effect of intraruminal infusion of different proportions of palmitic (saturated fatty acid) and linolenic (unsaturated fatty acid) on rumen degradability of organic matter fraction of Pennisetium purpureum, total volatile fatty acid and total methane productions in West African Dwarf sheep. Five combination proportions of palmitic and linolenic acids viz: 70 % palmitic acid + 30 % linolenic acid, 30 % palmitic acid + 70 % linolenic acid, 50 % palmitic acid + 50 % linolenic acid, 100 % palmitic acid + 0 % linolenic and 0 % palmitic acid + 100 % linolenic acid designated treatments A-E respectively served as the experimental treatments. These treatments were intraruminally infused into five (5) adult WAD sheep of average body weight of 13.49 ± 1.63 kg and the trial performed in a 5 x 5 latin square experimental design. A sixth group of four sheep, that did not receive any fatty acid infusion, served as the control group. The in-sacco technique for degradability studies was adopted in the determination of organic matter disappearance from the rumen at time intervals of 4, 8, 12, 24 and 48 hours in both the experimental and control groups. Appropriate mathematical model for estimation of total volatile fatty acid (VFA) and total methane production were used for determination of VFA and methane productions. The result of the study showed that organic matter degradability was significantly (p < 0.01) highest in treatment A (70 % palmitic acid + 30 % linolenic acid) at 24 hours (84.63 ± 8.6 %) and 48 hours (88.42 ± 4.8 %) compared to other treatments and the control. Higher proportion of linolenic acid (treatments B and E) significantly (p<0.01) reduced potential OM degradability at 48 hours with values at 41.08 ± 5.5 % and 23.92 ± 2.4 % respectively. Total VFA production was significantly (p <0.01) increased in treatment A at 24 hours (3.59 ± 0.07 m mol/l) and 48 hours (3.62 ±0.04 m mol/l) compared to other treatments and the control. At same time post incubation, total methane production was significantly (P<0.01) decreased in treatments B (0.39 ± 0.01 mol/hr) and E (0.34 ± 0.006 mol/hr) compared to treatments A (0.52 ± 0.01 mol/hr) which recorded a significant (P<0.01) increase. The study revealed that high proportion of unsaturated fatty acid suppressed rumen fermentation with resultant decrease in organic matter degradability, total VFA and methane productions. The reverse was however the case with high proportions of saturated fatty acids.

Keywords: Fatty acids, Degradability, Volatile fatty acid, Methane, WAD sheep

INTRODUCTION

Fat and fatty acid metabolism and digestion in ruminants particularly the dairy cows are of considerable interest. This renewed interest is based on several reasons, first, the use of dietary fat supplements by nutritionists to increase the energy density of diets to meet requirements of the high producing dairy and beef cows; second, it is now recognized that fatty acids, both of dietary and rumen origin, can have specific and potent effects on ruminant metabolism and human health (Doreau *et al*., 1997) and third, we now recognize that specific fatty acids produced in the rumen are potent regulators of rumen function and milk fat synthesis in the mammary gland (Bauman and Grinari, 2003). These therefore pose a great challenge in the understanding of the optimal and satisfactory dietary level of fats *vis-à-vis* saturated and unsaturated fatty acid proportions, within the rumen and probably in

the postruminal segments of the gastrointestinal tract, that would not be detrimental to rumen functions and other physiologic indices of fat digestion and utilization particularly in the West African Dwarf sheep.

Energy value of fat supplements varies (Shingfield *et al*., 2003). The variability in net energy for lactation (NEL) among fat supplements has been described as a function of the long chain fatty acid content and digestibility (Borsting and Weisberg, 1989). Digestibility of these fatty acids can be influenced by dry matter intake and amount of fat consumed as well as the characteristics of the supplemental fat (degree of saturation) (Elliot *et al.,* 1999). Degree of fatty acid unsaturation is probably the most important characteristic that influence rumen fermentation and intestinal digestibility of fats (Grummer *et al.,* 1990; Nestle *et al*., 1994). Iodine value (IV) is an indicator of the degree of unsaturation. The higher the IV, the greater the degree of unsaturation. Digestibility may decrease if the IV is below 45 (Firkins and Eastridge, 1994). Maximal digestibility of fats with an IV greater than 40 was 89 % compared with 74 % for fats with IV less than 40 (Jenkins, 1994). Thus saturated fatty acids are less digestible (within the intestine) than unsaturated fatty acids and the difference is greater when predominantly saturated fats are supplemented (Borsting *et al*., 1992). These indicated that unsaturated fatty acids may have synergistic effect on the digestibility of saturated fatty acids.

There are also possibilities that some synergistic interaction between saturated and unsaturated fatty acids occur within the rumen and may exert influence on rumen functions such as microbial population and activity, rumen fermentation (VFA, methane, carbon dioxide and other intermediates) rate of passage and fatty acids pool (Harfort and Hazlewood, 1988). If so then, the extensive metabolism of lipids in the rumen (hydrolysis and biohydrogenation of polyunsaturated fatty acids) would be affected by the proportions of the saturated to unsaturated fatty acids in the rumen at any given time and thus may have major impact on the profile of fatty acids available to the ruminant animal in milk and tissues.

Unsaturated fatty acids are toxic to many rumen bacteria (Baumgard *et al*., 2000). This is however checked by rumen biohydrogenation of the polyunsaturated fatty acids (PUFA). Therefore, the understanding of this toxic level of unsaturated fatty acids is very pivotal to the understanding of the influence of degree of saturation of fatty acids in ruminant nutrition. This therefore refers to the quantitative intake, digestion and metabolism of fatty acids in ruminants with respect to dietary saturated and unsaturated fats. This quantitative model could be useful in studying the physiologic effect of fatty acids in different segments of the gastrointestinal tract of ruminants. Against this background, this study was aimed at investigating the effect of different combination proportions of saturated and unsaturated fatty acid on rumen functions with emphasis on organic matter degradability, total volatile fatty acid and total methane productions in the West African Dwarf sheep.

MATERIALS AND METHODS

Nine West African Dwarf sheep were purchased from Ibagwa market in Igbo-Eze Local Government Area of Enugu State. They were weighed, dusted with an ectoparasite powder (pif-paf) and introduced into the Veterinary Farm, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were further dewormed using levamisol and ivermectin. They were also given prophylactic treatment for trypanosomosis. They were adapted on free range grazing for 21 days. After the adaptation period their body weights were recorded before implantion of the rumen fistula. Following a 24 hour fasting, rumenotomy was performed as described by Remi-Adewumi *et al*. (2006), and rumen fistulation as described by Santra and Karim (2002) and Aka and Kamalu (2005).

Pennisetium purpureum which was oven dried and milled, fine granules were used for the *in-sacco* degradability of organic matter (Aregbede *et al*., 2002). The experiment was designed in a 5 X 5 Latin square model of five sheep, five treatments and three replicates of *P. purpereum*. The proportions of fatty acids used are as follows: 70 % palmitic acid + 30 % Linolenic acid (treatment A), 30 % palmitic acid + 70 % Linolenic acid (treatment B), 50 % palmitic acid + 50 % Linolenic acid (treatment C), 100 % palmitic acid + 0 % Linolenic acid (treatment D), 0 % palmitic acid + 100 % Linolenic acid (treatment E). Three WAD sheep without any fatty acid infusion served as the control group. Each of the fatty acid treatments was infused daily for 10 days and the study performed during the last 2 days. A cross over period of 5 days was allowed for the Latin square model. The chemical composition of the forage sample *P. purpereum* was determined by the AOAC (1990).

The disappearance of the OM fraction from the nylon bags at various incubation time was fitted to Orskov and McDonald (1979) equation thus: $P = a + b (1-e^{ct})$, where p = level of potential degradability, a = immediate soluble fraction, b = water insoluble

but rumen fermentable fraction on time (t), c = rate of degradation of b and t = duration of incubation.

Total rumen volatiles fatty acid production was determined as described by Kennedy and Milligan (1978). Volatile fatty acid production and methane production in the rumen were related to the amount of organic matter (OM) apparently degraded in the rumen (g/d) using the equation: V = 0.00809D + 2.903 and M = 0.00277D + 0.273, where D = OM degradability, M = quantity of methane produce, V = volatile fatty acid, M is measured in mol/hr and V is measure in mMol/l of rumen fluid.

In this experiment the OM for rumen degradability of *P. purpereum* was used to estimate the total VFA and methane production at various time intervals of incubation following the intraruminal infusion of the various proportions of the fatty acids

Statistical Analysis: The results were analyzed statistically by the one-way analysis of variance (ANOVA). Treatment means for OM degradability, VFA and methane productions at 48 hours were tested for significant variation at $p < 0.01$ using the least significant difference (LSD) of mean comparison (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Table 1 showed the chemical composition of the forage (*P. purpureum)* used for the study. The organic matter content of *P. purpureum* was 80.53 %. The fraction of 80.53 % that was soluble in water (i.e. 'a' fraction) and the rumen degradable fraction (i.e. 'b' fraction) out of the 80.53 % organic matter of the forage sample (*P. purpureum*) are shown in table 2.

Table I: Chemical composition (% of dry matter) of *Pennisetium purpureum*

Chemical Constituent	Percentage proportion of Dry matter
Dry matter	92.03
Crude protein	12.80
Organic matter	80.53
Ash	11.50
Crude fiber	22.13
Ether extract	4.56
Gross energy (kcal/g)	3.47
Nitrogen free extract	43.78
Calcium	0.30
Phosphorus	0.36
Potassium	5.34
Sodium	1.86

The immediate soluble fraction 'a' was the same for all the treatments. That is, 2.16 ± 0.12 % of OM of *P. purpureum* was soluble in water, and since same forage was used for all treatments, the value was constant. The percentage disappearance of organic matter from the rumen varied between 4 to 48 hours of incubation. The rumen degradable OM was least in treatment E (100 % linolenic) with a value of 21.76 ± 2.41 at 48 hours post incubation. This was followed by 39.72 ± 4.04 in treatment B (70 % linolenic + 30 % palmitic) compared to other treatments that had 86.26 ± 4.75 % (treatment A – highest); 67.86 ± 3.961 % (treatment C – 50 % palmitic + 50 % linolenic), 58.17 ± 4.57 % (treatment D – 100 % palmitic + 0 % linolenic) and 68.24 ± 3.65 % (control). The general observation here was that organic matter degradability was reduced as the proportion of unsaturated fatty acid increased.

The potential degradability of *P. purpureum* when 70 % palmitic + 30 % linolenic acid was infused was significantly ($p < 0.01$) higher than all treatment groups and control, with a value of 84.63 ±8.6 % and 88.42 ± 4.8 % at 24 and 48 hours respectively (Table 3). This was followed by a potential degradability of 64.72 ± 5.0 % and 66.22 ± 4.9 % at 24 and 48 hours respectively, when 50 % palmitic + 50 % linolenic acid (treatment C) was infused. There was no significant difference in the potential degradability (PD) with treatment C and the control group, which had a PD of 61.80 ± 4.9 % and 70.40 ± 3.7 % at 24 and 48 hours respectively. The results show that higher levels of unsaturated fatty acids (30 % palmitic + 70 % linolenic and 100 % linolenic acids) significantly reduced PD with values at 41.08 ± 5.5 % for treatment B and 23.92 ± 2.4 % for treatment E. At 100 % saturated fatty acid infusion potential degradability at 60.33 ±4.6 % was significantly reduced compared to treatments A, C and the control. This trend indicated that organic matter degradability was markedly improved at high proportion of saturated fatty acid than at high proportions of unsaturated fatty acids. Equal proportions of saturated and unsaturated fatty acids gave rise to improved rumen degradability especially at prolonged incubation period compared to the control and treatment A.

These observations probably was as a result of these proportions of fatty acids on the: cellulolytic bacterial population in the rumen; accumulation of lactic acid in the rumen; and changes in intramminal pH. The addition of fats and oils to animal diets particularly ruminants has produced conflicting result with regard to rumen degradability of feed fractions and energy utilization (Van Soest, 1963). In this study where potential rumen degradability was significantly reduced in treatments B and E (high level of unsaturated fatty acid), it is probable that the activity of cellulolytic bacteria were inhibited by high

Table 2: Water solubility ('a') and rumen degradable ('b') organic matter content of *Pennisetium purpureum* in WAD sheep intraruminally infused with varied ratios of palmitic and linolenic acids

Fatty Acid proportions	a	4 hours	8 hours	12hours	24 hours	48 hours
A	2.16 ± 0.12	46.95 ± 2.47	60.43 ± 1.62	70.08 ± 8.75	80.47 ± 6.43	86.26 ± 4.75
B	2.16 ± 0.12	8.74 ± 0.84	19.83 ± 2.99	21.48 ± 4.57	30.67 ± 6.29	39.72 ± 4.04
C	2.16 ± 0.12	37.74 ± 0.49	42.80 ± 2.27	54.87 ± 4.22	62.56 ± 5.01	67.86 ± 3.96
D	2.16 ± 0.12	26.78 ± 1.29	30.75 ± 2.84	33.64 ± 3.29	48.17 ± 4.41	58.17 ± 4.57
E	2.16 ± 0.12	4.63 ± 0.49	6.78 ± 2.10	8.86 ± 1.97	16.73 ± 4.48	21.76 ± 2.41
Control	2.16 ± 0.12	43.47 ± 2.82	44.87 ± 3.09	56.61 ± 4.41	59.44 ± 5.09	68.24 ± 3.65

Table 3: Potential degradability (%) (a+b) of OM fraction of *Pennisetium purpureum* in WAD sheep intraruminally infused with varied combination ratios of palmitic and linolenic acid

Fatty Acid Proportions	% potential degradability of P. purpureum OM at time t				
	4 hours	8 hours	12 hours	24 hours	48 hours
A	40.51 ± 20.5	62.59 ± 1.6	73.64 ± 7.3	84.63 ± 8.6^{a}	88.42 ± 4.8^{a}
B	10.96 ± 0.8	22.00 ± 3.0	24.23 ± 3.6	32.83 ± 6.3^{ab}	41.08 ± 5.5^{ab}
C	39.90 ± 0.5	44.96 ± 2.3	57.03 ± 4.2	64.72 ± 5.0^{b}	66.22 ± 4.9^{b}
D	28.94 ± 1.3	32.91 ± 2.8	35.80 ± 3.3	50.33 ± 4.4^{abc}	60.33 ± 4.6^{abc}
E	6.79 ± 0.5	8.94 ± 2.1	11.10 ± 2.1	18.89 ± 4.5^{c}	23.92 ± 2.0^{4c}
Control	45.76 ± 2.8	47.03 ± 3.1	58.77 ± 4.4	61.80 ± 4.9^{b}	70.40 ± 3.7^{b}

a, ab, b, abc, c= means with different superscript are significantly different at p<0.01

proportions of unsaturated fatty acids either by a coating effect or direct toxic effect. Increased dietary unsaturated fatty acids have been associated with reduced fermentative activity (Leng, 1993). Eastridge (2002) had pointed out that unsaturated fatty acids inhibit cellulolytic bacteria and rumen fermentation. It is also a known fact that cellulolytic bacteria have a low metabolic rate and hence population changes are also slow (Leek, 2004). Therefore, rate of regeneration once inhibited is slow. It could be that once the coating effect or toxic effect were exerted on the cellulolytic bacteria their rate of regeneration became reduced and hence their decreased ruminal population and activity.

Another possible mechanism by which the potential degradability was decreased by high proportions of unsaturated fatty acids could be by changes in rumen pH. The pH optimum of celluloytic bacteria is 6.2 to 6.8 (Leek, 2004). Under the experimental condition, it could be that rumen pH reduced to a level that compromised optimal activity of the cellulolytic bacteria at high unsaturated fatty acid levels. At reduced rumen pH, the conversion of lactic acid and metabolic acids to propionate was reduced (Yang *et al.,* 2002). A situation that could lead to further reduction in rumen pH and possibly negative impact on cellulolytic bacteria. Since experiments with high fat diets have noted ketosis and inefficient use of energy (Van Soest, 1963), accumulation of lactic acid in situation of high dietary or supplemental level of unsaturated fatty acid could be responsible for poor OM degradability in the rumen. Increased supplemental fat or when grains are fed in high amount have been demonstrated to increase intraruminal acidity (Huhtanen and Svseinbjornsson, 2006). Changes in intraruminal pH are known to decrease ruminal microbial population of both the cellulose fermenting and non-fermenting bacteria such as *S. ruminantium, P. ruminocola and B. fibrisolvens* (Russell and Wallace, 1988). *S. ruminantium* also occurs in quite high proportions in the rumen, especially when high amounts of cereal grains or fats are fed (Russell and Wallace, 1988). This species does not degrade cellulose or hemicellulose, but utilize the intermediary products such as cellodextrins and xylo-oligosaccharides (Paynter and Elsden, 1970). Vitamin depletion has been shown to affect proliferation of desirable groups of bacteria in the rumen of animals fed poor quality feeds of high fibre and fatty acid content (Van Gylswyk, 1994). The major cellulose-digesting bacteria, as well as others, have absolute requirement for a range of vitamins (Stack and Hungate, 1984). Low concentration of these vitamins in the rumen could have resulted from moderate to high level of unsaturated fatty acid and very high (100 %) level of saturated fatty acid infusion. Low concentration of vitamins in the rumen has been shown to limit fibre degradability (Harfoot and Hazlewood, 1988). Fatty acid content of diets has been shown to affect the rate of capture of vitamins and minerals contained in ruminant diets (Komisarczuk-Bony *et al.,* 1994).

The cellulolytic bacteria incorporate the straight and branch chain volatile fatty acid (Bc-VFA) mainly into n-C13 to n-C7 straight and branch chain

Table 4: Total volatile fatty acid (VFA) in (mol/hr) in WAD sheep fed *Pennisetium purpureum* and intraruminally infused with varied combination ratios of plamitic and linolenic acid

Fatty Acid Proportions	Total VFA (mmol/l) at time t				
	4 hours	8 hours	12 hours	24 hours	48 hours
A	3.30 ± 0.02	3.41 ± 0.01	3.50 ± 0.06	3.59 ± 0.07^{a}	3.62 ± 0.04^{a}
B	2.99 ± 0.006	3.08 ± 0.01	3.10 ± 0.03	3.17 ± 0.05^{b}	3.24 ± 0.03^{b}
C	3.23 ± 0.004	3.2 ± 0.02	3.36 ± 0.03	3.43 ± 0.04^{c}	3.44 ± 0.04^{c}
D	3.14 ± 0.010	3.17± 0.02	3.19 ± 0.03	3.32 ± 0.04^{c}	3.39 ± 0.04^{c}
E	2.96 ± 0.004	2.97 ± 0.02	2.99 ± 0.02	3.06 ± 0.04^{b}	3.10 ± .02^{ab}
Control	3.27 ± 0.022	3.28 ± 0.05	3.38 ± 0.04	3.40 ± 0.04^{c}	3.47 ± 0.03^{c}

a, ab, b, c, d, = means within column (24 and 48 hours) with different superscripts are significantly different at p<0.01.

Table 5: Total methane production in WAD sheep intraruminally infused with varied combination ratios of palmitic and linolenic acid

Fatty Acid proportions	Methane Production (mol/d)				
	4 hours	8 hours	12 hours	24 hours	48 hours
A	0.41 ± 0.007	0.4 ± 0.004	0.48 ± 0.02	0.50 ± 0.01	0.52 ± 0.01^{a}
B	0.30 ± 0.002	0.33 ± 0.008	0.34 ± 0.009	0.36 ± 0.02	0.39 ± 0.01^{b}
C	0.38 ± 0.001	0.40 ± 0.006	0.43 ± 0.01	0.45 ± 0.01	0.46 ± 0.01^{c}
D	0.35 ± 0.004	0.36 ± 0.007	0.37 ± 0.009	0.41 ± 0.01	0.44 ± 0.01^{c}
E	0.29 ± 0.001	0.29 ± 0.02	0.30 ± 0.006	0.34 ± 0.05	0.34 ± 0.006^{d}
Control	0.40 ± 0.008	0.40 ± 0.008	0.44 ± 0.01	0.44 ± 0.01	0.47 ± 0.01^{c}

a, b, c, d= means within column at 48 hours with different superscript are significantly different at p<0.01.

fatty acids and aldehydes as part of the lipid component of bacterial cells (Wegner and Foster, 1963). It has been suggested that the long-branch chain acids and aldehydes lend fluidly to the lipids of the cellulolytic rumen bacteria as is the case for unsaturated long chain straight acids in aerobic organisms (Allison and Byrant, 1963). In the rumen and other anaerobic environment, there is a strong tendency towards saturation of double bounds due to reducing conditions. The special need for fluidity in the lipids of the cellulolytic bacteria could indicate that it is concerned with cellulolysis. Thus increased infusion of unsaturated fatty acid perhaps incorporate high branch chain fatty acids into the microbial fatty acid pool, which could have led to cellulolysis hence a decreased OM degradability.

Finally, increased unsaturated fatty acid infusion or saturated fatty acid infusion beyond 70 % intraruminally could have exerted undesirable effect on OM degradability by obstructing the uptake of minerals by the rumen bacteria. Growth and activity of *F. succinogenes* and *R. flavefaciens* are known to be dependent on availability and uptake of phosphorus, calcium and manganese within the rumen (Komisarezuk-Bony *et al.,* 1994). Concentrations below 15mg/l for P and 5mg/l for Mg reduced growth and cellulose degradability by *R flavefaciens* (Komisarczuk-Bony et *al*; 1994). This could still be another mechanism by which the fatty acid infusion, at the observed level of reduced OM degradability, exerted their effects.

Though these suspected mechanism discussed here were not studied in this research work, the fact that optimal infusion level of saturated fatty acid (70 %) and unsaturated fatty acid (30 %) had positive effect on OM rumen degradability, beyond which OM degradability was reduced, has been established in this study. Efforts should be made to closely study the underlying mechanism by which high proportions of unsaturated fatty acid reduces organic matter degradability in the rumen.

The total volatile fatty acid (VFA) production was highest is treatment A (70 % palmitic + 30 % linolenic) with a value of 3.59 ± 0.07mmol/l and 3.62 ± 0.04mmol/l at 24 and 48 hours respectively (Table 4). These values were significantly ($p < 0.01$) different from all other treatment groups and control. The VFA in control with a value of 3.47 ± 0.03 mmol/l though significantly less than treatment A, was significantly higher than treatment B (30 % palmitic + 70 % linolenic) and treatment E (100 % linolenic acids). Thus at high unsaturated fatty acid proportions VFA production was significantly reduced compared to other treatments and control. There was no significant difference in VFA production between treatment C with a value of 3.44 ± 0.04 mmol/l compared to treatment D, with a value of 3.39 ± 0.04 mmol/l and the control with a value of 3.47 ± 0.03 mmol/l. These trends suggest that higher proportions of unsaturated fatty acids, beyond 50 %, depressed total VFA production while high proportion of saturated fatty acid not in excess of 70 % increased

total VFA production. It was however observed that at 100 % proportion of saturated fatty acid total VFA was depressed. This, in comparison with 70 % proportion of saturated fatty acids, shows that an optimal level of saturated fatty acids (probably 70 %) is required to support increase in total VFA production in the rumen. That is to say that all saturated or all unsaturated fatty acids were inimical to volatile fatty acid production. Therefore, some forms of interactions between these two fatty acids, are probably required for improved volatile fatty acid production in the rumen... These findings indicate a direct and definitive effect of degree of saturation of dietary fatty acids on total rumen VFA. The more saturated the free fatty acid concentration in the rumen are, the more rumen VFA are produced. This trend which was same for OM degradability indicating a direct relationship between organic mater degradability and rate of volatile fatty acid production. This observation agreed with report of Reynolds *et al*. (2003) and Kristensen (2005). These workers established linear relationship between OM degradability and rumen volatile fatty acid production in steers intraruminally infused with volatile fatty acids. In the analysis of Banik *et al.* (2006), ruminally digested starch was on the average 2.64 and 3.11 kg/d for roughage and concentrate diets respectively and the concentration of VFA in the rumen was 0.22 and 0.31 mol/day for the respective diets, thus indicating a direct relationship. Brown *et al.* (2002) observed high VFA concentration with increase starch degradation with higher propionate and lower acetate proportions. Yang *et al*. (2002) have also demonstrated even higher propionate proportions (740 mMol) VFA when the rate of rumen degradation of starch was improved by defaunation. In the light of these, the suggested possibilities for increased or decreased OM degradability probably apply to increased or decreased volatile fatty acid respectively in this study, thus suggesting a direct relationship between OM degradability and rumen VFA production. Furthermore, VFA production in the rumen has been related to the microbial yield in the rumen (Leng, 1993). The most important concept that bears on the feeding strategies used for ruminants is that microbial protein available and total volatile fatty acids produced in the rumen are inversely related (Brown *et al*., 2002). This arise because under the anaerobic condition of the rumen, the feed nutrients provide both the substrate for microbial cell synthesis and also the potential energy as ATP generated through conversion of feed nutrients to VFA. The efficiency of microbial growth in the rumen appears to be highly dependent on the feeding conditions. The factors that affect microbial growth efficiency and therefore the protein relative to VFA available for digestion and absorption are:

a) A deficiency of any microbial factor (e.g. ammonia, sulfur, phosphorus, amino acid etc.) in the feed or induced some time after feeding in rumen liquor because of rapid absorption of nutrient (Leng, 1993).

b) The relative amounts of carbohydrate and protein that are fermented (fermentative degradation). A high protein to carbohydrate ratio in the diet can lead to a relatively low microbial protein to VFA ration in the end products of fermentative digestion where the dietary protein is easily and rapidly fermented in the rumen (Orskov and McDonald, 1979).

The observed differences in the VFA at different proportions of saturated and unsaturated fatty acids probably resulted from the effects of those fatty acid proportions on the microbial growth factors in the rumen as well as relative fermentative degradation of carbohydrate and protein fractions of the feed.

The relative decrease in VFA production in the control group compared to treatment A showed that in the control condition, the proportions of these fatty acids in the rumen does not support maximum VFA production. In general, changes in rumen pH at different fatty acid combination proportions could be the primary reason for the observed effect on OM degradability and VFA production. The pH at which bacteria grow can affect the fermentation pattern (Hobson and Summer, 1972) and this affects different bacteria differently (Mueller-Harvey and Reed, 1992). Low rumen pH not only in part but sometimes totally abolishes cellulose fermentation (Khazoal and Qrskov, 1994) leading to poor VFA production. It also exposes the animals to problems of acidosis, acetonaemia, laminitis and other feed associated problems. Increase in VFA production stimulates the growth of cellulolytic microorganisms (Palmquist and Eastridge (1991). This again may lay credence to the direct relationship between OM degradability and VFA production as observed in this study.

It has therefore been demonstrate from this study that unsaturated fatty acids are more inhibitory to cellulolytic activity than saturated fatty acids especially in relation to OM degradability and VFA production. This agreed with the observations of Eastridge (2002), Palmquist and Eastridge (1991) and Firkins and Eastridge (1994), that positive ruminal function in relation to OM degradability and VFA production was exhibited at a maximum rumen infusion level of 70 % saturated fatty acid and 30 % unsaturated fatty acid of the NRC (1996)

recommended fat intake (i.e. 3-5 % DM intake/day). Beyond or below this combination proportions OM degradability and VFA production are likely to be inhibited.

At 48 hours, total methane production was highest at 0.52 ± 0.01 mol/ hr when 70 % palmitic + 30 % linolenic (treatment A) was infused (Table 5). This was significantly ($p < 0.01$) different from the total methane production in the control that had a value of 0.47 ±0.01 mol/hr. Total methane production was however significantly reduced in treatment B (30 % palmitic + 70 % linolenic acids) and treatment E (100 % linolenic acid) compared with the control. At the same 48hr incubation period, there was no significant difference in the total methane produced between treatment C (50 % palmitic + 50 % linolemic), treatment D (100 % palmitic) and the control. The general observation was that methane production was suppressed at high proportions (100 % and 70 %) of unsaturated fatty acids.

Unsaturated fatty acids have been shown to be toxic to rumen bacteria particularly the methanogenic bacteria (Philipson, 1970; Eastridge, 2002). This has been evidenced in this study. The relative decrease in methane production at high proportion of unsaturated fatty acids suggests reduction in energy loss and vice-versa. This is so because methane is a high energy compound and its increased production and elimination represents the loss of about 8 percent of the total digestible energy of the diet (Leek, 2004). These results show that methane production was suppressed at high proportions of unsaturated fatty acids. Unsaturated fatty acids have been shown to be toxic to rumen bacteria particularly the methanogenic bacteria (Eastridge and Firkins, 2000). In an earlier report, Eastridge and Firkins (2000) has reported that increasing dietary unsaturated fatty could be beneficial to the animals as it reduces methane production. Though they did not describe the level of unsaturated fatty acid associated with this effect. Though this has been demonstrated in this study, inclusion of unsaturated fatty acid beyond 50 % of the recommended dietary intake should not be considered entirely beneficial as it could adversely affect other important digestive indices especially as it bothers on digestion and absorption at the post ruminal segments of the gastrointestinal tract.

The relative increase in methane production in treatments A is considered wasteful because methane is a high energy compound and its elimination from the body as a waste product represents the loss of about 8 percent of the total digestible energy of the diet (Leek, 2004).

The methane production in treatment C, D and control occur as intermediates. The mechanisms surrounding the suppressive effect of unsaturated fatty acids on rumen bacteria and particularly the methanogenic bacteria has not been clearly studied and this cannot be clearly explained here. However, it may be related to the inhibitory effect of biohydrogenation products or intermediates, which probably exert selective lethal effect on methanogenic bacteria. Ruminal microbes rapidly hydrolyze dietary lipids and using the unsaturated fatty acids as hydrogen acceptors quickly convert most of them to stearic acid. It is probable that methanogenic bacteria have need for hydrogen ions in the rumen and these ions are mopped up at high level of dietary or intraruminal infusion of unsaturated fatty acid by a competitive effect for hydrogen between unsaturated fatty acids and the methanogenic bacteria. When the available hydrogen become depleted and thus not readily available for the unsaturated fatty acids and the mathanogenic bacteria both rate of biohydrogenation and rate of methane formation will be reduced. In this situation, both number and activity of methanogenic bacteria become reduced leading to reduced methane production. Philipson (1970) noted that C 18 unsaturated fatty acids, particularly the polyunsaturated fatty acids, inhibited methanogenic bacteria and that the nature of the inhibition was one of direct toxicity towards the bacteria, resulting from the adsorption of fatty acids on the bacterial surface. Czerkawski *et al*. (1986) found that intraruminal infusion of unesterified C 18 fatty acids, particularly the unsaturated homologues, caused a marked reduction in methane production. They attributed this effect to the inhibition of gram-positive *Methanobactrium ruminatium.* The variations in methane production at different proportions of fatty acids, especially on the decreasing effect of unsaturated fatty acids, agrees with the report of these worker, who stated that the effects of fatty acids on the growth and metabolic activity of microorganisms is dependent not only on the nature of the added fatty acid but also, *interalia,* on the concentration of the added fatty acid in a culture media thus laying credence to the varied effects at different proportions of the unsaturated fatty acids. Except in treatment A (i.e. 70 % saturated fatty acid versus 30 % unsaturated fatty acid), methane production was reduced when compared with the control, thus suggesting optimum level of bacterial tolerance for saturated fatty acids beyond which (treatment D) population and activity decrease. On the other hand, at any dietary or ruminal infusion level of unsaturated fatty acids exceeding 30 % of

recommended fat intake, methanogenic bacterial activity wanes.

In conclusion, this study has demonstrated that the proportion of saturated and unsaturated fatty acids in the ruminant diet is critical to the functional efficiency of the rumen particularly as it concerns rumen fermentation of feed nutrients (carbohydrates, proteins, lipids). It is particularly important therefore, that the proportions of these fatty acids should be carefully regulated in the formulation of fat supplements for the improvement of ruminant milk and meat productions.

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