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RUMEN FERMENTATION AND MICROBIAL CHARACTERISTICS OF GRAZING CATTLE ADMINISTERED COCONUT OIL

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ABSTRACT

The study evaluated the effects of coconut oil (CNO) on rumen fermentation and microbial population of grazing cattle. Fifteen (15) White Fulani cattle were allotted to five treatment groups of CNO administration (0 g/day, 50 g/day, 100 g/day, 150 g/day and 200 g/day). The animals were grazed and supplemented with concentrate for twelve weeks. The experiment was set out in a completely randomised design. Data on rumen pH, temperature, volatile fatty acids (VFA), ammonia nitrogen and rumen microbes were analysed using one-way analysis of variance. Results showed that rumen pH was not affected ($p>0.05$) by CNO while VFA concentration and rumen protozoa population decreased ($p<0.05$) from 4.57 mM to 1.25 mM and 650 FEC/g to 350 FEC/g respectively when CNO supplementation was increased from 0 g/day to 200 g/day. In conclusion, administration of coconut oil to grazing cattle did not affect rumen pH, decreased rumen ammonia volatile fatty acids, rumen protozoa while the counts of *Clostridium spp.* and fungi increased.

Keywords: Rumen fermentation, coconut oil, cattle, methane, microbes

INTRODUCTION

Digestion of feed in the rumen occurs by a combination of microbial fermentation and physical breakdown during rumination. The microbial attack is carried out by a mixed population of bacteria and ciliate protozoa, together with a smaller, but possibly metabolically important, population of anaerobic fungi (Dehority, 2003). Though needful for nutrient utilization, rumen microbial feed degradation leads to dietary energy loss of up to 15% in the form of methane, and protein losses in form as ammonia (Johnson and Johnson, 1995; Moss *et al.*, 2000; Aderinboye *et al.*, 2017). Methane, a greenhouse gas, globally contributes about 22% of the total from all of the long-lived and globally mixed greenhouse gases. The high impact of methane on climate changes is due to being 310 times higher in climate warming potential than carbon dioxide (Sommer, 2005). Owing to this ecological significance of methane, considerable efforts are being made to develop methods to reduce its emission from domesticated animals through rumen manipulation. In addition, reducing methane production is of direct economic benefit because it coincides with greater energy use efficiency of the feed by the animals (Sharma, 2005). Strategies for manipulation include provision of additives (chemicals, organic acids and probiotics) and oils in diets, feeding diets rich in unsaturated fatty acids.

Coconut oil is a source that is rich medium-chain fatty acids. *In vitro* studies have shown that coconut oil reduced methane production in both faunated and defaunated rumen fluid (Dohme *et al.*, 1999). In another study reported by Dohme *et al*. (2000), coconut oil reduced methane by 21%, it However, it is important to note, that *in vivo* studies involving oil supplementation are often accompanied by a reduction in dry matter intake, which can also result in reduced methane production

(Machmüller *et al.*, 2000). This study was therefore carried out to evaluate the effectiveness of coconut oil at manipulating rumen fermentation and microbial population while maintaining the performance of grazing cattle.

MATERIALS AND METHODS

Description of the Study area

The experiment was carried out at the Cattle Production Venture Farm, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The site is located in the rain forest vegetation zone of South-Western Nigeria on Latitude 7^{o} 13′ 58.19″ N, longitude 3^{o} 25′ 10.36″ E and an elevation of 145m above the sea level (Google Earth, 2013). The climate is humid with a mean annual rainfall of 1,037mm and mean temperature and relative humidity of 34.7^{0}C and 83% respectively. This experiment was carried out in the rainy season of the year.

Experimental design

The experimental design for this field trial was a completely randomized design (CRD). The factors are five (5) levels of coconut oil administration (0 g/day, 50g/day, 100g/day, 150g/day and 200g/day).

Experimental ingredient and animals

Weights of individual treatments were measured using a sensitive scale at first while volume for weight measurements was used for on toward daily administration. The sample ingredient was at room temperature before administration to animals.

The animals for this experiment were sourced from the herd maintained under the Cattle Production Venture scheme of Federal University of Agriculture, Abeokuta. Fifteen (15) White Fulani cattle with average weight of 138±2.21 kg were used for this trial that lasted for 12 weeks.

The animals were classified into 5 treatment groups according to the levels of coconut oil that make up each treatment. The animals were drenched daily between 0700 and 0830 hours with coconut oil before being released for grazing at 0930 hours. Concentrate supplement was made available to the animals in stall feeding prior and after grazing periods for *ad libitum* intake.

Collection of data, samples and laboratory analyses

Determination of volatile fatty acid concentrations, ammonia, pH and microbial content of rumen fluid

One hundred millilitres (100ml) of rumen fluid sample was obtained from each cattle at the end of 12 weeks of oil administration. Samples were collected by means of a stomach tube thrust directly into the rumen compartment through the oesophagus of the animals. Immediately after collection, the pH of the rumen samples was measured using a portable pH and temperature meter (RoHS pH-618). The samples were freed of coarse particles by filtration through cheese cloth and were divided into three portions.

The first portion sample (50ml) of the sample filtrate was acidified with 1ml of a 5% (v/v) orthophosphoric acid solution and stored frozen at -20^{o}C in air tight bottle containers for subsequent determination of volatile fatty acid (VFA) concentrations. Total volatile fatty acids distillate concentration was determined by titration of sample with 0.1N NaOH solution and expressed as volatile fatty acid content. The procedure was a modified protocol that replaced the conventional titration with the potentiometric titration system. The concentration of NaOH solution was matched with volatile fatty acid content in samples for all the samples (Siedlecka *et al.*, 2008).

The second portion (10ml) was used for the determination of ammonia concentration as described by Lanyansunya *et al.* (2007). Methane gas production was calculated from the various proportions of the volatile fatty acids using the formula prepared by Ørskov *et*

al. (1968). The formula is expressed as:

Methane = 0.5 (Acetate) – 0.25 (Propionate) + 0.5 (Butyrate)

Rumen microbial analysis

The last portion (40ml) of rumen fluid samples was fixed with 10% formalin solution (1:9 v/v, rumen fluid: 10% formalin) for measuring microbial population by total direct count of bacteria, protozoa and fungal zoospores (Galyean, 1989). A further identification and isolation analysis was carried out using the Anaerogen pack for anaerobes.

Statistical analysis

The data from this experiment were analysed separately using one-way analysis of variance option of the SPSS (IBM SPSS Statistics 20) software. Treatment means were statistically compared using Duncan's Multiple Range Test to identify differences between means. Significant differences were declared if $p<0.05$.

RESULTS

The effects of coconut oil (CNO) on the rumen temperature, pH, ammonia nitrogen and total volatile fatty acids (VFA) of grazing White Fulani cattle are presented in Table 1. It can be observed that the administration of coconut oil maintained the rumen pH of cattle across all treatments as no significant ($p<0.05$) variation was recorded in the values obtained. Rumen ammonia nitrogen values of cattle obtained from this study showed that the effect of coconut oil peaked ($p<0.05$) in cattle maintained on 100g/day CNO (5.43mg/dL) while the lowest ($p<0.05$) was obtained from those on 200g/day CNO (3.58 mg/dL). Rumen total volatile fatty acids (VFA) of cattle decreased ($p<0.05$) with CNO administration. In Table 2, the concentration of various VFAs generally declined ($p<0.05$) with increasing CNO levels derived methane values also decreased but acetate: propionate ratio remained unchanged. The effects of various levels of coconut oil (CNO) on the rumen ecology of grazing cattle are shown in Table 3. The population of the rumen anaerobic bacteria in the genus *Bacteroides spp*. and *Lactobacillus spp.* was not significantly affected by levels of administration of coconut oil.

The population of *Clostridium spp*. was however significantly affected by the administration of coconut oil with highest ($p<0.05$) observed in cattle maintained on 200g/day CNO (7.15×10^6cfu/ml) while the lowest was recorded in cattle on 100g/day CNO (2.70×10^6cfu/ml).

Rumen fungi population increased ($p<0.05$) with increasing coconut oil. 0.10×10^6cfu/ml was obtained in cattle on 100g/day CNO administration while 0.35×10^6cfu/ml was recorded in those 200g/day CNO. Rumen protozoa in cattle was reduced ($p<0.05$) by coconut oil administration. The highest ($p<0.05$) population was obtained in the control group (650.00 FEC/g) while the lowest ($p<0.05$) was obtained from cattle maintained on 100g/day CNO (150.00 FEC/g).

DISCUSSION

The rumen pH of cattle did not vary with coconut oil levels. A similar observation was made by Bhatt *et al.* (2011) which reported that the rumen pH of lambs remain unchanged when fed increasing coconut oil levels of up to 75g/kg of concentrate with *ad libitum* forage intake. The rumen pH values of cattle in this study were slightly higher than the normal range (6.0-7.0) reported for optimal microbial digestion of protein and fibre (Van Soest, 1982). This can be adduced to the fact that the animals were predominantly grazed and only offered concentrate as supplement.

Some studies have reported contrasting observations in rumen ammonia-nitrogen with the use of coconut oil. Wanapat *et al*. (2005), Phengvilaysouk and Wanapat (2008) reported that oil supplementation did not affect ammonia-nitrogen concentration but increased

when urea (up to about 4%) was added as part of the feeding ingredients. However, as obtained in this current where ammonia nitrogen levels reduced with increasing levels coconut oil, the rumen ammonia nitrogen and trichloroacetic acid precipitable nitrogen of lambs were found to decrease as the level of dietary coconut oil supplementation increased (Bhatt *et al.,* 2011). These effects could be due to the numerous medium-chain saturated and long-chain unsaturated fatty acids content of coconut oil which exert dramatic effects on ruminal fermentation. As noted in a study carried out by Hristov *et al.* (2004), the authors found that myristic acid did not affect ammonia concentrations except at high levels of inclusion of up to 0.5% of an incubation set up.

The effect of coconut oil on VFA production observed here was consistent with those of Machmüller *et al*. (2003) and Kongmun *et al*. (2011). This result is also supported by the observations made in a study conducted on sheep fed chestnut tannins and coconut oil by Liu *et al.* (2011). The report observed that the lone addition of coconut oil decreased total VFA concentration in rumen fluid, but recorded no effects when combined with tannin. Unlike the current study, no differences were however observed in the proportions of VFA among treatment groups. In a contrasting result, Bozzolo *et al*. (1993) did not find any alterations in VFA patterns in lambs supplemented with 50 g/kg coconut oil for a period of two weeks directly after weaning. Also, Pilajun *et al*. (2010) reported that the supplementation of coconut at 50 g/kg did not affect rumen fermentation in terms of fermentation end products. The results could be due to the inability of animals to consume sufficient coconut oil in the forms supplemented. The administration of coconut oil in this current study was done by directly drenching the animals to ensure full administration of oil.

The volume of individual VFA concentrations are critical in assessing rumen fermentation and manipulation strategies. Rasmussen and Harrison (2011) in a review on methane abatement strategies concluded that reductions in total VFA concentration was due to a decline in the acetate and butyrate concentrations of rumen fluid obtained from lambs fed with coconut oil supplementation. Khorasani *et al*. (1992) reported that fat supplementation depress ruminal digestion through an inhibitory effect of fatty acids on fibre digestion, which was also reflected in decreased VFA production. The authors therefore opined that the dietary inclusion of fats reduces the amount of energy available to bacteria because digestion of fat in the rumen is limited and, hence, decreases microbial protein synthesis and VFA production.

The variations noted in VFA concentration can also be traced to the effect of coconut oil on rumen microbial population of grazing cattle. It is observed here that that lipid administration depressed the population of protozoa in the rumen, as also reported by Males and Purser (1970) that the concentration of VFA is correlated with protozoa numbers. Later studies also reported that a decrease in the concentration of these rumen fermentation metabolites might be due to reduced bacterial and protozoal numbers with increased coconut oil supplementation (Bhatt *et al.,* 2011). Hristov *et al*. (2004) reported that long chain unsaturated fatty acids decrease protozoal counts, ammonia and VFA concentrations while increasing concentrations of free amino acids, reducing concentration of sugars and soluble protein. Dohme *et al*. (1999) reported that the number of methanogen and protozoa per millilitre of rumen fluid was reduced by using coconut oil as against the use of protected fat. Reductions in methanogen and protozoa populations have been suggested to be due to the toxicity of unsaturated fatty acid to methanogen and protozoa (Zhang *et al*., 2008). Also, other studies have shown that saturated fatty acids such as those found in abundant quantity in coconut oil have been shown to

have a toxic effect on protozoa and methanogens (Dong *et al*., 1997; Machmüller and Kreuzer, 1999). Feeding 3.5 and 7% coconut oil reduced ciliate protozoa to 1.0×10^5 mL^{-1} and 0.3×10^5 mL^{-1}, respectively, compared to the controls (1.7×10^5 mL^{-1}) (Machmüller and Kreuzer 1999). Reductions in protozoa population was also obtained by Liu *et al.* (2011) which observed reduced populations of rumen methanogen and protozoa upon adoption of coconut oil for sheep feeding. Similar results were obtained by Kongmun *et al*. (2011) when 7% coconut oil supplementation was fed to swamp buffalo and found decreased total methanogen and protozoa populations in rumen fluid.

CONCLUSION

Administration of coconut oil to grazing cattle did not affect rumen pH. However, it resulted in decreased rumen ammonia nitrogen, total and individual concentration of volatile fatty acids. Rumen ammonia concentration of animals were not affected. Coconut oil depressed the population rumen protozoa, and while the counts of *Clostridium spp.* and fungi increased, the population of *Bacteroides* and *Lactobacillus* were not affected.

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Table 1: Rumen characteristics, ammonia nitrogen and total volatile fatty acids of grazing cattle fed various levels of coconut oil

	Coconut Oil Levels						
Parameters	**0g**	**50g**	**100g**	**150g**	**200g**	**SEM**	**P value**
Rumen temperature (°C)	31.05[a]	23.39[c]	26.18[b]	30.19[a]	25.83[b]	0.697	0.06
Rumen pH	7.22	7.24	7.26	7.31	7.26	0.032	0.95
Rumen ammonia (mg/dL)	4.87[ab]	4.82[ab]	5.43[a]	4.45[ab]	3.58[b]	0.215	0.04
Total VFA (mM)	4.57[a]	2.33[b]	2.34[b]	1.70[b]	1.25[b]	0.359	0.00

[abc] Means on the same row with the different superscripts are significantly different ($P < 0.05$)

Table 2: Volatile fatty acids concentrations, ratios and methane of grazing cattle fed various coconut oil levels

	Coconut Oil Levels						
Parameters	**0g**	**50g**	**100g**	**150g**	**200g**	**SEM**	**P value**
Acetate (g/dL)	3.04 [a]	1.55 [b]	1.56 [b]	1.14 [b]	0.84 [b]	0.239	0.00
Propionate (g/dL)	2.03 [a]	1.03 [b]	1.04 [b]	0.75 [b]	0.55 [b]	0.159	0.00
Butyrate (g/dL)	3.08 [a]	1.84 [ab]	1.59 [b]	1.15 [b]	0.84 [b]	0.254	0.00
Methane	2.56 [a]	1.43 [b]	1.31 [b]	0.96 [b]	0.70 [b]	0.205	0.00
C_2:C_3	1.50	1.51	1.50	1.53	1.53	0.006	0.07
Valerate (g/dL)	0.77 [a]	0.39 [b]	0.39 [b]	0.28 [b]	0.21 [b]	0.061	0.00
Isobutyrate (g/dL)	2.38 [a]	1.45 [ab]	1.06 [b]	0.99 [b]	0.74 [b]	0.184	0.00
Isovalerate (g/dL)	0.73 [a]	0.35 [b]	0.35 [b]	0.26 [b]	0.18 [b]	0.059	0.00

[ab] Means on the same row with the different superscripts are significantly different (P < 0.05)

Table 3: Rumen microbial count of grazing cattle fed various levels of coconut oil

	Coconut Oil Levels						
Parameters	**0g**	**50g**	**100g**	**150g**	**200g**	**SEM**	**P value**
Clostridium (× 10^6cfu/ml)	5.35[ab]	4.00 [ab]	2.70 [b]	4.15 [ab]	7.15 [a]	0.548	0.01
Bacteroides (× 10^6cfu/ml)	5.65	4.50	4.50	5.85	4.75	0.473	0.75
Lactobacillus (× 10^6cfu/ml)	0.15	0.10	0.20	0.10	0.25	0.026	0.35
Fungi (× 10^6cfu/ml)	0.30 [a]	0.20 [ab]	0.10 [b]	0.25 [ab]	0.35 [a]	0.031	0.01
Protozoa (FEC/g)	650.00[a]	500.00[ab]	150.00[c]	350.00[b]	350.00[b]	86.740	0.28

[ab] Means on the same row with the different superscripts are significantly different (P < 0.05)

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