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ORIGINAL RESEARCH ARTICLE

Effects of coconut oil on *in vitro* gas production characteristics and blood chemistry of West African dwarf sheep fed a total mixed diet

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ABSTRACT

The study was conducted to evaluate the effects of coconut oil supplementation on rumen fermentation, in vitro gas production characteristics and blood indices of west african dwarf (WAD) sheep fed a total mixed diet. Twelve sheep aged between 14 and16 months and weighing 11.22 ± 1.22kg were randomly selected for the study. The animals were divided into three groups of 4 per group. Each group received a total mixed diet containing 0%, 1.5% or 3.0% of coconut oil. The experimental design was completely randomised design. The study lasted 70 days. Results of this study showed that there was a significant (P<0.05) linear increase in rumen pH with increase in the level of coconut oil supplementation: 7.03, 7.09 and 7.15 for 0%, 1.5% and 3.0% coconut oil respectively. However, rumen ammonia-N concentration was not affected by coconut oil supplementation Gas production parameters (gas production from fermentable fraction b, rate of gas production c and 24-hr gas production) decreased significantly (P<0.05) with coconut oil supplementation. There was also a significant (P<0.05) reduction in organic matter digestibility and metabolisable energy. Coconut oil reduced enteric methane production by about 34.01% to 40.21%. While blood urea was not affected by coconut oil, there was a significant (P<0.05) increase in total cholesterol and high density lipoprotein (HDL). Addition of coconut oil to the diet of WAD sheep has the potential to effectively inhibit methanogenesis and reduce methane production. However, its addition can also negatively impact on the performance of the animal by reducing digestibility and therefore the amount of energy available for productive function.

Keywords: WAD sheep, coconut oil, rumen fermentation, in vitro gas production, blood indices

INTRODUCTION

Lipids, such as fatty acids and oils, are options for feed supplementation that have been investigated both *in vitro* and *in vivo* for their effects on rumen fermentation and particularly methanogenesis. The inclusion of lipids is one of the most feasible techniques that have been used by producers, due to the anti-methanogenic properties of these compounds (Bauchemin *et al*., 2008; Rasmussen and Harrison, 2011). Among the most used vegetable oils are linseed, coconut, canola, radish, sunflower and soybean (Machmüller and Kreuzer, 1999; Beauchemin and McGinn, 2006; Beauchemin *et al*., 2008 and Mao *et al*. 2010). Coconut oil has high content of fatty acids, mainly myristic, palmitic, stearic, oleic and linoleic (Blas *et al*., 2003; Kobayashi, 2010). However, Kongmun *et al.* (2011) reported low organic matter digestibility when diets were supplemented with 7% coconut oil. Jordan *et al*. (2006 a, b) found that high levels of coconut oil (42 % of the DM) in beef cattle fed with forage-concentrate 50:50, reduced the consumption and digestibility of the diet, but lower levels of oil (between 10 - 28 % of the DM) did not affect these indicators. They also reported a decrease in daily enteric methane output when expressed both as litres per day or per kilogram of dry matter intake. Lee *et al*. (2011) reported that adding coconut oil to the diet even-though diminished the protozoa population the methanogenic population was not affected. Galindo *et al*. (2009) also reported that coconut oil reduced cellulolytic and methanogenic population in the rumen. Machmuller and Kreuzer (1999) reported that there was a decrease of about 63.8% of methane production when 7% of coconut oil was used. Marked differences in responses to supplementation with lipid sources in respect to the reduction of the ruminal methanogenesis have however, been reported (Beauchemin *et al*., 2008)

MATERIALS AND METHOD

Experimental site

The experiment was conducted at the Sheep Unit of the Teaching and Research Farm, University of Ibadan, Ibadan. The location is 7º 27'N and 3º 45'E at altitude 200-300 m above sea level. The climate is humid

tropical with mean temperature of 25-29ºC and the average annual rainfall of about 1250 mm.

Animal management, diets and experimental design

Twelve WAD sheep aged 14-16 months and weighing 11.22 ± 1.22kg were randomly selected for the study. The animals were divided into three groups of 4 per group and acclimatized to individual pens for at least 15 days before the start of the experiment. Each group received 1 of 3 total mixed diets containing 0%, 1.5% and 3.0% of coconut oil (Table 1). The animals were fed at 5% of their body weight on dry matter bases. The animals were fed once a day at 08.00 h. Feed intake was recorded daily and feeding lasted 70 days including the 15 days for acclimatization. The experimental design was completely randomised design.

Table 1: Gross composition of experimental diets fed to West African Dwarf sheep

Ingredients	0%	1.5%	3.0%
Wheat offals	50.0	48.5	47.0
Cassava peels	25.0	25.0	25.0
Coconut oil	0.0	1.5	3.0
Groundnut haulms	20.0	20.0	20.0
Urea	1.0	1.0	1.0
Dicalcium phosphate	1.5	1.5	1.5
Limestone	1.0	1.0	1.0
salt	0.5	0.5	0.5
Multi-vitamin premix	1.0	1.0	1.0

Growers premix: Vitamin A (7500000IU), Vitamin D3 (1000000IU), Vitamin E (1800mg), Vitamin B1(500mg), Vitamin B2 (1000mg), Vitamin D- Pantothenic acid (3200mg), Vitamin B6 (180mg), Vitamin B12 (5mg), Vitamin C (5000mg), Vitamin K (700mg), Nicotinic acid (4000mg), Folic acid (50mg), Choline chloride (63000mg), Manganese (35000mg), Cu (1500mg), Cobalt (180mg), Iron (10000mg), Iodine (720mg), Zinc(1500mg).

Collection of rumen liquor

On day-28 fresh liquor was collected from the rumen of the 12 WAD sheep, using stomach tubes as described by Santra *et al*. (2012). Before the collection, the animals were starved of feed overnight but adequate water was provided. The rumen liquor was taken to the laboratory immediately after collection in thermos flasks to avoid contamination of the rumen microbes and to maintain anaerobic condition. The pH of the rumen liquor was determined immediately after collection using a portable digital pH meter.

***In vitro* gas production characteristics determination**

Gas production characteristics were determined as described by Menke and Steingass (1988) and Yusuf *et al* (2013) over a 24-hr period at 3-hourly intervals. Ground maize was used as the substrate for the *in vitro* determination.

Calculations

The values obtained for gas production were fitted into the exponential equation, $Y = a + b (1 – e^{ct})$ to estimate gas production characteristics as described by Ørskov and McDonald (1979)

Where
Y = Volume of gas produced,
t = time of rapid change in gas production,
a = intercept (gas produced from the soluble fraction),
b = gas produced from insoluble fraction,
c = gas production rate from the insoluble fraction (b)

The following parameters were calculated:
Metabolisable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) were calculated as:

Table 2: Chemical composition of experimental diets fed to West African Dwarf sheep

	Treatments		
Parameters (%)	0%	1.5%	3.0%
Dry matter	92.10	92.00	92.20
Crude protein	11.80	12.20	12.50
Ether extract	2.70	2.80	2.90
NFE	30.07	27.58	27.80
NDF	71.31	73.55	71.70
ADF	53.86	55.31	54.30

NFE- nitrogen free extract; NDF- neutral detergent fibre; ADF- acid detergent fibre

ME = 2.20 + 0.13*GV + 0.057*CP + 0.0029*CF (Menke and Steingass, 1988)
OMD = 14.88 + 0.889*GV + 0.45*CP + 0.651XA
SCFA = 0.0239*GV ¬ 0.0601(Getachew *et al*., 1999).

Where GV, CP, CF and XP are Total gas production (mL/200mgDM), crude protein, crude fibre, and ash of the incubated samples respectively.

Haematology and serum biochemical analysis

On day 36, 5mL each of blood samples were collected from the experimental animals according to standard procedures for haematology and serum biochemical analyses. This was done early in the morning before the animals were fed. Blood samples were collected from the jugular vein into sterilized glass tube containing EDTA (ethylene - diamine - tetra - acetic acid) and another glass tube without anti-coagulant for haematological and serum biochemical assays,

Table 3: Rumen fermentation and *in vitro* gas production characteristics of West African dwarf sheep fed experimental diets

	Treatments			
Parameters	0%	1.5%	3.0%	SEM
pH	7.03[b]	7.09[ab]	7.09[ab]	0.058
NH_3-N (mg/L)	7.82	8.7	7.45	0.91
a (mL)	7.00[a]	3.67[b]	2.67[b]	1.83
b (mL)	50.00[a]	37.67[b]	36.33[b]	10.05
a+b (mL)	57.00[a]	41.33[b]	39.00[b]	11.29
c (mL/hr)	0.08	0.08	0.05	0.03
t (hrs)	12.00[c]	16.00[a]	14.00[b]	1.77
Y (mL)	35.50[a]	29.83[ab]	21.67[b]	8.87
ME (MJ/kgDM)	10.19[a]	8.15[b]	7.85[b]	0.47
OMD (g/100gDM)	70.90[a]	56.97[b]	54.90[b]	7.04
SCFA (µmol)	1.30[a]	0.94[b]	0.87[b]	0.27
CH_4(mM/gDM)	28.00[a]	22.50[b]	15.00[c]	4.35
CH_4 (% GV)	51.03	52.27	49.7	2.41
CH_4 (mmol/gOMD)	0.41[a]	0.36[ab]	0.27[b]	0.08

abc: mean along the same row with different superscripts are significantly ($p<0.05$) different
Y = Volume of gas produced, t = time of rapid change in gas production, a = intercept (gas produced from the soluble fraction), b = gas produced from insoluble fraction, c = gas production rate from the insoluble fraction (b) CH_4 – methane

respectively. Blood samples for serum assay were centrifuged and serum was decanted and freeze stored at -10^0 C until analysis.

Haematology

The packed cell volume (PCV) and haemoglobin (Hb) were determined using microhaematocrit method and cyanmethaemoglobin method, respectively (Schalms *et al.*, 1975). Erythrocyte count (RBC) and leukocyte count (WBC) were determined using the improved Neubauer haemocytometer after the appropriate dilution (Schalms *et al.*, 1975). Other blood corpuscular constants (MCV, MHC and MCHC) were calculated.

Serum biochemistry

Serum glucose was determined by the O-Toluidine method using acetic acids (Cooper and McDaniel, 1970). Serum urea was determined by urease method and creatinine by Folin-wu filtrate methods (Toro and Ackermann, 1975). Serum total protein was determined by Biuret method (Kohn and Allen, 1995), while albumin was determined using the BCG (Bromocresol green) method (Peter *et al.*, 1982). Serum cholesterol and triglycerides were measured using appropriate laboratory kits (Gowenlock *et al.*, 1988), aspartate aminotransferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) activities were determined using spectrophotometric methods (McComb *et al.*, 1988; Rej and Hodder, 1983).

Chemical analysis

Proximate composition of the feeds was determined according to AOAC (1995) and detergent fibre fractions by the method of Van Soest *et al*. (1991). Rumen ammonia nitrogen was determined by steam distillation (AOAC, 1995)

Statistical analysis

Data collected were subjected to ANOVA according to the procedure of SAS (2009) and means were separated using Duncan's Multiple Range F-test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Chemical composition

The gross and chemical compositions of the experimental diets are shown in Tables 1 and 2. Crude protein concentration was within the range recommended for optimal rumen function.

Rumen fermentation

There was a significant ($P<0.05$) linear increase in rumen pH with increase in the level of coconut oil supplementation (Table 3). However, rumen ammonia-N concentration was not affected by coconut oil supplementation (Table 3). Newbold *et al*. (2004) and Castillejos *et al*. (2006) have suggested that essential oil may increase ruminal pH with no effect on rumen fermentation. Hristov *et al.* (2009) and Kongmun *et al.* (2011) observed that rumen ammonia nitrogen concentration was not influenced by coconut oil for swamp buffalo.

Table 4 : Haematology and serum biochemistry indices of West African dwarf sheep fed experimental diets

	Treatments			
Parameters	0%	1.5%	3.0%	SEM
PCV (%)	31.00	33.25	35.25	4.66
Hb (g/dL)	10.35	11.09	11.10	0.72
WBC ($x10^9$/mm3)	7.83	8.21	7.58	0.98
Total protein (g/dL)	5.8	5.71	4.83	0.63
Urea (mg/dL)	31.29	35.79	35.57	4.22
Triglyceride (mg/dL)	37.82	28.85	37.82	10.49
Total cholesterol (g/dL)	26.52^c	54.77^b	71.76^a	8.60
HDL(g/dL)	44.21^b	57.73^b	109.96^a	13.33
LDL(g/dL)	60.37	54.70	60.37	8.62

abc: mean along the same row with different superscripts are significantly ($p<0.05$) different
PCV- packed cell volume ; Hb- haemoglobin ; WBC- white blood cell ; HDL- high density lipoprotein ; LDL- low density lipoprotein

Similar observation was also reported by Phengvilaysouk and Wanapat (2008).

***In vitro* gas production characteristics**

Gas production parameters measured in this study decreased significantly ($P<0.05$) with coconut oil supplementation (Table 3). Gas production is a consequence of dry matter and organic matter fermentation by rumen microbes and in this study there was a significant ($P<0.05$) reduction in organic matter digestibility (Table 3). Jordan *et al.* (2006a,b); Manso *et al.* (2006); Dutta *et al.* (2008) and Kongmun *et al.* (2011) have reported reduction in dry matter and organic matter digestibility with coconut oil supplementation. This might have caused the reduction in amount of gas produced with coconut oil supplementation in present study.

The Metabolisable energy decreased ($P<0.05$) with increased coconut oil supplementation. This is in line with the observation of Machmuller e*t al.* (2000) that coconut oil reduces organic matter digestibility and metabolisable energy by causing a negative effect on ingestion and digestibility of nutrients. Coconut oil has been reported to reduce enteric methane production. Significant ($P<0.05$) decreases (34.01- 40.21%) were observed for methane output with increased level of coconut oil supplementation. This is in agreement with previous reports (Lovett *et al.,* 2003; Jordan *et al.,* 2006a,b; Odongo *et al.,* 2007; Beauchemin *et al.*, 2008; Rasmussen and Harrison, 2011). It is however contrary to the report of Lee *et al*. (2011) that coconut oil had no effect on methane production.

Haematology and serum biochemistry

Haematology and serum indices were not significantly ($P<0.05$) affected except for total cholesterol and HDL that increased with coconut oil supplementation. Phengvilaysouk and Wanapat (2008) reported that no effect of coconut oil supplementation was found on blood urea nitrogen. However, Bindel *et al.* (2000) and Bhatt *et al.* (2011) reported increased cholesterol level and plasma triglycerides with increased coconut oil but blood urea nitrogen was also not affected according to their reports. The increases observed in cholesterol level may be due to an increase in its synthesis (Bindel *et al.,* 2000).

CONCLUSION

Addition of coconut oil to the diet of WAD sheep has the potential to effectively inhibit methanogenesis and reduce methane production. However, it is important that the level of supplementation that will not negatively impact animal performance be established.

CONFLICT OF INTEREST

There is no conflict of interest with regards to the publication of this study.

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