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ORIGINAL RESEARCH ARTICLE

Complementary effects of raw garlic and water-washed neem fruit on *in vitro* gas production characteristics and blood chemistry of West African dwarf sheep

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ABSTRACT

The study was conducted to evaluate the effects of raw garlic (Allium sativum) and neem (Azadirachta indica) fruit and their mixture on rumen fermentation, in vitro gas production characteristics, organic matter digestibility, metabolisable energy, short chain fatty acids and blood indices of West African Dwarf (WAD) sheep. Twelve WAD sheep with an initial live weight of 15.66±0.75 kg were randomly assigned to a completely randomised design over a 36-day feeding period after 4 weeks of adaptation. Results showed that there was a significant (P<0.05) difference in rumen pH among the treatments means. Inclusion of garlic either alone or in a mixture with neem fruit reduced ruminal pH. Rumen ammonia-N concentration was not affected by garlic or neem fruit. However, there was a significant (P<0.05) decrease in rumen ammonia concentration when the mixture of garlic and neem fruit were added to the diet. Gas production parameters (gas production from fermentable fraction b, rate of gas production c and 24-hr gas production) measured in this study increased significantly (P<0.05) with the supplementation of garlic, neem fruit or their mixture. There was a significant (P<0.05) increase in organic matter digestibility, metabolisable energy and short chain fatty acids. Significant (P<0.05) decreases in methane output were observed with garlic and neem fruit supplementation Haematology and serum indices were not significantly (P<0.05) affected except for white blood cell, blood urea, total cholesterol and glucose. The mixture of garlic and neem fruit resulted in the greatest (P<0.05) reduction in blood urea observed in this study. Significant (P<0.05) reduction was observed for blood cholesterol with supplementation with neem fruit and garlic-neem fruit mixture while garlic supplementation was the most effective to improve serum glucose. The results of this study indicate the potency of garlic and neem fruit to improve rumen fermentation and energy utilization in WAD sheep.

Keywords: WAD sheep, garlic, neem fruit, in vitro gas production, blood indices

INTRODUCTION

Livestock production currently faces challenges posed by the demand for 'clean, green and ethical' animal production systems (Martin et al., 2004). This concept involves limited use of drugs, chemicals and hormones, while reducing the impact of food production on the environment and considering animal welfare. It refers also to increased demand for high-quality animal products manufactured with fewer synthetic chemical inputs, in particular routine use of antibiotics in livestock feeds (Barton, 2000; Goldsmith and Schur, 2002) as bacteria in the animal gut develop antibiotics resistance over time, which may transfer to humans with animal products (Piddock, 2002). In recent years, aromatic plants and their extracts have received increased attention as potential alternatives to growth promoters because they contain several secondary compounds that can be used for rumen manipulation (*e.g.* defaunation, decreased methane production, decreased ruminal degradation of dietary proteins, *etc.*) which can be a cost-effective way to improve the performance of small ruminants. Effects of some medicinal plants, plant extracts or essential oils on ruminal fermentation have already been reported (Evans and Martin, 2000; Broudiscou *et al.*, 2000, 2002; Wallace *et al.*, 2002). In this regard, effects of garlic and its bioactive components have been partly demonstrated on rumen manipulation(*e.g.* defaunation, decreased methane production, decreased ruminal degradation of dietary proteins, reducing the proportion of acetate and increasing that of propionate) and consequently on animal production and performances. Garlic has a complex mixture of secondary plant products including allicin, diallyl sulfide, diallyl disulfide and allyl mercaptan among others (Lawson, 1996). These compounds can manipulate rumen fermentation, increase dietary energy efficiency by decreasing the proportion of acetate and increase the proportion of propionate and butyrate, inhibition of methanogenesis and decrease in methane emission: volatile fatty acid ratio (Busquet *et al.*, 2005). The effect of garlic supplementation on blood chemistry has also been reported. Yeh and Liu (2001) reported that supplementation of aged garlic extract in animal diets reduced plasma concentrations of total cholesterol and triacylglycerol by 15 and 30%, respectively. Neem (*Azadirachta indica*) fruit has been shown to have

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

	Diets			
Ingredients (%)	I	II	III	IV
Cassava peels	60.00	60.00	60.00	60.00
Soyabean meal	20.00	20.00	20.00	20.00
Pineapple wastes	10.00	10.00	10.00	10.00
Corm bran	10.00	8.75	0.00	0.00
Garlic	0.00	1.25	0.00	1.25
Neem fruit	0.00	0.00	10.00	8.75

Treatments: I (0% neem and 0% Garlic); II (0% neem and 1.25% Garlic); III (10% neem and 0% Garlic); IV (10% neem and 1.25% Garlic)

anti-microbial properties similar to those of salinomycin (Tipu *et al.,* 2002; Tipu *et al.,* 2006). Therefore, it has a potential to be used as natural replacement for ionophores as a rumen manipulating agent. The objective of this study was to evaluate the effects of neem fruit, raw garlic and their mixture on rumen fermentation, in vitro gas production characteristics and blood chemistry of West African dwarf sheep.

MATERIALS AND METHODS

Experimental site

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

Experimental design, animal management and diets

Twelve WAD sheep weighing 15.66 ± 0.75kg were randomly divided into four groups in a complete randomized design and acclimatized to individual pens for at least 4 weeks before the start of the experiment. Four (4) supplementary diets were formulated from a mixture of cassava peels, corn bran, soyabean meal, pineapple wastes, raw garlic and water-washed neem fruits as shown in Table 1. The animals were fed at 3% of their body weight on dry matter bases. *Panicum maximum* constituted the basal forage and was supplied at 50% of the total dry matter requirement while the supplementary diets supplied the remaining 50%. The animals were fed once a day at 08.00 h. The study lasted 36 days.

Collection of rumen liquor

On day-28 fresh liquor was collected from the rumen of the 12 WAD sheep, using stomach tubes. Before the collection, the animals were starved 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) as modified by Babayemi et al. (2005) 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

	Diets			
Parameters (%)	I	II	III	IV
Dry matter	92.17	91.16	93.01	91.68
Crude protein	10.18	11.01	10.91	10.22
Ether extract	2.50	2.34	2.15	2.64
NFE	43.07	41.22	43.01	42.04
NDF	71.31	69.73	69.71	70.40
ADF	43.18	45.31	44.09	46.11

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

Treatments: I (0% neem and 0% Garlic); II (0% neem and 1.25% Garlic); III (10% neem and 0% Garlic); IV (10% neem and 1.25% Garlic)

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 28, 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, 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 (1999) and means were separated using Duncan's Multiple Range Test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Gross and chemical composition of supplementary diets
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) difference in rumen pH between the treatments (Table 3). Inclusion of garlic either alone or in a mixture with neem fruit reduced ruminal pH. Cardozo *et al.* (2005) suggested that as pH decreases, acids tend to be more undissociated and hydrophobic thereby interacting easily with cell membranes and exerting their antimicrobial effect.

Table 3: The rumen fermentation characteristics and 24-hr in vitro gas production characteristics of a standard substrate incubated in rumen liquors obtained from West African dwarf sheep fed experimental diets

	Diets				
Parameters (%)	I	II	III	IV	SEM
pH	7.00^a	6.63^b	6.83^a	6.30^c	0.08
Ammonia-N(mg/L)	9.18^a	9.01^a	9.13^a	7.76^b	0.33
b (mL)	50.06^c	67.07^b	77.47^a	78.58^a	2.25
c (mL/hr)	0.02^c	0.04^b	0.05^b	0.08^a	0.0005
t (hr)	0.40^c	3.90^a	1.60^b	1.43^b	0.18
24hr GV	9.00^d	30.11^c	31.50^b	33.97^a	0.11
CH_4(mM/200mgDM)	15.00^a	10.02^b	8.00^c	6.03^d	0.29
ME(MJ/kgDM)	3.10^b	7.07^a	7.20^a	7.35^a	0.23
OMD (%)	28.40^c	48.30^b	48.40^b	50.40^a	0.19
SCFA (µmol)	1.60 ^{c}	6.91^b	6.93^b	7.54^a	0.02

Means with different superscript along the same row are significantly different (P<0.05)
Treatments: I (0% neem and 0% Garlic); II (0% neem and 1.25% Garlic); III (10% neem and 0% Garlic); IV (10% neem and 1.25% Garlic). **KEY:** b (Gas production of potentially degradable but insoluble fraction); c (Rate of gas production); t (time of most rapid increase in gas production); GV (total volume of gas produced); CH_4 (Methane); ME (Metabolizable energy); OMD (Organic matter digestibility); SCFA (Short chain fatty acid); SEM (standard error of the mean)

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

	Parameters				
Parameters	I	II	III	IV	SEM
PCV (%)	30.00	32.00	30.33	29.33	1.82
Hb (g/dL)	10.00	10.55	10.17	9.78	0.60
WBC ($x10^9$/mm3)	12.20^b	7.93^d	9.60^c	17.37^a	0.98
Total protein (g/dL)	4.54	5.40	5.49	4.78	0.37
ALB (g/dL)	3.83	3.57	3.51	4.46	0.34
Urea (mg/dL)	16.66^b	15.71^b	27.63^a	2.37^c	1.49
CRT (mg/dL)	0.12	0.12	0.11	0.13	0.01
AST (U/L)	29.03	27.67	25.03	27.72	1.94
ALT (U/L)	1.62^a	0.96^b	1.76^a	0.98^b	0.11
ALP (U/L)	303.80^a	265.80^a	293.90^a	191.60^b	27.45
CHOLE	53.16^a	59.17^a	44.00^b	46.85^b	3.53
TRY (mg/dL)	35.82	28.47	31.46	35.51	3.44
GLU (g/dL)	84.05^b	116.70^a	83.31^b	87.33^b	8.76

^{a,b,c,d} Means with different superscripts along the same row are significantly different (P<0.05)

Treatments: I (0% neem and 0% Garlic); II (0% neem and 1.25% Garlic); III (10% neem and 0% Garlic); IV (10% neem and 1.25% Garlic)

However, Yang *et al*. (2007) reported that pH and volatile fatty acids concentration were unaffected by garlic addition in diet. Rumen ammonia-N concentration was not affected by garlic or neem fruit. However, there was a significant (P<0.05) decrease in rumen ammonia concentration when the mixture of garlic and neem fruit were added to the diet (Table 3). Castillejos *et al*. (2006) and Wanapat *et al.* (2008) reported that garlic powder had no effect on ruminal fluid concentration of ammonia nitrogen. Busquet *et al.* (2005) however, suggested that garlic oil could increase ruminal ammonia nitrogen.

***In vitro* gas production characteristics**

Gas production parameters measured in this study increased significantly (P<0.05) with the supplementation of garlic, neem fruit or their mixture (Table 3). There was a significant (P<0.05) increase in organic matter digestibility, metabolizable energy and short chain fatty acids (Table 3). Yang *et al.* (2007) reported increased ruminal digestibility of dry and organic matter with garlic or juniper berry. They suggested that the increased rumen fermented organic matter with garlic supplementation suggests increased availability of energy to ruminal microorganisms. Significant (P<0.05) decreases in methane output were observed with garlic and neem fruit supplementation. This is in agreement with the report of Busquet *et al.* (2005) that garlic oil and its compound diallyl disulfide and allyl mercaptan reduced methane production. It was observed in this study that the activity of garlic to improving rumen fermentation and gas production characteristics was enhanced in mixture with neem fruit. This is probably due to the antimicrobial activity of neem fruit which is similar to that of salinomycin (Tipu *et al.,* 2002; Tipu *et al.,* 2006), an ionophore used for rumen modulation to enhance feed efficiency.

Haematology and serum biochemistry

Haematology and serum indices were not significantly (P<0.05) affected except for white blood cell, blood urea, total cholesterol and glucose (Table 4). The mixture of garlic and neem fruit was observed to have produced the greatest reduction in blood urea. Cardozo *et al.* (2004) suggested that deamination was inhibited by garlic oil and that essential oil contained in garlic powder affected ammonia nitrogen and blood urea nitrogen. Busquet *et al.* (2005) reported that there is substantial evidence that essential oils may affect rumen microbial fermentation and protein metabolism. Also, numerous studies have demonstrated the inhibitory effects of garlic organosulphur compounds on cholesterol biosynthesis in the hepatocytes by the inhibition of the HMG-CoA reductase (Gebhardt and Beck, 1996; Cho and Xu, 2000). No effect of garlic on cholesterol was observed in this study probably because of the level of supplementation. However, significant (P<0.05) reduction was observed for neem fruit and garlic-neem fruit mixture (Table 4). Similar effects of neem fruit on cholesterol level have been reported (Gangopadhyay *et al.,* 1981; Gowda *et al.,* 1996).

In the present study serum glucose was significantly (P<0.05) improved by garlic supplementation. This result is however, in contrast to Chaves *et al.* (2008) who reported no difference in serum glucose concentration of growing lambs fed diet supplemented with garlic. This may be due to the level of garlic supplementation in this study or the type of diet fed to the animals. The glucose level in the blood is considered as a reflection of gluconeogenesis. Therefore, garlic may help to improve the efficiency of energy utilization in the rumen

CONCLUSION

The results showed that garlic had positive effects on rumen fermentation, gas production characteristics and blood indices of WAD sheep. However, these effects were enhanced when garlic was mixed with neem fruit. Therefore, garlic and neem fruit have the potential to be used as additives for ruminants in order to improve rumen function and feed efficiency.

CONFLICT OF INTEREST

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

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