Predicting Rumen Microbial Population and Volatile Fatty Acids in Growing Rams Fed Avocado Seeds with Orange Peels Meal as Replacement for Guinea Grass

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Abstract

The West African Dwarf (WAD) growing rams, aged between 8 and 9 months with mean body weight of 8.00 ± 0.12kg were used to predict rumen microbial population and volatile fatty acids (VFAs) for 84days. The WAD growing rams were randomly allotted to three dietary treatments with eight rams per treatment group in a completely randomized design. The compared experimental diets were; diet A (solely 60% guinea grass which serves as the control group), diet B (avocado seeds with orange peels in a ratio of 25:35) and diet C (avocado seeds with orange peels in a ration of 20:40). Concentrate supplement of 40% was added to each of the experimental diet. The results showed that rumen fungi (40.26mm^2), rumen pH (6.78), acetic acid (46.77%) and acetic: propionic ratios (3.89%) were significantly (P < 0.05) highest in growing rams on diet A compared to other treatment diets. Rumen ammonia nitrogen concentration (18.04mg/100ml), rumen protozoa (14.01 x 10^{3}ml), rumen bacteria (16.02 x 10^{9}ml), propionic acid (21.51%) and butyric acid (12.85%) were significantly (P < 0.05) better in growing rams on diet B, whereas rumen temperature (38.95^{0}C) and total volatile fatty acids (88.01mmol/litre) were significantly (P < 0.05) highest in growing rams on diet C. It is concluded that avocado seeds with orange peels meal in a ratio 25:35 respectively, has the potential to replace guinea grass in the diet of WAD growing rams.

Keywords: avocado seeds, orange peels, rumen microbes, volatile fatty acids, rams

Introduction

Small ruminants are important aspects of ruminant livestock sector in the tropics. They are kept world wide for their numerous economic and social contributions to humanity. The peasant farmers derive a large proportion of their income from small ruminant livestock in Nigeria. Taiwo et al. (2005) have earlier identified exorbitant cost of cattle production and encourage the production of small ruminants (sheep and goats) which cost less in terms of feeding and other management practises. Recently, more attention have been paid to small ruminants in developing countries like Nigeria as their advantages are becoming more understood than ever before, particularly for their ability to produce meat, milk, skin and wool even in hostile environment (Konlan et al., 2012). The systems of small ruminant production in Nigeria are usually characterised by limitation posed by non-availability of all year round feed resources due to usually prolonged dry season. They are raised predominantly on grasses which are inherently poor in nutritive values and unavailable during the off – season. The stiff competition for conventional feeds by man and other livestock species also reduce the scale advantages in tracking the fundamental problems of small ruminant feeds in Nigeria which in turns increase the cost of ruminant and their products (Oladotun et al., 2003). These limitation posses the challenge to search for alternative cheaper and locally available feed sources to reduce the dwindling small ruminant industry in the nation that result from feed shortage. This search has attracted a closer look at lesser known agro-industrial by-products such as avocado seeds and orange peels.

Avocado (*Persea americana*) seeds and orange (*Citru sinensis*) peels have been identified as potential feedstuffs for livestock based on their nutrient contents and acceptability. However, they also require form of processing before use as feeds for livestock to reduce phenolic content that was found to be greater than 70% in avocado seeds (Al-Hassan et al., 2012) and d – limonene in orange peels that are toxic to pigs and poultry (Fung et al., 2010).

Notwithstanding, the rumen that form the largest part of the reticulorumen, serves as the primary site for microbial fermentation of ingested feed components. Gerard and Frederique (2006) reported that the nature of feed given to ruminant to support productivity is one of the several abiotic factors that can alter the balance of rumen microbial population and their activities which may lead to either decrease in performance or increase the risk of health problems. The proportion of partials volatile fatty acid concentration in the rumen also depends largely on the type of feeds consumed by the animals. Therefore, there is paucity of information describing the rumen microbiology and volatile fatty acid in sheep that dependent upon this type of feed source. Hence, the objective of this study was to predict rumen micro-organism population and volatile fatty acids in growing rams fed avocado seeds with orange peels meal as replacement for guinea grass.

Materials and Methods

Experimental site: The experiment was conducted at the Small Ruminant Unit of the Teaching and Research Farm, Ambrose Alli University, Ekpoma, Nigeria. Ekpoma is located on longitude 6.09^0E and latitude 6.42^0N with a unimodal rainfall pattern which starts from April and end in October. The mean rainfall and temperature are about 1556mm and 31^0C respectively.

Experimental Diets: Guinea grass was obtained from the Teaching and Research Farm and manually chopped into smaller sizes of about 4cm. Orange peels were collected fresh in convergence of local processors located within Ekpoma, before they are chopped into small sizes of about 4cm and sun dried. Avocado seeds were obtained from a plantation vicinity and market areas within Ekpoma. The fresh seeds were carefully examined, selected, sliced into approximately 3cm cubes and sundried. Dried orange peels and avocado seeds were then milled and stored in airtight containers separately. The composition of the concentrate were as follows: 78% wheat offal, 20% brewery dried grain, 0.75% oyster shell, 0.5% bone meal, 0.5% salt and 0.25% vitamin premix respectively. The experimental diets constituted guinea grass and avocado seeds with orange peels. The concentrate was used as supplementary diet to the experimental diets. The treatment diets were offered at the rate of 5% (DM basis) of the animals' body weight. The experimental diets and concentrate supplement were given in the ratio of 60:40 respectively. Diet A which served as control group constituted 60% solely guinea grass, while diets B and C comprised combination of avocado seeds with orange peels in a ratio of 25: 35 and 20: 40 respectively. Concentrate supplement of 40% was added to each of the experimental diet.

Experimental Animals and Management: Twenty four growing West African Dwarf (WAD) rams, age between 8 and 9 months with average initial body weight of 8.00 ± 0.12kg were used for the study. The rams were purchased at Ekpoma market and randomly allotted to the three dietary treatments in a completely randomised design. Each treatment was replicated with eight animals. On arrival, the WAD rams were given prophylactic treatments against ecto and endo parasites and allowed a period of 21days for acclimatization. Thereafter, they were individually housed in demarcated pens. The pens were adequately ventilated; cleaned daily and wood shavings were changed fortnightly. The experimental diets were fed to rams once daily at about 8:00am in the morning with concentrate supplement first followed by the experimental diets. Drinking water and mineral salt lick were provided *ad-libitum* throughout the experimental period. The experiment lasted for 84days excluding the 21days of adaptation period.

Rumen Study: Rumen fluid sample (40ml) was taken at one hours post – feeding once every one week from six rams per treatment throughout the experimental period. The rumen fluid sample was collected by means of suction tube thrust into the rumen compartment. As soon as the sample was obtained, rumen fluid temperature and pH were determined within two minutes of collection by using thermometer and digital pH meter, respectively. The digital pH meter was stabilised in distilled water with specific pH recommendation before used for the reading. 20ml of the rumen fluid samples was stored in 40ml 10% formal saline prior to the direct microscopic counts of rumen protozoa, bacteria and fungi. While the other 20ml sample of rumen fluid was bulked for each animal before made free of coarse particle by filtration with cheese cloth. Thereafter, 5ml sample of the filtrate was then acidified with 1ml of a 5% (v/v) orthophosporic acid solution and stored frozen in the airtight plastic bottle container for determination of volatile fatty acid concentration and its fractions. The other 15ml of the filtrate sample was added to 10% sulphuric acid solution before they were stored freeze for analysis of ammonia nitrogen (NH_3 – N) concentration.

Laboratory Analysis: Samples of the experimental diets (guinea grass, avocado seeds and orange peels) and concentrate supplement offered to rams were analysed for proximate composition using the procedure AOAC (1990). Microscopic counts of rumen fluid bacteria, protozoa and fungi were performed with the improved Neubauer and Mod Fuchs Rosenthal type counting chambers as reported by Morrison et al. (1990). Total volatile fatty acids production was determined by steam distillation process using Markham micro-distillation apparatus as reported by Yusuf et al. (2013). Individual volatile fatty acids were determined using gas chromatography (Mebrahtu and Tenaye, 1997). Rumen ammonia nitrogen concentration was determined as described by Lanyansunya et al, (2007).

Statistical Analysis: Data obtained from rumen microbial population and volatile fatty acids studies were subjected to analysis of variance (ANOVA) and significant difference between means were separated using Duncan Multiple Range Test (SAS, 1999)

Results and Discussion

The proximate composition (% dry matter) of the experimental feedstuffs (guinea grass, avocado seeds, orange peels) and concentrate supplement diet are shown in Table 1. The result indicated that, the dry matter (DM) contents of the experimental feedstuffs and concentrate supplement that ranged from 70.82% to 86.02% were relatively high, suggesting the feeds can be stored for a longer period of time without spoilage. The crude protein value of experimental feedstuffs (guinea grass 7.00%, avocado seeds 5.00% with orange peels 7.49%) were below the 10% crude protein level recommended by Bengaly et al. (2007) for maximum growth in ruminant animals. Thus, the 20.01% crude protein of concentrate supplement was added to the feedstuffs to provide adequate nitrogen requirement for rumen microbes to maximally digest the components of dietary fibre leading to the production of volatile fatty acids (Okoruwa and Igene, 2014). Crude fibre and ash contents were considerably different in values, being highest in guinea grass (38.00 and 10.00%) and lowest in avocado seeds (4.20 and 2.70%), respectively. This implies that the total crude fibre with mineral content present in guinea grass is highest compared to other feedstuffs used in the experimental diets. Ether extract had similar low values with exception of orange peels (5.60%) that is higher in value, indicating high fats and oil in orange peels compared to avocado seeds and concentrate supplement with guinea grass. Nitrogen free extract values that ranged from 44.11% to 57.92% were considerably high and reflect high energy content of the feeds.

Table 1: Proximate Composition (% DM) of the Experimental Feedstuffs and Concentrate Supplement

Parameters	Experimental feedstuffs			Concentrate Supplement
	GG	AS	OP	
Dry Matter	78.44	70.82	74.30	86.02
Crude protein	7.00	5.00	7.49	20.01
Crude fibre	38.00	4.20	25.01	13.00
Ash	10.00	2.70	6.72	7.98
Ether extract	0.90	2.00	5.60	1.09
Nitrogen free extract	44.11	56.92	55.18	57.92

GG = Guinea Grass, AS = Avocado Seeds, OP = Orange Peels

Table 2: Rumen Microbial Population in Growing rams fed Experimental Diets

Parameters	Diets			SEM ±
	A	B	C	
Rumen temperature (^{0}C)	37.99^b	38.64^a	38.95^a	0.34
Rumen NH_3 – N (mg/100ml)	13.05^c	18.04^a	16.47^b	0.07
Rumen fungi (mm^2)	40.26^a	34.35^b	36.69^b	0,41
Rumen protozoa ($x10^3$ml)	10.06^b	14.01^a	9.94^b	0.64
Rumen bacteria ($x10^9$ml	10.58^c	16.92^a	11.42^b	0.81

NH_3 – N = ammonia nitrogen concentration

^{a,b,c} Means within the same row with different superscripts differ significantly ($P < 0.05$), SEM = standard error of means

Presented in Table 2, is the rumen microbial population in growing rams fed experimental diets. The rumen fluid temperature (^{0}C) level was significantly (P<0.05) higher in rams on diets B (38.64^{0}C) and C (38.95^{0}C) compared with diet A (37.99^{0}C). This observed difference might be a reflection of the difference in evolution of heat from microbial fermentation activity in the diets. This is in agreement with the finding of Cabrita et el. (2006) who reported that the trend at which temperature rise in the rumen following ingestion of feeds is due to the evolution of heat in the fermentation process which has been used as a measure of microbial fermentation rate for bacteria in the rumen. Notwithstanding, the rumen temperature values obtained in this study were almost within the relative constant range values (38.00 to 40.00^{0}C) for continues microbial fermentation as reported by Cabrita et al. (2006). The rumen ammonia nitrogen (NH_3 – N) concentration was significantly (P < 0.05) highest in diet B (18.04 mg/100ml), followed by diet C (16.47mg/100ml) before diet A (13.05mg/100ml). This variation obtained in NH_3 – N concentration might be due to the crude protein combination from the test ingredients inclusion levels in the diets, specifically increased within avocado seed inclusion in the experimental diets which influenced the nitrogen uptake by the rumen microbes. The rumen NH_3 – N concentration values (13.05 – 18.04mg/100ml) obtained in this study fell in the range of values reported by Satter and Syster (1974). These authors reported that rumen NH_3 – N concentration had a good profile, with values between a minimum of 2mg/100ml and a maximum of 30mg/100ml suggested for maximum microbial growth in the rumen. Similarly, Lindela and Lewis (1995) also reported that ruminal NH_3 – N concentration has a good profile with values between 2 and 5mg/100ml as a minimum rumen fluid for maximize rumen microbial synthesis, 15mg/100ml rumen fluid to maximum fibre digestion and 20mg/100ml rumen fluid to maximize intake. However, the reported optimum rumen ammonia concentration (RAC) ranging 5 – 20mg/100ml for the most suitable microbial activities by Leng and Nolan (1984) is also in consonance with the values reported in this study but contradict the suggested ruminal NH_3 – N concentration above 20mg/100ml that is required for sufficient voluntary intake of low quality roughage as reported by Yusuf et al. (2013).

Microbes yield in the rumen is very important because is an index or a function of the amount of microbial protein made available to the ruminant daily. Anaerobia fungi are reported to be the first to reduce the tensile strength of feed particles and increase the particles breakdown in rumination, thus they are important initiators of fermentative breakdown of insoluble plant cell wall materials (Okoruwa et al., 2013). However, rumen fungi population that ranged from 34.35 to 40.26mm^2 was significantly (P < 0.05) highest in diet A and lowest in diets B and C. This variation could primarily influenced by the rate of attaching plant particles of the feed to size reduction. This agrees with the findings of Morrison et al. (1990) that enhanced fungal activity can cause a significant decrease in the resistance of plant particles to size reduction and then, the weakening and /or fragmentation of plant particles would also perhaps increase the surface area suitable for bacteria colonization and attack.

Major changes in rumen protozoa population counts were observed in the study due to different levels of test ingredients inclusion in the diets. Marked significant (P < 0.05) higher difference was observed in rumen protozoa counts for rams on diet B (14.01 x 10^{3}ml) compared to rams on diets A (10.06 x 10^{3}ml) and C (9.94 x 10^{3}ml). This increment in rumen protozoa counts with higher rumen pH in diet B, undoubtedly result in an increased outflow of volatile fatty acids in the rumen as testify in Table 3. This further confirms the previous findings of Getachew and Makkar (2002) that volatile fatty acids are the major fermentation end products of increased outflow of protozoa population counts in the rumen. In addition, ruminal pH could be stabilize by stimulating ciliate entodiniomorphid protozoa which are known to engulf starch granules very rapidly and thus compete effectively with amylolytic bacteria for their substrate. Hence, starch is fermented by protozoa as a slower rate that by amyloytic bacteria and the main end product is volatile fatty acid not lactate. However, rumen bacteria are the principal agents for fermenting plant cell wall carbohydrates, being the largest population of micro-organism in the rumen than protozoa and fungi. The rumen bacteria count values that ranged from 10.58 to 16.02 x 10^{9}ml was significantly (P < 0.05) highest in diet B and lowest in diet A. the highest number of rumen bacteria counts observed in diet B, might be responsible for high digestion of more protein and fibre by attaching the plant particle to provide more NH_3 – N concentration and total volatile fatty acids which enhance microbial activities. Bacteria count of rumen fluid is dependent in rumen NH_3 – N concentration and pH of rumen fluid and both depend on the type of diet (Yusuf et al., 2013). This implies that, increasing lactate utilizing bacteria species (Megashaera alsdenii and Selenomonas ruminantium) population outnumber the lactating producing bacteria species (Streptococcus bovis) in this diet B, leading to less accumulation of lactate in the rumen that would have led to metabolic acidosis in the rams (Gozho et al., 2005).

Chaucheyras Durand et al. (2008) also reported that the major – fibre degrading bacterial species which are Fibrobacter succinogenes, Ruminoccus albus and Ruminoccus flavfaciens are particularly increase in population under high rumen pH but sensitive to low rumen pH. However, the low bacteria counting diet A might indicate ammonia is limiting for bacteria growth and present of high protozoa count that encourage defaunation (Okoruwa et al., 2013).

Tables 3: Rumen Volatile Fatty Acids (VFAs) Parameters of Growing Rams fed Avocado Seeds with Orange Peels Meal as Replacement for Guinea Grass

	Diets			
Parameters	A	B	C	SEM±
Rumen pH	6.78[a]	6.65[a]	5.98[b]	0.66
Total VFAs (mmol/litre)	77.95[b]	85.16[a]	88.01[a]	0.96
Molar (%)				
Acetic acid	46.77[a]	43.06[b]	40.61[c]	1.02
Propionic acid	13.01[c]	21.51[a]	16.62[b]	0.72
Butyric acid	12.02[a]	12.85[a]	9.43[b]	0.49
Acetic: Propionic ratio	3.89[a]	2.00[b]	2.44[b]	0.05

a,b,c means within the same row with different superscripts differ significantly ($P < 0.05$)
SEM = standard error of mean

Presented in Table 3, is the rumen volatile fatty acids (VFAs) parameters of growing rams fed avocado seeds with orange peels meal as replacement for guinea grass. The rumen pH values that ranged from 5.98 to 6.78 were significantly ($P < 0.05$) higher in diets A and B but lower in diet C. This difference could be attributed to the different inclusion levels of the test ingredient in the diets, specifically, increased in orange peels inclusion levels in the experimental diets would have reduced the rumen pH values. Ruminal pH is an important factor that measure the acidity and alkalinity of rumen content in ruminants and for optimum rumen microbial fermentation, the rumen pH should ranged between 6.00 and 6.80 (Ososanya et al., 2013). Gozho et al. (2005) also reported that when the ruminal pH is low, microbial diversity is reduced as protozoa numbers may sharply declined and the bacterial population is altered and largely reduced. However, the rumen fluid pH values observed in diet B (6.65) compared favourably with the range values (6.00 to 6.70) for maximum microbial growth as reported by Lindela and Lewis (1995). Total volatile fatty acids was significantly ($P < 0.05$) higher in diets C (88.01mmol/litre) and B (85.16mmol/litre) than diet A (77.95mmol/litre). This variation in the result might be connected with the inclusion levels of the test ingredients in the experimental diets, which increase the fermentation of the feeds offered which result in the production and accumulation of more total volatile fatty acids at low pH. It has been reported (Lindela and Lewis, 1995) that high production of volatile fatty acid in the rumen is linked with ruminal lysis of microbes and fermentation of microbial cell. Yusuf et al. (2013) also reported that, if the volatile fatty acids production rate exceeds the clearance rate, volatile fatty acids will accumulate in the rumen; this may lower rumen pH and cause the metabolic disturbance known as rumen acidosis. However, there was no case of rumen acidosis in this study, meaning that the rumen pH was still within the normal range for the rams. Nagaraja and Lechtenberg (2007) reported that ruminal pH value that drop below 5.2 to 5.5 should be considered as the threshold for rumen acidosis in ruminants fed high concentrate diet.

Volatile fatty acids are classified as one of the universal end – product of anaerobic microbial fermentation of carbohydrates in the rumen that contribute about 70% for the calories requirement of ruminants and the proportion of major partials of volatile fatty acid concentration in the rumen depends largely on the type of feed consumed by the animals (Dung et al., 2011). The significant ($P < 0.05$) higher value of acetic acid observed in rams on diet A (46.77%) compared to diets B (43.06%) and C (40.61%) might be due to the fact that they depend solely on guinea grass as their experimental diet. Widiawati and Thalib (2009) reported that feeds resulting in increase of acetate production will promote an increase of methane and carbondioxide production, which will represent a net loss of feed energy as well as inefficiency in feed utilization. The proportion of propionic acid was significantly ($P < 0.05$) highest in diet B (21.51%), followed by diet C (16.62%) before diet A (13.01%). Butyric acid proportion did not follow the same pattern of variation as observed in propionic acid. The butyric acid values ranged between 9.43 to 12.85% was significantly ($P < 0.05$) highest in diets A (12.02%) and B (12.85%) but lowest in diets C (9.43%).

However, the highest proportion of propionic acids obtained in diet B revealed the better rumen fermentation of feeds by the rumen microbial activity to yield energy, while the low propionic acid of diet A could constrain rams productivity as propionic acid has been reported to be increased by concentrate diet and classified as the major precursors of glycogenic fatty acid in ruminants (Vasta et al., 2009). The inverse relationship showed between acetic and propionic acids in this study further buttress the fibre and energy contents in the experimental diets. Notwithstanding, the significant ($P < 0.05$) reduction in acetic and propionic ratio in diets B (2.00) and C (2.44) compared to diet A (3.89) could probably be an indicative of increased bacteria activities. Moreover, it is interesting to note that the overall microbial counts in the rumen fluid for rams on diet B could be an indicative of normal rumen environment (normal pH, availability of NH_3 – N and volatile fatty acids) for microbial growth. This would be invariably led to increase in the production of microbial protein.

Conclusion

Avocado seeds with orange peels meal are potential source of readily available feeds that would fill the gap of forage availability shortage for growing rams during the dry season. However, base on the result obtained in this finding, it was therefore concluded that feeding of avocado seeds with orange peels meal as replacement for guinea grass offered a balance of essential nutrient requirement for growing rams. Thus, the combination of avocado seeds with orange peels meal in a ratio of 25: 35 respectively as replacement for guinea grass can be recommended as an appropriate feeding strategy to improve growing rams' performance without any negative effects on rumen microbial population and volatile fatty acids in growing rams.

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