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Live Performance and Rumen Microbial Composition of Yankasa Rams with Supplemented Levels of *Zingiber officinale*

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Authors' contributions

This work was carried out in collaboration between all authors. Author NM designed the study, wrote the protocol, analyse the data, provided PG training for author UMI and finalized the manuscript. Author UMI managed the experimental process, wrote the first draft of the manuscript. Author SAM critiqued the manuscript. Author IAA managed the literature searches and reviewed the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The experiment was conducted to evaluate the effect of graded levels of *Zingiber officinale* on performance and rumen microbial composition of Yankasa rams.
Methodology: A complete experimental diet was formulated while ginger was supplemented at 0, 2.5, 5 and 7.5 g/kg inclusion levels to serve as treatments. The four treatment diets were fed to twenty (20) intact male animals with an average live weight of 34.2±1.07 kg. A completely randomized experimental design (CRD) was used in the experiment with four treatments replicated

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five times. Each animal serves as replicate and graded levels of ginger representing treatments.
Results: Results indicated significant difference (P<0.05) in animal's final weight, live weight gain and average daily gain. Ginger supplementation beyond 2.5 g/kg resulted in decreased bacterial specie composition in the rumen. *Bacillus* sp was found to be persistent even at the highest level of supplementation.
Conclusion: It was concluded that for efficient animal performance, ginger supplementation should not exceed 2.5 g/kg.

Keywords: Performance; rumen microbes; Yankasa Rams; Zingiber officinale; supplementation.

1. INTRODUCTION

Artificial growth promoters in animal feeds have transmissible resistance factors that may compromise the use of therapeutic antibiotics in humans [1]. Livestock farmers are generally faced with challenges of improving livestock performance in order to ensure more net returns [2]. A lot of research and production strategies have been employed, including the use of antibiotics to achieve this aim [3]. Although antibiotics achieved good performance, their potential side effects became a real public health concern globally [4]. This triggered an explosion of interest in the use of herbs and spices and their products as supplements in animal rations [5]. Modification of rumen fermentation using feed additives, such as organic sources like spices has proved to be a useful strategy for improving production efficiency in ruminants. The use of ginger as feed additive is a useful tool in reducing energy and nitrogen losses by ruminants [6].

Manipulation of rumen microbial ecosystem for enhancing fibrous feed digestibility and reducing nitrogen excretion to improve animal performance are some of the most important goals of animal nutritionists. Plant extracts are good materials for achieving these objectives [7]. Therefore, the use of spices that are rich in plant secondary metabolites (PSM) in many parts of the tropics is increasing [8,9]. Plant herbs (such as ginger and garlic) have been shown to improve rumen ecology [10,11]. However, limited data are available on the effects of ginger on rumen microbial fermentation and subsequent utilization by farm animals. The present study evaluated the effect of ginger supplemented levels on productive performance and rumen microbial composition of Yankasa rams.

2. MATERIALS AND METHODS

2.1 Experimental Location

The experiment was conducted at the National Animal Production Research Institute (N.A.P.R.I.), Ahmadu Bello University (ABU), Shika, Zaria, Nigeria. Shika is geographically located at latitude 11º12'N and longitude 7º33'E at an altitude of 640 meters above sea level [12]. It is about 20 km from Zaria city along Zaria - Sokoto road in Kaduna State, Northern Nigeria. Zaria has three distinct climatic seasons which include cold dry season (November-February), hot dry season (March-May) and wet season (June-October). The total annual rainfall ranges from 617 to 1365 mm with a 50-year average of 1041 mm [13]. Most of the rains fall between July and September. Zaria falls within guinea savanna vegetation zone [13]. The mean maximum temperature varies from 26℃ to 35℃ depending on the season, while the mean relative humidity during harmattan period is 21% [13].

2.2 Experimental Animals and Their Management

Twenty apparently healthy intact male Yankasa rams of between 34.2±1.07 were used for the study. The animals were obtained from Small Ruminant Research Programme unit of N.A.P.R.I and quarantined for two weeks. They were orally dewormed with albendazole (2.5% solution) against gastro-intestinal parasites and treated with Oxytetracycline (a broad-spectrum antibiotic) based on manufacturer's recommendation.

2.3 Sanitation and Health Management

Feces and urine were removed daily from the feeding pens to ensure adequate hygiene, less ammonia accumulation, optimal cleanliness of the experimental pens and minimum discomfort of the experimental animals. This practice was maintained for the entire period of the study.

2.4 Experimental Feed Preparation

A complete experimental diet was formulated (Table 1) with varying supplemented levels of

ginger at 0, 2.5, 5 and 7.5 g/kg feed dry matter to represent treatments. The diets were designated as diets 1, 2, 3 and 4 in the experiment. The ginger was sourced from local market and ground before inclusion.

2.5 Experimental Design and Feeding Procedure

A completely randomized experimental design (CRD) was used in the experiment with number of animals representing replication and graded levels of ginger supplementation representing treatments. Five animals were allocated to each treatment. The initial weights (34.2±1.07) of the animals were balanced at the start of the experiment. Each group was assigned to one of the experimental diet (based on ginger supplementation) and fed for 84 days. The experimental diet was fed according to the body weights of the animals. The basal diet (*Digitaria grass*) was offered three (3) hours after feeding the concentrate (Table 1). Clean drinking water was offered *ad-libitum*.

2.6 Proximate Analysis of the Experimental Diet and the Test Ingredient

The experimental diet and test ingredient were analyzed for proximate components at the N.A.P.R.I. They were examined for dry matter (DM), crude protein (CP), nitrogen free extract (NFE), crude fibre (CF), ether extract (EE), and ash. The analysis procedure was carried out as outlined by Association of Official Analytical Chemists [14].

2.7 Phytochemical Analysis of the Test Ingredient / Determination of Metabolites

Phytochemical screening were carried out for all the extracts (alkaloids, saponins, phenols, tannins, flavonoids and oxalates) as per the standard methods as described by Prashant et al. [15] and Solomon et al. [16].

2.7.1 Determination of alkaloids, saponins, Phenols, tannins and flavonoids

Extracts of the test ingredient were dissolved individually in dilute Hydrochloric acid and filtered. The sample was then subjected to Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids. For the determination of saponins, froth Test was involved; Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins. Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols. In the gelatin test (for detection of tannins), 1% gelatin solution containing sodium chloride was added to the sample. Formation of white precipitate indicates the presence of tannins. Alkaline Reagent Test was used for the determination of flavonoids. Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

2.7.2 Determination of oxalates

3 ml portion of extracts were added a few drops of glacial ethanoic acid. A greenish black colouration indicates presence of oxalates.

2.8 Data Collection

The data were collected in two phases as follows:

2.8.1 Phase I

2.8.1.1 Feeding trial

Body weight of the animals was taken at the beginning of the experiment (day 0). Subsequently, the animals were weighed weekly for body weight changes. Feed intake was measured and recorded daily by subtracting the left over from the quantity of feed offered to the animals the previous day. Feed conversion ratio was determined using feed intake and body weight gain. Nutrient intake was obtained from feed intake and chemical composition of the feed material. Feed and nutrient intake as % body weight was obtained from live weight and feed intake of the experimental animals.

2.8.2 Phase 2

2.8.2.1 Rumen fluid collection

Ten (10) mls of rumen liquor was withdrawn individually from three animals of each treatment using rubber stomach tube before feeding at the beginning, middle and last week of the experiment.

Table 1. Gross composition of the experimental diets (%)

Ingredients	Treatments (Ginger supplementation level (g/kg)			
	1 (0)	**2 (2.5)**	**3 (5.0)**	**4 (7.5)**
Maize	20.46	20.46	20.46	20.46
Cowpea husk	12.55	12.55	12.55	12.55
Cotton seed cake (CSC)	10.98	10.98	10.98	10.98
Rice offal	12.65	12.65	12.65	12.65
Cowpea hay	39.86	39.86	39.86	39.86
Salt	0.50	0.50	0.50	0.50
Bone meal	2.50	2.50	2.50	2.50
Premix	0.50	0.50	0.50	0.50
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated nutrient contents				
Crude Protein (%)	12.00	12.00	12.00	12.00
Energy (kcal/kg)	2600	2600	2600	2600
Crude Fibre (%)	23.82	23.82	23.82	23.82
Supplemented ginger level (g/kg)	0	2.5	5.0	7.5

Collected samples were immediately taken to the Microbiology laboratory of the Department of Microbiology, Faculty of Science of Ahmadu Bello University, Zaria and analyzed for identification of Microbial species. Sterile glass bottles were used for storage of the samples. The bacterial colonies were observed and identified based on their colony morphology, cell morphology, Gram's reaction and reaction to some biochemical tests (citrate utilization, methyl red and Triple sugar iron agar test respectively) [17].

2.9 Statistical Analysis

The data generated from the experiment were subjected to analysis of variance (ANOVA). Least significant difference (LSD) was used to separate the means. The data was analysed Using Statview Statistical Package [18].

3. RESULTS AND DISCUSSION

3.1 Proximate Composition of the Experimental Diet and Test Ingredient

Results (Table 2) indicated that DM, Ash CF were higher in the concentrate compared to ginger and the basal diet (grass) whereas CF was higher in the grass. Other nutrients (EE, N, CP and NFE) were higher in ginger compared to the concentrate and the grass.

The value of dry matter is similar to the values of 95.52-99.35% reported by Ahmad et al. and Obe et al. [19,20]. The high DM content of the formulated diet and the ginger was as a result of high dry matter content of the ingredients used in the diet formulation which is the characteristic of most tropical crops as observed by Aduku [21]. The crude protein content of the concentrate diet and the ginger was adequate to meet the optimum microbial need in the rumen as it is above 7% protein required for optimum microbial growth [22]. The EE values are comparable to the range of 4.30 to 5.50% reported by Maigandi [23] while ash contents are within 7.91 to 12.81% indicated by Oloche et al. [24]. The NFE composition of the concentrate diet is within the range of 44.60-49.80% obtained by Adeniji et al. [25] while CF content is similar to the range of 25.50 to 26.20% reported by Aruwayo et al. [26].The similarities in nutrient composition of the feeds might be due to the fact that the ingredients might originate from the same sources (possibly from similar climatic and soil conditions). The Ash content of the ginger is slightly less than the 8.84% reported by Olukotun et al. [27] But the EE and NFE contents are similar to the value of 5.62% and the range of 54.27% - 64.46% respectively observed for *Ocimum gratissimum* by Faniyi et al. [28]. The CF content of the test ingredient (12.28%) is similar to the value of 12.50% reported by Faniyi et al. [28] for *Anacardium occidentale*. The CP composition observed for ginger in the present study is less than 17.56% indicated by Faniyi et al. [28]. This variation could be attributed to varietal difference, soil conditions and agronomic practices.

Table 2. Proximate composition of the experimental diet and test ingredient

Nutrients	Grass	Concentrate	Ginger
DM	90.04	96.16	96.09
Ash	2.65	11.21	7.37
EE	0.54	4.94	5.90
CF	30.05	25.13	12.28
CP	5.36	9.20	11.36
NFE	61.40	49.52	63.09

DM=Dry Matter, EE=Ether Extract, CF=Crude Fiber, CP=Crude Protein, NFE=Nitrogen Free Extract

3.2 Phytochemical Constituents of Ginger and Their Intake by Yankasa Rams

Phytochemical analysis indicated a higher oxalate content followed by flavonoid, phenols, alkaloids and saponins in that order. Tannins were the lowest constituents in the sample material (Table 3). Intake of all the phytochemicals was significantly higher for animals fed diets containing higher ginger levels and lower for the control diet ($p<0.01$) (Table 4).

Table 3. Quantitative Phyto-chemical analysis of the test ingredient (ginger)

Constituents	Composition (mg/100 g ginger)
Tannin	0.26
Saponin	0.80
Oxalates	4.56
Alkaloids	1.20
Flavonoids	2.40
Phenols	1.52

The alkaloids, saponins and tannins contents of ginger are similar to the values of 1.44, 0.18 and 0.24 mg/100 g respectively reported by Ogundun et al. [29]. However, the value for flavonoids (2.40 mg/100 g) obtained in the present study is higher than 0.22 mg/100 g obtained by Denman and McSweeney [30]. Animals fed higher ginger supplementation (T2 and T3) were observed to consume more of tannins and saponins than animals on control diet (T1) (Table 4). Although animals fed higher ginger supplementation have a reduced DMI, DMI/concentrate intake and DMI/grass intake, their LWG and ADG were not significant from those fed no ginger supplementation (Table 6). This might be due to the beneficial effects of some phyto-chemicals taken along with other nutrients. This is because low to moderate concentrations of tannins have been reported to improve the digestive utilization of feed due to reduction of protein degradation in the rumen and a subsequent increase in amino acid flow to small intestine have been reported [31]. Saponins have also been found to enhance protection of protein from degradation in the rumen and increase availability of protein post-ruminally [31]. Saponins also form complexes with protein and could decrease protein degradability [32]. Increased intake of alkaloids by the animals in the present experiment also might not have affected the performance of the animals. It was observed that sheep, goats and cattle are much more resistant and tolerant to even much higher alkaloid dosages which are thought to be due to thorough detoxification via pyrrolizidine alkaloid-destroying rumen microbes [33]. The same reason could be attributed to increased flavonoids and phenols intake as the dietary supplementation of ginger increased from treatment 1 to treatment 4 (Table 4). Similarly, flavonoids rich plants are bio-active regulator for ruminants [34]. Phenols are also known to improve live weight gain, milk yield and protein concentration and ovulation rate [34]. They prevent bloat in ruminants; reduce gastrointestinal nematode numbers and methane production [34]. Oxalates recorded the highest ($P<0.01$) (4.56 mg/100 g) phyto-chemical in the present study. As reported by Ramasastri [35], certain spices contain exceptionally high levels of oxalates and when ruminants are exposed to a diet high in oxalate, the population of oxalate-degrading bacteria in the rumen increases sufficiently to prevent oxalate-poisoning [36]. In the present study, *Bacillus species* was the only bacteria found in treatment 4 (7.5 g/kg ginger) with the highest oxalates intake (Table 5). *Bacillus subtilis* possesses induced oxalate de carboxylase enzyme that could prevent oxalate toxicity [37].

3.3 Effect of Ginger Supplementation Levels on Rumen Microbial Composition

Results (Table 5) showed that, the number of isolated organisms decreased with increasing level of ginger (Fig. 1). *Bacillus* sp population increased with increased ginger level. Results indicated that only *Bacillus sp* was found at higher ginger supplementation (Table 5). Colony forming unit (cfu/ml of rumen content) were significantly higher ($p<0.01$) for animals fed diets containing 2.5 g/kg ginger (Table 5). Animals fed diets containing higher ginger level (treatment 4) had a significantly lower cfu (cfu/ml) (Fig. 2).

Table 4. Intake of phyto-chemicals Based on ginger supplementation (mg/Kg)

Parameter	Treatments (Ginger supplementation level (g/kg)				SEM
	1 (0)	2 (2.5)	3 (5.0)	4 (7.5)	
Tannin	0.00^{d}	19.05^{c}	38.23^{b}	52.34^{a}	2.93
Saponin	0.00^{d}	58.60^{c}	117.64^{b}	161.05^{a}	9.02
Oxalates	0.00^{d}	334.04^{c}	670.55^{b}	918.01^{a}	51.43
Alkaloids	0.00^{d}	87.91^{c}	176.46^{b}	241.58^{a}	13.53
Flavonoids	0.00^{d}	175.81^{c}	352.92^{b}	483.16^{a}	27.07
Phenols	0.00^{d}	111.35^{c}	223.52^{b}	306.00^{a}	17.14

Means within the same row with different subscripts are significantly different (p<0.01)

Table 5. Effect of ginger inclusion level on microbial composition of the rumen

Treatment (Ginger supplementation level (g/kg)	Colony forming unit (cfu/ml)	Isolated organism
1 (0)	$2.15 \times 10^{5\ b}$	*Salmonella* sp, *Klebsiella* sp, *Bacillus* sp *Escherichia coli*
2 (2.5)	$3.0 \times 10^{5\ a}$	*Salmonella* sp, *Klebsiella* sp, *Bacillus* sp *Escherichia coli*
3 (5.0)	$2.3 \times 10^{5\ b}$	*Escherichia coli* *Bacillus* sp
4 (7.5)	$3.8 \times 10^{4\ c}$	*Bacillus* sp

sp=species

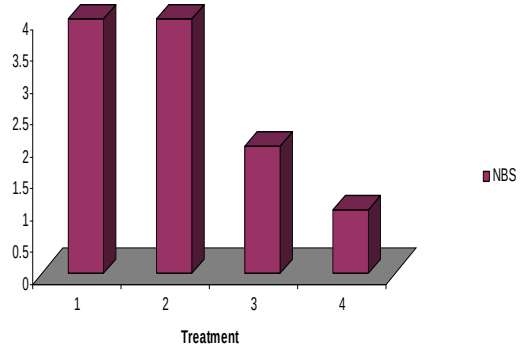

Fig. 1. A graph showing the number of bacterial species according to treatments
NBS= Number of Bacterial Species

It was observed that rumen bacterial specie composition decreased (P<0.01) with increased ginger supplementation from 2.5g/kg. However, the CFU was observed higher at 2.5g/kg (T2). Of all the isolated organisms, only *Bacillus* sp was found at the highest level of supplementation (7.5 g/kg) (T4) supporting the antimicrobial activity of ginger. It further suggests that *Bacillus* sp is more viable and adaptive to rumen environment. It was reported that *Bacillus sp* is extremely tolerant to heat and disinfectant and lives in a wide range of habitats. It also synthesizes important enzymes [38]. The highest CFU together with higher specie composition observed from animals in Treatment 2 (2.5 g/kg ginger) is an indication that ginger could influence CFU of retculo-rumen as observed by Sivakumaran et al. [39]. Decrease in bacterial specie composition and CFU beyond 2.5 g/kg supplementation indicates that 5 and 7.5 g/kg levels are beyond optimum. Decreasing level of bacterial *sp* with increased level of ginger suggests the suppressive effect of the test material on rumen microbes.

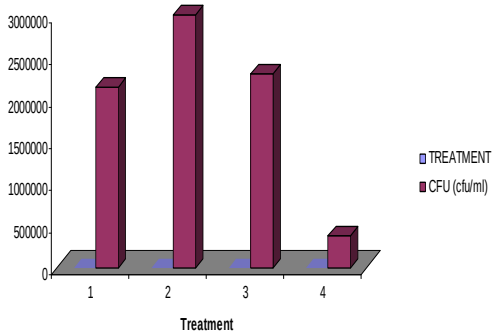

Fig. 2. Bacterial colony forming unit (CFU)

Table 6. Growth Performance of Yankasa rams fed graded levels of *Z. officinale*

Parameter	Treatments (Ginger supplementation level (g/kg)				SEM
	1 (0)	2 (2.5)	3 (5.0)	4 (7.5)	
Initial body weight (kg)	34.20	34.80	34.20	34.20	1.07
Final body weight (kg)	44.60 [a]	43.60 [ab]	42.30 [b]	41.50 [b]	2.03
Weight gain (kg)	10.40	8.80	8.10	7.30	1.21
Average daily weight gain (g/day)	123.81 [a]	104.77 [b]	96.43 [bc]	86.91 [c]	14.37
Feed conversion ratio	12.49	13.42	13.07	3.77	0.58
Feed intake (kg/day)	1.87	1.86	1.76	1.69	0.08
Concentrate intake (kg/day)	1.53	1.47	1.38	1.34	0.083
Grass intake (kg/day)	0.34	0.38	0.39	0.35	0.036
Overall feed intake (kg)	156.69	155.84	148.18	142.09	8.16
Overall concentrate intake (kg)	128.16	123.61	115.83	112.57	6.49
Overall grass intake (kg)	28.54	32.23	32.35	29.53	2.81
Feed intake as % body Weight (kg)	4.18	4.24	4.17	4.08	0.09
Concentrate intake as % Body weight (kg)	3.42[a]	3.36[ab]	3.26[ab]	3.23[b]	0.06
Grass intake as % body Weight (kg)	0.76	0.88	0.91	0.85	0.07

a,b Means within the same row with different subscripts are significantly different (p<0.01)

3.4 Growth Performance of Yankasa Rams fed Graded Levels of *Z. officinale*

There were no significant difference in concentrate intake (kg) (overall and daily), grass intake (kg) (overall and daily), feed intake (kg) (overall and daily) and feed intake as % body weight (p=0.05). Concentrate intake as % body weight was significantly higher in treatment 1 (3.42 kg) (p<0.01) compared to treatments 2, 3 and 4 (Table 6).

The non-significance difference (P=0.05) in FCR and dry matter intake (DMI) was responsible for the non-significance difference in LWG. The decrease in ADG as a result of decrease in feed intake might be as a result of the ginger's pungent properties induced by the phyto-chemicals as observed by Akoachere et al.; Sarica et al. and Greathead [40,41,42]. The animals ate more of the basal diet as the level of ginger supplementation increased to compensate for lower concentrate intake. Thus, increase in grass intake with increasing level of ginger supplementation might be due to the fact that the animals compensate for lower concentrate intake from high grass intake. This phenomenon of compensation was also observed by Muhammad et al. [43]. Decreased feed intake for animals in treatment 4 (7.5 g/kg ginger) might be attributed to high supplementation of ginger which have affected microbial composition. Antimicrobial properties of ginger have been reported [44,45,46]. However, the non-significant difference in final live weights, live weight gain and ADG among treatments 1 and 2 shows the efficiency of the animals in utilizing the feeds with supplementation of the test ingredient. Although the microbial specie composition decreases with increased ginger level, the live weight gain of the animals was not significantly affected at the lower level of supplementation (2.5 g/kg). The persistence of the *bacillus sp* suggests that it is more viable in the rumen of the animals compared to other species.

4. CONCLUSION

It was concluded that level ginger supplementation did not have significant impact on growth performance of Yankasa rams. Above 2.5 g/kg supplementation level, rumen microbial specie composition decreases whereas *Bacillus sp.* population increased. For optimum animal performance and microbial specie composition, ginger supplementation should not exceed 2.5 g/kg.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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