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Dietary impact of soybean haulms and/or garlic powder on rumen fermentation and microbial biomass of sheep

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Abstract
The need to assess the fermentation pattern in the rumen is important in evaluating alternative feeding resources that can be used for sustainable feeding regime in ruminants. This research assessed the rumen fermentation and microbial populations as affected by dietary soybean haulms and/or garlic powder in the diets of sheep. A total of fifteen (15) rams from crosses between Yankasa and Uda breeds were used for this experiment. The rams were balanced for weight and randomly divided into three (3) groups of five animals each and assigned three treatment diets in a completely randomised design. The three treatment diets were T1 (Control: no inclusion of soybean haulms), T2 (30% inclusion of soybean haulms in the diet), and T3 (30% inclusion of soybean haulms + 0.5% inclusion of garlic powder). Each animal served as a replicate and the animals were fed experimental diets for 77 days, thereafter, rumen samples were collected from all animals for sampling. Data were collected on rumen pH, volatile fatty acids and its proportions and microbial count were subjected to one-way analysis of variance using SPSS (version 23). Results obtained indicates that the rumen pH and metabolites measured, total counts for bacteria, fungi and protozoa were not affected ($p>0.05$) by inclusion of soybean haulms and/or garlic powder in the diet of sheep. However, populations of *Bacillus spp*. reduced ($p<0.05$) in group of sheep fed soybean haulms only (T2) compared to control (T1). Also, *Isotricha spp*. decreased ($P<0.05$) in group of sheep on soybean haulms only (T2) compared to those on soybean haulms and garlic powder T3). Some of the bacteria and protozoa species assessed in this experiment were not affected ($p>0.05$) by inclusion of soybean haulms in diet of sheep. Also, the addition of garlic to diet with soybean haulms did not further influence the fermentation pattern in the rumen. In conclusion, 30% of soybean haulms in diets of sheep without addition of garlic powder are recommended to maintain healthy fermentation pattern in the rumen.
Keywords: Sheep, soybean haulms, garlic, microbial counts, rumen metabolite

Introduction

Nutrition in ruminant production is key because feeds have capacity to manipulate rumen microbiota and improve animal growth performance. This is because the rumen is a complex environment comprising diverse microbes that assist the host in digesting and utilizing feed energy. Rumen microbes produce various glucanases and xylanases to digest solid fibre by adhering to its surface, finally converting it into volatile fatty acids (VFAs), which includes acetate, propionate, and butyrate [8,11]. The VFAs are the primary source of energy in ruminants, as they provide 70–80% of the body's energy needs of the animals, thereby aiding in growth and production performance. The VFAs produced in the rumen are absorbed and transported through the rumen epithelium into the blood by VFA transporters [15]. This confers on ruminant the advantage of utilizing even fibrous materials as alternative feedstuff that cannot be used by non-ruminants and humans. Some of these alternatives include crop residues with soybean haulms as potential feedstuff for feeding ruminants. A good understanding of rumen fermentation pattern and microbiota under different feeding regimes is important for exploiting the microbiota to enhance feed efficiency [1,7]. Therefore, the understanding of the fermentation pattern and microbiota in sheep fed soybean haulms will better appreciate the efficiency of using crop residue as an important alternative feed resource in sheep husbandry to improve economics of meat and milk production [14]. The use of other feed additives with potential to manipulate the rumen environment is expected to further improve the efficiency of feed utilization of soybean haulms. One of such feed additives that have been reported to manipulate rumen environment to improve efficiency of feed utilization is garlic powder [5]. Dietary soybean haulms and garlic powder in diet of sheep have shown promising results in the improvement of antioxidant capacity of the animals. Similar response in terms of growth performance compared to control were reported when soybean haulms were included in diet of sheep at 30%, whereas packed cell volume, haemoglobin and total protein reduced in the same experiment [4]. However, little

information is available regarding the impact of feeding soybean haulms and/or garlic powder on dietary energy levels dynamics in the rumen during fermentation and microbiota of sheep. The objective of this study was to evaluate the effect of dietary soybean haulms and/or garlic powder on rumen fermentation, microbial composition and fungi diversity of sheep.

Materials and Methods

Experimental site

The study was conducted at the Animal Science Teaching and Research Farm of Joseph Sarwuan Tarka University, Makurdi, Benue State, Nigeria. Makurdi lies between latitude 7°50.89'N and 7°51.56' N and 8°35.94'E and 8°36.780' E in the guinea Savannah zone of Nigeria. The arear is characterized by about 6-7 months of rainy season ranging from 1317-1323 mm annually, between April to October and 5 months of dry season (Novermber-March). The temperature ranges from 17.58-38.44 °C and it is highest in the month February and March [13].

Experimental animals and management

The fifteen (15) rams used for the experiment were purchased from Lafia market, Nasarawa State. They animals were quarantined for six weeks to allow for proper acclimatization before commencement of the experiment. During the quarantine period, PenStrep (1 ml/10 kg) was administered intramuscularly to the animals for prophylactic treatment against bacterial disease for three days. They were also treated for ecto and endoparasite using Ivermectin (0.5 ml/10 kg) subcutaneously. Prior to the arrival of the rams, the pen was thoroughly cleaned and disinfected using izal solution. The rams were housed in separate pens measuring 1 x 1.5 m each. The house was constructed to have free flow of air, the floor of the pen was a slatted floor and made with wood with a zinc roof. The rams were fed in the morning and afternoon (7:00am and 12:00pm) respectively and fresh clean water was provided *ad-libitum*. The rams were kept under hygienic condition and were confined throughout the experimental period of 77 days.

Experimental Design and Diet Arrangement

The experimental design was a completely randomized design (CRD). Three (3) experimental diets (concentrate diets) were formulated as follows (Table 1):

T1(No inclusion of soybean haulms in the diet)
T2 (30% inclusion of soybean haulms)
T3(30% inclusion of soybean haulms + 0.5% inclusion of garlic powder)

Data collection

Collection of rumen sample

Rumen samples were collected six hours post-feeding from the animals from the feeding trial using a suction tube that was passed through the oesophagus into the rumen. The rumen pH was determined immediately after collection using a pH meter, samples were thereafter filtered with four-layer cheese cloth and divided into two sub-samples, which were taken to the laboratory for determination of rumen metabolites, enumeration and identification of microbes.

Fungi and bacterial count

Potato dextrose agar (PDA) was used for total fungi count while Nutrient agar was used for total bacterial count. The media were prepared according to specifications and sterilized at 121 °C for 15 mins., then 0.1 ml of the 6th dilution factor was pipetted and inoculated into the PDA plates and incubated at 25 °C (room temperature) for 24 hrs. Thereafter, the various colonies were counted. The results of colony count were expressed as fungi count per ml of the rumen sample. Colony-forming units/ml (CFU/ml) of both bacteria and fungi were obtained with the pour plate technique using nutrient algae (NA) and potato dextrose agar (PDA), respectively. All colonies appearing at the end of the incubation period were counted using a digital illuminated colony counter.

Protozoa count

The rumen fluid was added to 5 ml of formalin solution (1:1) dilution. 2.0 ml aliquot of the well mixed formalized sample was pipetted into a test tube containing 18 ml glycerol-buffer- methyl green mixture (1:20) final dilution. The mixture was allowed to stand for 4 hours before counting tubes were made in duplicate. The counting chamber (Sedgwick-rafter cell) was filled with the mixture and a cover slip was carefully placed on the chamber without causing formation of air bubbles. The slide was then set for counting in 10 Minutes to allow settling of protozoa. The eye-piece was used to count the number of organisms per 50 fields.

Protozoa count/ml of rumen sample = number of organism X dilution X organism per count (constant)
Where dilution factor =20
Constant =46.6235

Statistical analysis

The result obtained for pH, total volatile fatty acids, acetic acid, butyric acid, propionic acid, microbial count, bacteria and protozoa isolates were analysed by one-way analysis of variance as outlined in general linear model procedure of SPSS (version 23). Significant differences among the different treatment groups where applicable were separated using Duncan multiple range test contained in the statistical software. Probability significance was declared at 5%.

Results and Discussion

Rumen pH, and metabolites from sheep on diet containing soybean haulms and/or garlic powder

The results of rumen pH and metabolites from sheep fed complete diet containing soybean haulms and/or garlic powder is shown in table 2. Rumen pH and other metabolites measured were not affected ($p>0.05$) by feeding soybean haulms either alone or with garlic powder. Rumen pH was in the range of 5.20 to 5.44 across the treatment groups with marginal increase ($p>0.05$) in diets containing soybean haulms and/or garlic powder compared to control. Total volatile fatty acids marginally reduced ($p>0.05$) in T3 and then further in T2 compared to control diet. This ranged from 4.75 to 5.59 mol/100mol. Same trend observed in total volatiles fatty

acids was observed for the various proportion of fatty acid with marginal decreased (p>0.05) in treatment groups compared to control. Methane production was also not affected (p>0.05) by inclusion of soybean haulms and/or garlic powder in the diets.

Effect of feeding soybean haulms and garlic powder on rumen total microbial count in sheep

Results of feeding soybean haulms and garlic powder on rumen total microbial count are presented in table 3. Total bacteria, fungi and protozoa counts were not affected by inclusion of soybean haulms and/or garlic in the diets of sheep. Bacteria count ranged from 3.90 cfu/mL x 10^5 in treatment 2 to 4.49 cfu/mL x10^5 in treatment 1. Treatment 3 had total bacteria count of 4.16 cfu/mL x10^5. Fungi count ranged from 1.08 cfu/mL x10^5 in treatment 2 to 1.48 cfu/mL x10^5 in treatment 3, while treatment 1 had fungi count of 1.19 cfu/mL x10^5. For total protozoa count, treatment 3 had marginally higher (p>0.50) counts (28.20 ml/100mL) compared to the values obtained in treatment 1 (19.80 ml/100 mL) and treatment 2 (16.80 ml/100 mL).

Bacterial isolates from sheep on diet containing soybean haulms and/or garlic powder

Results of bacteria isolates from rumen of sheep fed complete diet containing soybean haulms and/or garlic powder is shown in table 4. *Bacillus spp*. reduced (p<0.05) in group with only soybean haulms (T2) (4.80 cfu/mL x10^5) compared to control (14.25 cfu/mL x10^5) but not group with soybean haulms and garlic powder (T3) (10.50 cfu/mL x10^5). Some of the bacteria species measured were not affected (p>0.05) by the inclusion of soybean haulms and or garlic in the diet of sheep.

Effect of feeding soybean haulms and garlic powder on different protozoa isolates

The results of different protozoa isolate from the rumen of sheep on diets with soybean haulms and garlic powder is shown in table 5. All isolates found except *isotricha spp.* was not affected (p>0.05) by the inclusion of soybean haulms and/or garlic powder in the diets of sheep. Higher (p<0.05) numbers of *isotricha spp.* observed in treatment 3 (7.80 cfu/mL x10^5) were similar to those found in treatment 1 (5.00 cfu/mL x10^5). However, *isotricha spp*. numbers decreased (p<0.05) in treatment 2 (2.80 cfu/mL x10^5), compared to treatment 3 but not treatment 1.

Table 1: Gross composition of experimental diets

Ingredient	T1	T2	T3
Maize offal	55	35	35
Soybean meal	15	10	10
Palm kernel cake	25	20	20
Soybean haulms	-	30	30
Bone meal	3	3	3
Salt	1	1	1
Vitamin/mineral premix	1	1	1
Total	**100**	**100**	**100**
Garlic powder	-	-	0.5
Determined analysis			
Dry matter	84.09	87.01	86.56
Crude protein	16.58	14.12	14.96
Ash	7.68	6.95	7.34
Ether extract	17.20	18.19	18.94
NDF	47.23	48.23	49.10
ADF	38.96	39.32	38.99

T1= Control (0% inclusion of soybean haulms)
T2= 30% inclusion of soybean haulms
T3= 30% inclusion of soybean haulms + 0.5% inclusion of garlic powder

Table 2: Effect of feeding soybean haulms and/or garlic powder on rumen pH and metabolites of sheep

Parameter	T1	T2	T3	SEM
pH	5.20	5.44	5.38	0.08
Total volatile fatty acids (mol/100mol)	5.59	4.75	5.02	0.20
Acetic (mol/100mol)	2.40	2.03	2.15	0.09
Propionic (mol/100mol)	1.60	1.36	1.44	0.06
Butyrate (mol/100mol)	0.24	0.20	0.22	0.01
Methane (mol/100mol)	0.54	0.46	0.49	0.02

T1= Control (0% inclusion of soybean haulms)
T2= 30% inclusion of soybean haulms
T3= 30% inclusion of soybean haulms + 0.5% inclusion of garlic powder
SEM= standard error of mean

Table 3: Total microbial count from rumen of sheep feeding on diet containing soybean haulms and garlic powder

Parameter	T1	T2	T3	SEM
Bacteria (cfu/mL x10^5)	4.49	3.90	4.16	0.25
Fungi (cfu/mL x10^5)	1.19	1.08	1.48	0.09
Protozoa (ml/100 mL)	19.80	16.80	28.20	2.45

T1= Control (0% inclusion of soybean haulms)
T2= 30% inclusion of soybean haulms
T3= 30% inclusion of soybean haulms + 0.5% inclusion of garlic powder
SEM= standard error of mean

Table 4: Effect of feeding soybean haulms and/or garlic powder on rumen bacteria isolates in sheep

Parameter	Control	SBH	SBHG	SEM
Bacillus spp. (cfu/mL x10^5)	14.25^a	4.80^b	10.50^{ab}	1.60
E.Coli (cfu/mL x10^5)	12.63	13.63	14.50	0.60
Staphylococcus spp. (cfu/mL x10^5)	12.00	12.25	3.67	1.83

^{a,b}Means with different superscript along the row differ significantly ($p<0.05$)
T1= Control (0% inclusion of soybean haulms)
T2= 30% inclusion of soybean haulms
T3= 30% inclusion of soybean haulms + 0.5% inclusion of garlic powder
SEM= standard error of mean

Table 5: Different protozoa isolates form the rumen of sheep on diet with soybean haulms and garlic powder

Parameter	T1	T2	T3	SEM
Endodinium spp. (cfu/mL x10^5)	9.00	10.60	11.80	0.90
Isotricha spp. (cfu/mL x10^5)	5.00^{ab}	2.80^b	7.80^a	0.96
Ostracodinum spp. (cfu/mL x10^5)	2.40	1.40	4.00	0.56
Diplodinium spp. (cfu/mL x10^5)	3.40	2.00	4.60	0.67

^{a,b}Means with different superscript along the same row are significantly ($p<0.05$) different
T1= Control (0% inclusion of soybean haulms)
T2= 30% inclusion of soybean haulms
T3= 30% inclusion of soybean haulms + 0.5% inclusion of garlic powder
SEM= standard error of mean

Discussion

The rumen environment may be affected by several factors including nutrition of the animals. A similar rumen pH and other metabolites indicate that inclusion of soybean haulms and/or garlic in diets of sheep did not affect fermentation pattern in the rumen.

Therefore, the use of soybean haulms as alternative feedstuff in sheep diets is advocated. Other reports on use of soybean haulms or crop residues in ruminant diets gave promising results.

The results of bacteria count in the rumen of sheep in this study is at variance with result obtained by [12] when different crop residues were used as roughages in diets of sheep. The report attributed the higher bacteria count in some diets compared to others to the presence of very high structural fermentable carbohydrate in corn cob and cowpea husk (major roughage components of diet) which when ruminally degraded could have resulted in the higher bacteria count. The non-significant difference in this study may be as a result of the high fibre in the soybean haulms compared to other residues such as cowpea husk and corn cob which may not contain highly structural fermented carbohydrates. Unlike this current study, fungi count in sheep on different crop residue diets were influenced by the type of residue used [12]. Like reasons adduced for bacteria count, same would have been responsible for the discrepancies between the two studies. This is consistent with findings of [12] where protozoa count was not affected at the use of different crop residues in diets of sheep. Similar counts for protozoa can be attributed to similar dietary levels of starch [2] and starch fermentation [3] across the treatment groups. This explains that soybean haulms in diet of sheep provided similar levels of starch and then fermentation of starch in the rumen compared to maize offal. The similar response for volatile fatty acids which are product of rumen fermentation supports the allusion that similar levels of starch would have been obtained when soybean haulms is used in diets of sheep compared to maize offal.

Reduced *Bacillus spp.* in the rumen of the sheep fed soybean haulms may indicate lower performance of the animals. This is because previous studies indicate that *Bacillus* improved animal growth performance, immune function, intestinal development, and also regulated intestinal microbial communities [9, 10]. It should be noted however that the inclusion of garlic powder in soybean haulms based diet addressed the problem or reduced *Bacillus spp.* bacteria as diet with garlic powder had similar *Bacillus* counts with control.

Bacteria isolates such as *E. coli* and *Staphylococcus spp.* observed in the research were not affected by the inclusion of soybean haulms and/or garlic powder in the diets of sheep.

Mixed ruminal populations of ciliate protozoa have a negative effect on the utilization of nitrogen by ruminants with report that a number of animal experiments showed decreased flow of non-ammonia nitrogen components from the stomach to the intestinal tract in defaunated ruminants inoculated with mixed protozoa populations [6]. The increased populations of *Isotricha spp.* in the garlic powder group compared to the soybean haulms only group can be traced to the activity of garlic on protozoa numbers in the intestinal tract of the animals. This is because report of [5] has affirmed that garlic powder inclusion

in diet of West African dwarf goats decreased protozoa populations in goats. It is likely that the decrease in protozoa population may be as a result of increases observed in a particular species of protozoa which is likely to reduce other species available in the rumen. It should be noted that in this current research, whereas total protozoa count remained unchanged in the rumen, the inclusion of garlic however, influenced the diversity of the protozoa species present. Even though not significant, other protozoa species were numerically higher in the garlic diet compared to sole soybean and control diets. The increase in *Isotricha* may result in higher flow of bacteria nitrogen, as was reported in wethers fed the haycrop diet [6]. This action may be to make up for the low nitrogen in soybean haulms residue. The presence of mixed ciliates specie in the rumen of the animals in this present study may be responsible for the non-significant differences observed in the total protozoa count. This is because deamination activity of rumen microbes was significantly decreased when *Isotricha spp*. were the only ciliate protozoa present in the rumen [6], with even larger negative impact on bacteria synthesis in the rumen in the presence of solely *Entodinium* spp.

Conclusion

The inclusion of 30% soybean haulms in the diet of sheep reduced the populations of *Bacillus spp*. and *Isotricha spp*. in the rumen of sheep without any effect on the pH, total microbial counts and fermentation pattern in the rumen. Addition of garlic powder to soybean haulms-based diets did not further improve fermentation characteristics in the rumen. Therefore, feeding soybean haulms at 30% may offer sustainable alternative to scarce and expensive feed resource base for sheep.

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Conflict of interest

The authors declare that no conflict of interest exist in the course of carrying out the research and writing manuscript

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