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DETERMINATION OF THE OPTIMUM INOCULUM CONCENTRATION AND FERMENTATION PERIOD AND THEIR EFFECT ON NUTRIENT COMPOSITION OF BREWERS' DRIED GRAINS.

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ABSTRACT

Brewers dried grains (BDG) a by-product of fermentation, was procured dried and subjected to a second stage fermentation using rumen liquor as inoculums. Five different concentrations of substrate (BDG) to inoculums ratios (10:1, 10:2, 10:3, 10:4, and 10:5 (w/w)) were used. The five inoculated samples were subjected to four periods of fermentation (2, 4, 6 and 8 days). Twenty (20) bottles of equal volume were arranged in five (5) sets (4-bottles each). The five sets of bottle were used to carry the same inoculated BDG sample. The four bottles of each set of the 5-inoculated BDG samples were buried under soil to maintain anaerobic condition and fermented for between 2 to 8 days. The refermented BDG samples were subjected to analyses for proximate nutrient composition and laboratory bacteria population count. The rumen liquor was microscopically examined for bacteria organism. Results in terms of dry matter (DM) percentage showed all BDG samples with substrate to inoculum concentration of 10:1. 10:2, 10:3 and 10:4 to be similar (P>0.05). The fermentation of all BDG samples generally resulted in increased crude protein (CP) and decreased crude fibre contents. The BDG sample on 2-day fermentation and 10:2 inoculum concentrations resulted in the highest crude protein. While the sample on 8 day fermentation with 10:4 concentration was least in CP content. The CP content of BDG samples on 2, 6, and 8 day fermentation did not differ significantly (P>0.05). The CP content of sample on 4 day fermentation was statistically similar (P>0.05) to those on 2 and 8 days fermentation but significantly lower (P<0.05) than those on 6-day fermentation. The crude fibre (CF) decreased from 37.90% to 14.16% on fermentation. BDG sample on 10:1 was significantly lower (P<0.05) compared to those on 10:2, 10:3 and 10:4 treatments. The ash content of samples on 10:2, 10:2, 10:3 and 10:4 were similar but significantly lower (P<0.05) than those on 10:5 fermentation. There were no definite trends observed in the bacteria population between samples in the different periods of fermentation. Rumen liquor contained *Streptococcus bovis* and *Ruminococcus albus on microscopic observation.* Second stage fermentation of BDG resulted in increased CP and decreased CF composition. For higher CP content, the optimum period of fermentation between 2, 4, 6 and 8 day is 2-day and the best substrate to inoculum ratio is 10:2.

Keywords: Brewers' dried grains, inoculums, fermentation, bacteria count, nutrient composition.

INTRODUCTION

The increase in the cost of ingredients used in compounding livestock feeds is unprecedented. The importance of identifying and utilizing cheaper non-conventional feeds has been emphasized time and again. Among such non-conventional feed ingredients as reported by Islam *et al*., (1994) are: marine waste, frog waste, broiler offal, kitchen waste, luceana leaf meal. Others from agro-based industrial by-products are cola nut cake meal, cotton seed cake, palm kernel cake, cassava peel, yam and potato peels. Maize bran, rice bran and brewers dried grains (Dairo, 1999). Some of the limiting factors associated with using crop residues and agro industrial by products as animal feeds include; procurement, storage, poor feed intake, high fibre content, toxic substances, low digestibility and low nutrient contents (Oluokun & Olalokun, 1995). BDG is a by-product of the brewing industry consisting of primarily the extract of residues of the grains used in the brewing industry (Pereira *et al.,* 1998). It has been reported to have the following chemical composition; ME (kcal/kg) 17.0, CP 25.3% (Church & Pond 1988) and CF, 3.12%; CP, 28.25%; EE, 6.70%; Ash, 7.36%; NFE, 44.57%; GE, 4.24 kcal/gm (Uchegbu & Udedibie, 1998) and cited as a potential substrate for the production of microbial protein during fermentation. Pido et *al* ., (1979) demonstrated detoxication of cassava products by fermentation, while improvement in protein quality from 70-83.7% with increased available lysine, luecine, methionine and reducing sugar reported by Kanazas & Field (1981) for fermented sorghum.

Oyegbile (1988) carried out a 7-day fermentation of spent sorghum mash and observed an initial proximate composition of 15.7% CP, 10% ether extract (EE), 2.0% Ash and 18.7% carbohydrate and values of 21.6% CP, 24% EE, 49.9% carbohydrate and 3% Ash after refermentation. The objectives of this study were to determine the optimum inoculum concentration and fermentation period and their effect on the nutrient composition of the refermented BDG.

MATERIALS AND METHODS

Sources and nature of brewers dried grains (BDG): The BDG used in this experiment was sorghum based. It was characteristically brownish in colour and appears fibrous. Dried samples are odourless with a sour taste. All samples used in this experiment were purchased wet from the International Beer and Beverage Industries (IBBI) makers of Kronengburg Lager beer located within Kaduna metropolis and sun- dried for 3 days.

Inoculum preparation: Rumen content of cow was collected fresh just after slaughter at the abattoir (Kawo, Kaduna). This was then mixed with water in a 2:1 ratio (w/w) and filtered through a mosquito net of pore size 2.78mm[2] (Tyokpat, 1989). The residue was discarded and the filtrate used as inoculum.

Two test tubes containing 5mls of the filtrate (inoculum) were preserved in a refrigerator for laboratory identification of the types of bacteria present in the rumen liquor (inoculum).

Inoculation: Five (5) different concentrations of substrate to inoculum ratios were used (10:1, 10:2, 10:3, 10:4 and 10:5, w/w). The 5 different inoculated samples were each subjected to four periods of fermentation (2, 4, 6 and 8 days). Twenty-one (21) bottles (empty jam containers with covers including one for uninoculated sample) of equal size were used. Each BDG inoculated sample (10:1, 10:2, 10:3, 10:4, and 10:5) was subjected

to 4 different periods of fermentation (2, 4, 6 and 8 days). Five (5) bottles were used to carry the same inoculated BDG sample (i.e. a set for each of the samples' concentration of 10:1, 10:2, 10:3, 10:4 and 10:5). Each set was then subjected to 4-different periods of fermentation (2, 4, 6 and 8 days). The quantity of water that will wet a given quantity of dried BDG was first determined. This was found to be in a ratio of 5:1 (w/w). The *5* different inoculum concentrations (10:1, 10:2, 10:3, 10:4 and 10:5) were first introduced into the water before wetting the BDG samples for uniformity of inoculation.

Quantitatively, 5 buckets were kept and 3kg of BDG were weighed into them. The following weights of inoculum (rumen liquor); 0.3kg, 0.6kg, 0.9kg, 1 .2kg and 1.5kg corresponding to the substrate to inoculum ratio of 10:1, 10:2. 10:3, 10:4 and 10:5 were weighed and introduced into equal volume of water. Properly mixed and each volume of water containing the different concentrations of inoculum was used to wet the 3kg of BDG in the bucket in a ratio of 1:5 (w/w). The different ratios used in the process are presented in the table below.

RATIOS OF SUBSTRATE TO WATER AND SUBSTRATE TO INOCULUMS CONCENTRATIONS

Sample label	Substrate:Water ratio	Substrate Inoculum	Fermentation period (Days)			
			2	4	6	8
A	1:5	10:1	A_2	A_4	A_6	A_8
B	1:5	10:2	B_2	B_4	B_6	B_8
C	1:5	10:3	C_2	C_4	C_6	C_8
D	1:5	10:4	D_2	D_4	D_6	D_8
E	1:5	10:5	E_2	E_4	E_6	E_8

Four of the bottles were allocated to each of the five different BDG inoculated samples (totaling 20 bottles) to which 0.5kg of the BDG inoculated samples were filled. The four bottles from each bucket were then subjected to four different fermentation periods (2, 4, 6 and 8 days).

Fermentation: The inoculated BDG samples were properly compacted into the four-bottle (500g) to exclude any possible air. The mouth of the bottles were covered with polythene materials and finally sealed with covers and buried under the soil so as to maintain anaerobic condition as much as possible. All bottles of the inoculated BDG samples were labeled accordingly and fermented for between 2 to 8 days. Another bottle with wetted BDG material was fermented for 8 days un-inoculated, making a total number of twenty-one (21) bottles used in this study.

After each period of fermentation, 10 gm of the re-fermented BDG (RBDG) sample was taken into text tubes. The twenty (20) test tubes containing wet re-fermented BDG material were properly labeled and kept in a refrigerator for bacteria count which was carried out at the medical laboratory, Yusuf Dantsoho Hospital, Tudun Wada, Kaduna.

The rest of the re-fermented BDG (21 samples) were removed from the bottles and sun-dried for 3 days after which samples were taken for chemical analysis. The proximate chemical determination for dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), ash and nitrogen free extract (NFE) were carried out at the National Animal Production Research Institute (NAPRI), Shika- Zaria, Nigeria. The results of the proximate analyses and bacteria population determined were as shown in tables 1, 2 and 6.

Chemical analysis: The analytical procedures (for DM, CF, CP, EE, Ash and NFE determination) employed in this experiment are in accordance with AOAC (1990) standard. Proximate analysis was carried out on the BDG sample and the 21 RBDG samples.

Microbiological analysis: The 5-ml rumen liquor that was preserved in the refrigerator was taken for the identification of the type of bacteria present in it at the laboratory. Likewise the twenty wet RBDG samples of the five different inoculums to substrate concentrations were taken for bacteria count. This was to identify the substrate to inoculum concentration that will result in the highest bacteria count.

Preparation of the dilution: 1 gm of each wet RBDG sample was weighed and mixed in 100ml sterile distil water and out of this, 10 ml, were taken in a test tube as the stock bacteria concentration. This contained 0.lg of the RBDG sample (0.1g/10ml).

Serial dilution: To reduce the bacteria concentration before inoculation of every sample, six (6) screw cap test tubes were kept, into which 9.9 mls of 0.1% peptone were put to serve as dilution blank. The test tubes were arranged in series from 1 to 6. From the stock concentration, 0.l ml was removed and transferred into the first dilution blank as shown in Fig. 1. These were mixed and from it 0.1ml was removed and discarded every time to allow equal concentration.

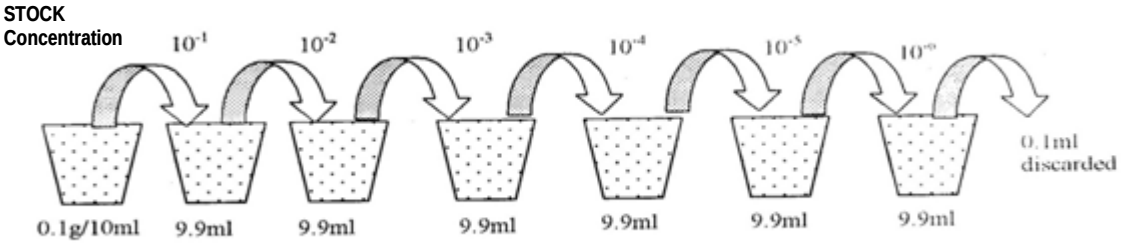

FIG 1. SERIAL DILUTION OF BACTERIA STOCK SOLUTION.

Inoculation of plates: The 5th and 6th dilutions were used for plate inoculation. In the process, I ml of each dilution (10^5 and 10^6) was removed with a pipette and introduced into the center of each sterile petri-dish. 10ml of nutrient agar (cooled to about 45 ^{0}C) were poured on each plate and mixed by moving the dish gently, six times in clockwise direction and repeated counter clockwise. After the media was allowed to set for 20 minutes the plastic dishes were inverted and incubated at 37 ^{0}C for 24hrs.

Bacteria count on plates: The plates were divided into four quarters as shown in Fig. 2. Each quarter was counted and the count of the four quarters added to obtain the total count (count per plate = a + b + c + d).

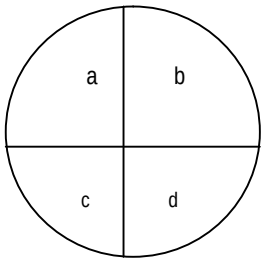

FIG 2. BACTERIA GROWTH PLATE DIVIDED INTO FOUR QUARTERS.

The results of the bacteria count are shown in Table 4

RESULTS

Nutrient composition of RBDG: The proximate composition of BDG analyzed before and after fermentation are as shown in Table 2. The wetted uninoculated BDG sample fermented for 8-days also revealed an increased crude protein content from 24.44 to 28.00% (Table 1). Compared to the crude protein content of all the re-fermented inoculated BDG samples (Table 2), the wetted uninoculated BDG sample was observed to contain the least crude protein content (28.0 %CP). The crude protein content of BDG was observed to increase from 24.44% before fermentation to between 29.28-36.28 % after fermentation (Table 2). The RBDG sample with the highest crude protein (36.28%) was observed among samples on 2-day fermentation with substrate to inoculum concentration ratio of 10:2

TABLE 1. PROXIMATE COMPOSITION OF BDG AND RBDG ANALYZED

Analysis *	Ingredients	
	BDG	[1]RBDG
[2]Dry Matter (%)	95.10	95.41
Ash (%)	4.98	4.98
Ether Extract (%)	9.44	9.44
Nitrogen Free Extract	23.28	30.95
Crude Fibre (%)	37.90	26.63
Crude Fibre (%)	24.44	28.00

*Values are means of two determinations
[1]Uninoculated fermented BDG sample.
[2]Analysis % of DM

The crude fibre content was observed to decrease from 37.90% before fermentation to 14.16% after fermentation. The least crude fibre content was observed among samples on 2-day fermentation and with the sample of substrate to inoculum concentration ratio of 10:1. The crude fibre content of the wet uninoculated fermented BDG sample was observed to be lower than the normal dried BDG sample but higher than all the inoculated samples.

TABLE 2. PROXIMATE COMPOSITION OF RBDG SAMPLES

Analysis	Control	Period of Fermentation															
		2 days					4 days					6 days				8 days			
	[1]0	10:1	10:2	10:3	10:4	10:5	10:1	10:2	10:3	10:4	10:5	10:1	10:2	10:3	10:4	10:5	10:1	10:2	10:3	10:4	10:5
DM	95.41	95.66	95.25	94.78	94.49	93.87	93.23	93.85	93.16	92.99	94.73	92.61	92.75	92.75	92.73	74.09	92.22	92.22	95.86	94.30	92.93
CP	28.00	34.47	36.28	33.26	29.75	33.88	37.69	33.44	34.64	35.94	32.41	33.88	35.00	34.94	35.00	34.10	34.76	35.16	33.40	29.28	31.00
CF	26.63	14.61	17.21	16.44	17.29	15.22	16.09	15.58	18.39	18.26	15.09	15.49	16.65	18.23	18.26	18.02	16.17	16.43	18.36	15.89	14.68
EE	9.44	9.65	11.05	7.49	8.02	6.87	16.22	16.04	17.07	10.48	10.52	16.76	9.92	10.89	10.49	10.91	15.95	7.49	11.16	7.67	12.10
ASH	4.98	4.27	4.07	4.51	4.11	4.29	4.53	5.64	4.38	4.58	4.30	4.11	4.41	4.00	4.88	4.21	4.27	4.51	4.74	4.53	5.56
NFE	4.48	5.52	5.81	5.32	5.44	5.42	5.55	5.35	5.54	5.63	5.19	5.42	5.60	5.59	5.61	5.64	5.56	5.63	5.35	4.70	4.39

Results are means of two determinations.
[1]0 =wetted uninoculated RBDG sample (Control)

Effect of different inoculum concentration on nutrient composition of refermented BDG: Table 3 shows the effect of BDG re-fermentation with different levels of inoculum concentrations on the nutrient composition of BDG. In terms of dry matter composition. All BDG samples with substrate to inoculum concentration of 10:2, 10:3 and 10:4 on fermentation had similar dry matter percentage. BDG samp1e on 10:5 and 10:1 substrate to inoculum concentration were similar but significantly higher (P<0.05) than samples on, 10:2, 10:3 and 10:4 substrate to inoculum concentration. The control sample was significantly higher in percentage DM than other samples on different inoculum concentration.

TABLE 3. EFFECT OF DIFFERENT BDG RE-FERMENTATION INOCULUM CONCENTRATIONS ON BDG NUTRIENT COMPOSITION.

Items	Substrate to inoculum concentration						
	control	10:1	10:2	10:3	10:4	10:5	*SEM
[1]DM,%	95.1[a]	93.59[b]	93.52[c]	93.13[c]	93.63[c]	94.41[b]	0.25
CP,%	24.44[c]	34.39[a]	34.97 [a]	34.11[a]	31.86[b]	33.91[a]	0.42
EE,%	9.04[c]	14.71 [a]	11.84 [b]	11.60 [b]	10.09 [b]	10.10 [b]	0.62
CF, %	39.0[a]	15.59[d]	16.69[c]	17.85[b]	16.98 [bc]	16.30[cd]	0.34
ASH,%	4.07[b]	4.03[b]	0.48 [b]	4.41 [b]	4.52 [ab]	4.99[a]	0.16

[a,b,c,d] are the means of the same row with different superscripts are significantly different (P<0.05)
[1]Analysis % of DM
*Standard error of means.

The crude protein content of all RBDG samples were significantly higher (P<0.05) than the unrefermented BDG sample. The crude protein content of samples on 10:1, 10:2, 10:3 and 10:5 were similar but significantly higher (P<0.05) than that of the BDG sample on 10:4. The percentage ether extract of all RBDG samples were significantly higher (P<0.05) than the control. The percentage ether extract of samples on 10:2, 10:3, 10:4 and 10:5 substrate to inoculum concentration were similar but significantly lower (P<0.05) than those on 10:1.

The percentage crude fibre of the control sample was significantly higher (P<0.05) than that of all RBDG samples. The crude fibre composition of samples on 10:2, 10:4 and 10:5 were similar (P>0.05). Samples on 10:3 substrates to inoculum concentration contained higher percentage crude fibre (P<0.05) than samples on 10:1, 10:2 and 10:5. Samples on 10:1 had significantly (P<0.05) lowest percentage of crude fibre which was similar to samples on 10:5 substrates to inoculum concentration**.**

The percentage ash for the control sample and that of samples on 10:1, 10:2, 10:3 and 10:4 substrate to inoculum concentrations were similar. The ash content of BDG sample on 10:5 was significantly higher (P<0.05) than those on 10:1, 10:2 and 10:3. Samples with 10:4 and 10:5 concentrations had similar percentage ash.

Effect of different periods of fermentation of BDG on nutrient composition: Table 4 shows the effect of different period of re-fermentation on the nutrient composition of BDG. The dry matter composition was significantly higher (P<0.05) for 2-day fermentation compared to those on 4, 6 and 8-day fermentation but significantly lower (P<0.05) compared to the control. The dry matter for samples on 6 and 8-days fermentation were similar but significantly lower (P<0.05) than that of 4-day fermentation.

There was no significant difference (P>0.05) in the ash composition across samples for the different period of fermentation. The percentage ash composition of the control sample was significantly lower than those of samples on different period of fermentation. The ether extract for 4-day fermentation was significantly higher (P<0.05) compared to those of 2, 6 and 8-day fermentation periods and the control. The ether extract content of 2-day fermentation was also significantly lower (P<0.05) than those on other fermentation periods. Samples on 2, 4 and 6-day fermentation had similar crude fibre content and were significantly lower (P<0.05) than the crude fibre content of the 8-day fermentation sample. The percentage crude of the control sample was significantly (P<0.05) higher than those of samples on different periods of fermentation. The crude protein content of samples on 6-day fermentation was similar to those on 2 and 8-day but significantly higher than those on 4-day fermentation and the control. The crude protein content of the control was significantly the lowest.

TABLE 4. EFFECT OF PERIOD OF RE-FERMENTATION ON NUTRIENT COMPOSITION OF BDG.

Items	Fermentation period (days)					
	0	2	4	6	8	SEM
DM (%)	95.1	94.81	93.99	92.98	92.83	0.22
ASH (%)	4.07[b]	4.76[a]	4.49	4.32[a]	4.38[a]	0.15
EE (%)	9.04[c]	8.66[c]	14.81[a]	11.75	11.45[b]	0.55
CF (%)	37.90[a]	16.28[c]	16.47[c]	16.23[c]	17.75[b]	0.30
N (%)	3.91[c]	5.42[ab]	5.32[b]	5.53[a]	5.38[ab]	0.06
CP (%)	24.44[c]	33.88[ab]	33.28[b]	34.59[a]	33.65[ab]	0.38

[a,b,c]Means within row with different superscripts are significantly different(P<O.05)

Table 5 shows the effect of BDG fermented for different periods with different inoculum concentration on crude protein and crude fibre compositions. The 2-days fermented samples on treatments 10:1 and 10:2 substrate to inoculum concentration did not differ significantly (P>0.05) in crude protein composition. Samples on 10:2 had higher (P<0.05) % CP compared to those that were on 10:3, 10:4 and 10:5. Samples on treatment 10:4 had the least % CP (P<0.05). BDG samples on 10:1, 10:2, 10:3, 10:4 and 10:5 inoculum concentration and of 4, 6 and 8-day fermentation had similar (P>0.05) crude protein content. On the basis of crude fibre composition, all RBDG samples on 2-day fermentation with different inoculum concentrations did not differ significantly (P>0.05). Similarly RBDG samples on 4, 6 and 8-day fermentation with different inoculum concentrations did not differ significantly (P>0.05) in crude fibre content.

TABLE 5. EFFECT OF FERMENTATION ON BDG CRUDE PROTEIN AND CRUDE FIBRE COMPOSITION

No days	Concentration					
	Crude Protein, %					
	10:1	10:2	10:3	10:4	10:5	SEM
2	34.47[ab]	36.38[a]	33.2	29.75[c]	33.89	0.29
4	34.69	33.44	34.63	33.94	33.10	0.98
6	33.	35.00	34.94	35.00	34.01	0.40
8	34.76	35.16	33.40	29.38	31.00	1.60
	Crude Fibre, %					
	10:1	10:2	10:3	10:4	10:5	SEM
2	14.61	17.21	16.43	17.29	15.89	0.69
4	16.09	15.56	18.36	16.08	16.22	0.64
6	16.26	16.65	18.23	15.71	15.09	0.98
8	16.17	17.34	18.	18.86	18.02	0.64

[a,b, c] Means within row with different superscript are significantly different (P<0.05)

Bacteria count on plates: The result of the laboratory bacteria count is as shown in table 6. There was no definite trend observed in the bacteria population between samples in the different fermentation periods (2, 4, 6 and 8 days).

TABLE 6. BACTERIA COUNT OF WET RBDG SAMPLES.

Period	RBGD sample	Bacterial count/ml
2 days	A	$77.7x10^6$
	B	$73.0x10^6$
	C	$50x10^6$
	D	$165 x10^6$
	E	$1.3x10^6$
4 days	A	$23.4x10^6$
	B	$0.75x10^6$
	C	$9.85x10^6$
	D	$12.45x10^6$
	E	$9.8x10^6$
6 days	A	$43.45x10^6$
	B	$4.85x10^6$
	C	$40.85x10^6$
	D	$24.0x10^6$
	E	$6.7x10^6$
8 days	A	$8.4x10^6$
	B	$15.05x10^6$
	C	$94.85x10^6$
	D	$2.55x10^6$
	E	$1.25x10^6$

Results are mean of two determinations.
Substrate to inoculums ratio; A = 10: 1, B = 10:2, C = 10:3, D = 10:4, E = 10:5

Microscopy: The result of the microscopic examination revealed the presence of gram-positive short chain cocci and gram-negative purple rod shaped bacteria.

DISCUSSION

The fibrous nature of BDG became smoother after fermentation signifying the physical transformation of BDG into a less fibrous material as was reported by Kherrati *et al*., (1997). They observed an increased level of fat and crude protein when slaughterhouse waste was recycled for animal feeding in a 15-day fermentation period. The smooth texture was as a result of the plant cell wall being degraded by anaerobic microorganism resulting in disruption of the feed structure.

The desirable sweet aroma perceived for the wet fermented samples were indications of good fermentation processes as stated by Mthiyane *et al*., (2001). Refermentation of BDG resulted in decreased DM% as the period of fermentation increased from 2 to 8-days. The decreased in DM% is in agreement with the findings of Odufa (1987) who reported a reduction in dry matter percentages during fermentation and contrary to the findings of Tyokpat (1989) who reported a non-significant effect on DM content after fermentation periods (and nature of the feed ingredients (Rymer *et al*., (1999).

The increase in CP of all fermented samples compared to the normal dried BDG samples is in agreement with the findings of Adeyemi & Adeleke (2000) who observed an increased CP (40.02-43.07) of the thevetia cake after fermentation and similarly with Ofuya & Obilor (1993) who observed an increased CP content of cassava peel from 5.4 to 16.9% after 20-day fermentation. Mthiyane *et al*., (2001) however observed a decreased (P<0.05) CP when sugarcane tops were ensiled with broiler litter with and without water.

The difference between the CP content of all inoculated and fermented samples are as a result of the activities of microorganism as confirmed by Macdonald *et al*.,(1998). This also

revealed the suitability of BDG as a potential substrate for the production of microbial protein as was observed for fermentation media with agro- industrial residues such as sugar cane bagasse (Pressoa as cited by Almaida *et al.,* (1995) and cassava root meal (Pido *et al*., 1979).

The highest CP content recorded with the sample on ratio 10:2 treatment after 2, 4,6 and 8-day fermentation signifies that fermentation of BDG at ratio 10:2 (substrate to inoculum) concentration will yield the highest CP content keeping other suitable conditions normal.

The insignificant difference in CP content of all samples on 10:2 treatment concentration and fermented for 2, 4, 6 and 8 days revealed that CP production through BDG fermentation on 10:2 substrate to inoculums will make no difference for any of the fermentation period used. This is in agreement with Wanderley *et al*., (1999) who observed that the digestion in rumen is related to the colonization of plant tissues by rumen microbes.

The significant decrease in crude fibre observed for uninoculated fermented sample also indicates the breakdown (fermentation) of BDG by anaerobic organism whose sources were not from the rumen but had the right environment for fermentation(MacDonald *et al*.,1998). The CF content of samples on different substrate to inoculums concentration was similar and there was no difference in the CF content between samples subjected to 2, 4, 6 and 8-days fermentation. This finding is in agreement with Rymer *et al*., (1999) who affirmed that the extent and rate of fermentation is largely determined by the nature of the feed and the environment respectively. Samples with lower CF content were not observed to contain higher CP content. Similarly Cone *et al.,* (1996) concluded that the rate of fermentation that are observed may be due to variation that is not necessarily due to differences in the nature of the feed being investigated but rather the type of methodology employed. Rymer & Givens (1997) observed that different types of apparatus produce different gas production profile with the same feeds.

The bacteria profile observed in Table 4 revealed bacteria population count of the different fermented samples following the six fold dilutions (from 10^{-1} to 10^{-6}). The bacteria range of 1x 10^6 to 165 x 10^6/ml is comparable to the findings of Kherrati *et al*., (1998) who reported that the total coliform count range from 4 x 103 to 10 x 106 cf u/g when slaughter house waste were inoculated with a starter culture of *lactobacillus planetarium* and incubated at 22-24ºc for 10-days. The population count observed in this study was only for viable bacteria (excluding yeast and mold cells count) which were reported to contribute to the crude protein values of the fermented samples (Virginia *et al.,* 1974). The bacteria count in this study did not increase with increased concentration of rumen liquor in all fermentation periods. Also the increase in crude protein did not correspond with increase cellulolytic bacteria as suggested by Weidmeier *et al*., (1987). The gram positive short chain cocci and gram negative rod shaped organism observed are characteristics of *streptococcus bovis* and *Ruminococcus albus* species of bacteria respectively which are typical rumen bacteria species as reported by Macdonald *et al*., (1998).

CONCLUSION

Results of this study indicate that refermentation of BDG between the periods of 2-8 days with rumen liquor as inoculum at concentrations of 10:1 to 10:5 resulted in increased CP and decreased in CF content with 10:2 test concentration being the best substrate to inoculum concentration for the highest % CP yield and 2-days optimum period of BDG refermentation.

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