

Chemical Composition, *in vitro* Gas Production and Microbial Quality of Rice Straw Supplemented with Pelleted or Unpelleted Dried Rumen Digesta Concentrate

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Abstract

The study sought to assess the chemical composition, microbial quality and *in vitro* gas production of rice straw supplemented with pelleted or unpelleted dried rumen digesta (DRD) concentrate in the savannah agro-ecological zone of Ghana. Four levels of DRD (0%, 5%, 10% and 15%) were incorporated into a concentrate and subjected to two methods of processing (unpelleted diet and pelleted diet). Processing methods and DRD inclusion levels had a significant ($P < 0.05$) interaction effect on crude protein (CP), ash, lactic acid bacteria (LAB) and *E. coli*. The unpelleted diet at a 15% inclusion level of dried DRD had the highest CP (14.22%). The highest *E. coli* count was recorded in the unpelleted diet at 0% inclusion of DRD. No *E. coli* was detected in the pellet diets. There was a decreasing trend of *E. coli* as the levels of DRD increased in the unpelleted concentrate diet. The highest ($p < 0.05$) *in vitro* digestible organic matter (IVDOM) and metabolisable energy was recorded in the 15% DRD pellet concentrate supplemented with 50% rice straw. The study concluded that DRD pellet can be used as a source of protein to enhance the utilisation of poor-quality feed resources during the dry season to improve the growth performance of ruminants.

Keywords: Dried rumen digesta; *In vitro* gas; Microbial; Pellet; Unpelleted

Composition chimique, production de gaz *in vitro* et qualité microbienne de la paille de riz additionnée de concentré de digesta de rumen séché granulé ou non granulé.

Résumé

L'étude visait à évaluer la composition chimique, la qualité microbienne et la production de gaz *in vitro* de la paille de riz supplémentée avec du concentré de digesta de rumen séché (DRD) granulé ou non granulé dans la zone agro-écologique de la savane du Ghana. Quatre niveaux de DRD (0 %, 5 %, 10 % et 15 %) ont été incorporés dans un concentré et soumis à deux méthodes de traitement (régime non granulé et régime granulé). Les méthodes de traitement et les niveaux d'inclusion de DRD ont eu un effet d'interaction significatif ($P < 0,05$) sur les protéines brutes (CP), les cendres, les bactéries d'acide lactique (LAB) et *E. coli*. Le régime non granulé avec un niveau d'inclusion de 15 % de DRD séché présentait la CP la plus élevée (14,22 %). Le nombre le plus élevé d'*E. coli* a été enregistré dans le régime non granulé à 0 % d'inclusion de DRD. Aucun

E. coli n'a été détecté dans les régimes à base de granulés. On a observé une tendance à la baisse du nombre d'*E. coli* à mesure que les niveaux de DRD augmentaient dans le régime concentré non granulé. La matière organique digestible *in vitro* (IVDOM) et l'énergie métabolisable les plus élevées ($p < 0,05$) ont été enregistrées dans les granulés concentrés à 15 % de DRD complétés par 50 % de paille de riz. L'étude a conclu que les granulés de DRD peuvent être utilisés comme source de protéines pour améliorer l'utilisation des ressources alimentaires de mauvaise qualité pendant la saison sèche afin d'améliorer les performances de croissance des ruminants.

Mots-Clés: Digesta de rumen séché; gaz *in vitro*; microbes; granulés; non granulés

Introduction

Ruminant livestock mostly derive their entire nutrition from natural pasture in most developing countries, including Ghana, with minimal supplementation (Konlan *et al.*, 2017; Ansah & Issaka, 2018). Heavy grazing, climate change, and land use patterns have led to decline in the quantity and quality of forage (Tessema *et al.*, 2011). This has been aggravated by the prolonged dry season, leading to the undernutrition of most ruminant livestock due to poor forage quality (Tessema & Baars, 2004; Murthy *et al.*, 2011). This phenomenon has led to the search for alternative feed sources. Several authors have reported appreciable levels of protein in dried rumen digesta and other micro-flora such as protozoa, fungi, and lactic acid bacteria and its utilisation as feed for livestock, especially ruminants (Adeniji & Balogun, 2002; Dairo *et al.*, 2005; Esonu *et al.*, 2006; Agbabiaka *et al.*, 2011; Agolisi *et al.*, 2020; Agolisi *et al.*, 2022). Dried Rumen digesta (DRD) is not regularly used in feeding livestock owing to its low palatability, high moisture content and certain quantities of indigestible fibre. Various processing techniques have been used, including oven drying, sun drying, mixing with blood, barley grain, and molasses to lessen these restrictions.

Depending on the processing method, the nutritional value of dried rumen digesta can vary (Makinde & Sonaiya, 2007; Sakaba *et al.*, 2017). Studies have demonstrated that pelleting increases apparent metabolisable

energy but does not affect amino acid digestibility (Svihus *et al.*, 2005; Roza *et al.*, 2018). Adding unconventional feedstuffs to complete diets and making them into pellets, makes it possible to increase the utilisation and flavour of these feedstuffs. Pelleting facilitates the handling of the feed material, improves equipment flow, and enables the use of alternative ingredients to lower the cost of formulation (Fairfield, 2003). Unfortunately, there is little data on how pelleting dried rumen digesta affects the nutritional content of rumen digesta as feed for livestock. The purpose of this study was to determine the nutritional value and microbial quality of pelleted and unpelleted dried rumen digesta-based concentrate.

Materials and Methods

Material collection and processing of the experimental feed

Rumen digesta from cattle was obtained from the Bolgatanga slaughterhouse. The rumen digesta was collected from cattle examined by veterinary staff to ensure that they were healthy. The rumen digesta were carefully collected into containers and transported to the experimental site, where they were placed in a sack and tied. A weight was placed on it for 3 hours to expel the liquid. The fresh rumen digesta (20 kg) was thoroughly mixed with 100 g of urea fertiliser and stored in white polythene bags for fourteen (14) days. The fermented product was spread on polythene sheets for further sun drying for

Table 1: Experimental diet formulation

Ingredients (% As fed basis)	DRD inclusion levels (%)			
	DRD 0%	DRD 5%	DRD 10%	DRD 15%
Maize bran	65.0	60.0	55.0	50.0
Cassava peels	13.5	13.5	13.5	13.5
Rice bran	15.0	15.0	15.0	15.0
Shea nut cake	5.0	5.0	5.0	5.0
Dried rumen digesta	0.0	5.0	10.0	15.0
Premix	1.0	1.0	1.0	1.0
Salt	0.5	0.5	0.5	0.5
Total	100	100	100	100

three days. The dried rumen digesta (DRD) was incorporated into the feed ingredients (Table 1). The formulated ration was then divided into two equal portions one portion pelleted and the other unpelleted.

Microbial load analysis of rumen digesta

The dried rumen digesta-based concentrate (pelleted vs. unpelleted) was analysed for total microbial load, lactic acid bacteria, *Escherichia coli* and *Salmonella enterica*. Enumeration of microbial load and lactic acid bacteria was done using a modified method described by Maturin & Peeler (2001) and Adzitey *et al.* (2019). Briefly, 10 g of each diet was added to 90 ml of 1% Buffered Peptone Water (BPW) to obtain the 'Neat'. Serial dilutions (10^{-1} - 10^{-5}) were made in 9 ml BPW using 1 ml of the 'Neat'. After which, 100 μ l of each serially diluted aliquot was spread plated unto duplicate Plate Count Agar (PCA) and de Man, Rogosa and Sharpe (MRS) plates for microbial load and lactic acid bacteria, respectively. The plates were then incubated at 37 °C for 24 hours, and colonies were counted using a colony counter.

Chemical and *in vitro* analyses

The dried rumen digesta-based concentrate (unpelleted or pellet diets) was ground in a

centrifugal mill and passed through a 1 mm sieve (Retseh GmbH, Hann, Germany) for chemical analysis and *in vitro* gas production. Dried rumen digesta was analysed for ash and crude protein (CP) using the procedures of the AOAC (2000). The Kjeldahl method was used to obtain the nitrogen concentration, which was multiplied by 6.25 to obtain the crude protein. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by the addition of sodium sulfite and alpha-amylase (Van Soest, 1991) using the Ankom²⁰⁰ fibre analyser. All nutrient composition results were reported on a dry matter basis.

The pelleted dried rumen digesta was selected based on the absence of *E. coli* for the *in vitro* gas study. The rice straw was supplemented with 50% graded levels of pelleted dried rumen digesta-based concentrate (Table 4). We adopted the technique and procedure of Theodorou *et al.* (1994) for the *in vitro* gas production at 24 hours and 72 hours. Dried rumen digesta samples (200 mg) were incubated in 50 ml test tubes containing buffered rumen fluid under anaerobic conditions. Fresh rumen fluid was obtained from slaughtered cattle at the Bolgatanga Slaughterhouse and filtered under continuous

flushing with carbon dioxide (Ansah *et al.*, 2016). The filtered rumen fluid was mixed with McDougall's solution and incubated in a water bath. The pressure from the fermentation gas in the incubation tubes was measured using a digital manometer over a 72 hours at regular intervals (0, 3, 6, 12, 18, 24, 36, 48, 60, 72 h).

The *in vitro* digestible organic matter (IVDOM) was computed from the 24-hour gas production using the equation:
 $IVDOM (\%) = 14.88 + 0.8893 GP + 0.0448 CP + 0.651\% Ash$ (Menke, 1988).

Where:

GP= 24-hour *in vitro* gas production

CP= crude protein.

The gas reading was then fitted to the exponential curve of Orskov and McDonald (1979) without an intercept using GraphPad Prism 7.9 edition. The degradation parameters (b and c) were derived from the exponential model: $Y = b(1 - \exp^{-ct})$, where Y

= gas volume at time t (ml/ 200 mg), b = asymptote gas production (ml/ 200 mg), t = time (h), c = fractional rate of gas production (ml/h).

Statistical analysis

The experimental design used for this study was the complete randomized design. The data generated were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of GenStat (18.2 edition). Means were compared using Tukey HSD at 5% probability ($p < 0.05$).

Results

The chemical composition of the pelleted and unpelleted rumen digesta concentrate is shown in Table 2. There was a significant main effect of the processing method on all chemical composition parameters. The effect of inclusion level was only significant for CP and EE whilst that of the processing inclusion level interaction effect significantly affected

Table 2: Chemical composition of pelleted and unpelleted DRD-based concentrate

Method	DRD inclusion level (%)	Parameters (% DM)					
		DM	CP	EE	ASH	NDF	ADF
Unpelleted	0	98.12	10.34 ^{ab}	12.51	7.20 ^b	52.9	17.22 ^{abc}
	5	97.55	11.78 ^c	12.47	8.61 ^b	57.7	19.01 ^{abc}
	10	98.06	12.88 ^d	13.36	10.45 ^a	59.3	23.24 ^{ab}
	15	97.19	14.22 ^c	13.43	10.69 ^a	64.2	25.18 ^a
Pelleted	0	95.27	10.06 ^c	11.54	7.20 ^b	44.6	13.52 ^c
	5	95.00	10.94 ^d	12.19	8.16 ^b	50.4	15.39 ^{bc}
	10	93.82	11.85 ^c	12.55	8.34 ^b	51.0	16.99 ^{bc}
	15	94.27	12.94 ^d	12.57	8.66 ^b	54.0	19.82 ^{abc}
SED P value	Method	0.613	0.075	0.20	0.43	4.10	1.741
	Level	<.001	<.001	<.001	<.001	0.003	<.001
	Method*Level	0.173	<.001	<.001	<.001	0.526	0.003
	Method*Level	0.248	0.005	0.284	0.012	0.064	0.233

DM= dry matter, CP= crude protein, EE= ether extract, NDF = neutral detergent fibre, ADF = acid detergent fibre

CP and Ash. The unpelleted DRD concentrate had a higher mean CP compared to the pelleted. The CP concentration increased with an increase in DRD inclusion, with the 15% DRD recording the highest CP in both pelleted and unpelleted diets. The NDF and ADF concentrations increased as the DRD inclusion level increased, with a higher concentration recorded in the unpelleted than the pelleted diets.

Table 3 shows the effect of the processing method and different inclusion levels on total microbial count (TBC), lactic acid bacteria (LAB) and *E. coli* of the DRD-based concentrate. There was no significant ($p>0.05$) interaction effect between the method and inclusion levels on TBC. The highest total bacteria count (TBC) was obtained in the unpelleted diet at a 15% inclusion level and the least in the pellet diet at a 0% inclusion level. There was a significant

interaction effect between the method and inclusion level on lactic acid bacteria and *E. coli*. Population counts on lactic acid bacteria increased as the level of DRD increased in the diet. The highest LAB (5.58 log cfu/g) was recorded in the unpelleted at a 15% inclusion level and the least in pelleted at a 0% inclusion level. Method and inclusion level had a significant interaction effect on *E. coli*. The unpelleted diet at 0% DRD inclusion recorded the highest *E. coli* (2.53 log cfu/g), with no *E. coli* detected in the pelleted diets at all levels. There was a decreasing trend of *E. coli* as the levels of DRD increased in the unpelleted diets.

The proximate composition of supplementing 50% of rice straw with 0, 5, 10, and 15% graded levels of dried rumen digesta pellet is shown in Table 4. The highest CP (14.0%) was obtained in 15% DRD pellet + RS. The control diet had the highest ash content

Table 3: Microbial assessment of pelleted and unpelleted DRD-based concentrate at different inclusion levels and processing methods

Method	DRD inclusion level (%)	Parameter (log cfu/g)		
		TBC	LAB	<i>E. coli</i>
Unpelleted	0	5.15	4.40 ^{dc}	2.53 ^a
	5	5.25	4.59 ^c	2.13 ^b
	10	5.92	4.69 ^{bc}	1.65 ^c
	15	6.16	5.58 ^a	1.39 ^d
Pelleted	0	4.77	4.09 ^f	0.00 ^c
	5	4.84	4.32 ^c	0.00 ^c
	10	5.59	4.54 ^{cd}	0.00 ^c
	15	5.78	4.78 ^b	0.00 ^c
SED	Method	0.04	0.049	0.050
	Level	<.001	<.001	<.001
	Method*Level	<.001	<.001	<.001
P value	Method*Level	0.518	<.001	<.001

TBC = Total microbial count (TBC), Lactic acid bacteria (LAB)

(10.12%), while the 15% Pelleted DRD + RS had the lowest (8.90%). Both the NDF and ADF all increased with higher level of DRD inclusion. They were, however, lower relative to the sole RS diet.

The results of the *in vitro* digestible organic matter and fermentation characteristics of DRD-based concentrate supplemented with 50% rice straw are presented in Table 5. The highest (52.14%) *in vitro* digestible organic matter (IVDOM) was recorded in the 15% Pelleted DRD concentrate + RS and the least in the control. The short-chain fatty acid (SCFA) did not differ ($P > 0.05$) among the

dietary treatments. The asymptote gas production (b) and the rate of gas production (c) did not differ ($P > 0.05$) among the treatments. The 15% pelleted DRD concentrate + RS had the highest (7.79 MJ/g/DM) metabolisable energy, with the least reported in the 10% pelleted DRD concentrate + RS.

Discussion

The high CP recorded in the unpelleted DRD concentrate compared to the pelleted may be due to the heating process involved in pelleting. This might have caused the protein to form an insoluble complex with other

Table 4: Mean chemical composition (\pm SD) of rice straw supplemented with 50% graded levels of pelleted dried rumen digesta-based concentrate (%).

Diet	DM	OM	CP	ASH	NDF	ADF
0% Pelleted DRD + RS	98.6 \pm 0.6	91.1 \pm 0.5	11.7 \pm 1.2	8.9 \pm 0.5	57.4 \pm 3.2	29.2 \pm 2.7
5% Pelleted DRD + RS	97.6 \pm 0.3	90.3 \pm 0.1	12.8 \pm 0.2	9.7 \pm 0.1	60.1 \pm 0.1	30.0 \pm 2.1
10% Pelleted DRD + RS	97.8 \pm 0.1	90.0 \pm 0.3	13.3 \pm 0.3	10.0 \pm 0.3	64.9 \pm 0.5	33.3 \pm 1.0
15% Pelleted DRD +RS	97.7 \pm 0.2	89.9 \pm 0.8	14.0 \pm 1.1	10.1 \pm 0.8	66.0 \pm 0.3	35.0 \pm 0.5

DM = dry matter, OM = organic matter, CP = crude protein, NDF = Neutral detergent fibre, ADF = acid detergent fibre, RS = rice straw

Table 5: Effect of supplementing 50% rice straw with graded levels of pelleted dried rumen digesta based concentrate (%) on *in vitro* digestibility and fermentation characteristics.

Diet	Parameter				
	IVDOM (%)	b (ml/gDM)	c (ml/h)	SCFA (mmol/l)	ME (Mj/gDM)
0% Pelleted DRD + RS	47.33 ^c	35.3	0.02	0.44	7.00 ^c
5% Pelleted DRD + +RS	51.04 ^a	31.8	0.03	0.50	7.79 ^b
10% Pelleted DRD + RS	49.36 ^b	34.3	0.02	0.43	6.98 ^d
15% Pelleted DRD + RS	52.14 ^a	28.3	0.02	0.50	7.86 ^a
SED	0.716	4.150	0.004	0.123	0.005
P. value	<.001	0.342	0.201	0.234	<.001

IVDOM: *In vitro* digestible organic matter; b: asymptote gas production; c: fractional rate of gas production; SCFA: Short chain fatty acid; ME: Metabolizable Energy

compounds like sugar. The difference in CP between the pelleted and the unpelleted was about 9%. The CP concentration in the DRD at 15% inclusion was above the 12% minimum CP required for the maintenance and growth of sheep (NRC, 2007). The results suggest that the addition of DRD to the concentrate has a positive effect on the CP concentration. The NDF which measured the concentration of cellulose, hemicellulose and lignin fraction of the concentrate increased with the addition of DRD. The increase may have been occasioned by the replacement of maize bran with DRD, which had a comparatively higher NDF (44.2% Vs. 52.9%) in the current study (Mondal *et al.*, 2008). The decrease in NDF concentration in the pelleted concentrate could be attributed to the effect of heat on the breakdown of fibre components. A similar result was reported by Bonfante *et al.* (2016) who found that the NDF concentration of a pelleted diet was lower than the mash diet.

The microbial load for the unpelleted and pelleted DRD-based concentrates were higher than the average 3.20 cfu/gDM reported earlier by Agolisi *et al.* (2022). The unpelleted diet had a higher total microbial count than the pelleted diet. A similar trend was observed in the lactic acid bacteria, and this was attributed to the thermal processing of the diet. The total microbial count increased as levels of DRD increased in the concentrate diet, which agreed with an earlier report by Agolisi *et al.* (2022). The unpelleted diet recorded higher *Escherichia coli* species even though no *Escherichia coli* species existed in DRD. The presence of rumen *E. coli*, especially those with virulence genes, has been associated with low rumen pH, often induced by the feeding of high grain diet (Khafipour *et al.*, 2011). In the current study, *E. coli* was absent in the rumen digesta, and their inclusion in the diet decreased the overall population of *E. coli*. This conforms to

the feeding practices of most ruminant farmers, where animals are fed more roughage than grain, leading to the absence of *E. coli* in the rumen. However, the pelleting process, which involves the use of heat, may have caused a reduction in the microbial load compared to the unpelleted.

The digestible organic matter was higher in the 15% DRD concentrate supplemented with 50% rice straw due to the relatively higher crude protein content in the diet, which supported cellulolytic microbes in digesting the dry matter. The asymptote gas production (b) showed no significant variation. However, the control diet recorded a higher asymptote gas production (b) but recorded the lowest IVDOM, suggesting a possible inefficiency in the digestibility of the feed. Higher gas production from microbial fermentation with a corresponding low digestible organic matter suggests a potential inefficiency in the digestibility of the diet in this study (Tenakwa *et al.*, 2022). When fed to ruminant livestock, enteric methane and carbon dioxide emissions could be released into the atmosphere. The ME recorded in this study for the supplemented diet fell within the ME recommended by NCR (2007). The ME reported in the current study conforms to previous values reported for DRD (Agolisi *et al.*, 2022).

Conclusion

The chemical composition, microbial population, and *in vitro* gas production of rice straw supplementation in the pellet concentrate suggests that DRD pellet can be used as a source of protein to enhance the utilisation of poor-quality feed resources during the dry season to improve the growth performance of ruminants.

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