Degradation of Crude Protein in Groundnut Cake, Guinea Grass (*Panicum Maximum*) and Rumen Epithelial Scraping based Diets by West African Dwarf Sheep

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Abstract

Three West African dwarf rams fitted with rumen cannula, were used in a completely randomized design for degradation of crude protein (CP) of groundnut cake (GNC), Panicum maximum, rumen epithelial scraping (RES), and diets containing increasing levels of RES. Concentrate diets were formulated such that 0% (A), 50% (B), and 100% (C) of groundnut cake were replaced with RES in a diet containing 20% GNC. The soluble fraction a, insoluble fraction b and rumen undegradable protein (RUP) obtained for GNC, grass and RES varied significantly ($p<0.05$). Significant ($p<0.05$) variations were also observed for a, b and RUP of the test ingredients. Effective degradability (ED) of CP in GNC, grass and RES (at outflow rate of 0.02) were 38.19, 19.63 and 2.13 respectively. The ED values obtained for the diets decreased significantly ($p<0.05$) with increased inclusion of RES. Panicum maximum and RES contained significantly higher ($p<0.05$) level of RUP when compared with GNC. The RUP values obtained for the diets were not influenced ($p>0.05$) by the inclusion of RES.

Keywords: *Rumen epithelial scraping, degradability, concentrate, cannulated sheep, supplement.*

Rumen epithelial scrapping as feed for sheep

Introduction

In feeding practices, the quality of protein fed to ruminants should not be entirely ignored. Before now, protein requirements and evaluation of feeds for ruminants (Akinsoyinu, 1974; Mba *et al*., 1974; Akinsoyinu *et al*., 1975; Akinsoyinu *et al*., 1976; Ikhatua and Adu, 1981; Onwuka and Akinsoyinu, 1985; Adu and Osinowo, 1985) have been largely predicated as outlined (Oyenuga and Akinsoyinu, 1976) on digestible crude protein. This arose largely because it was considered that cattle and sheep could obtain their essential amino acids from microbes produced in the rumen. Extensive use could thus be made of non protein nitrogen (NPN) in high rbohydrate feeds and that a special role of ruminants could be to convert NPN to high quality microbial protein.

These thoughts have however, been recently superceded by newer concepts (Preston and Leng, 1987; ∑rskov, 1991; Preston, 1995) which take into consideration that when amino acid requirements are high, insufficient digestible microbial protein is available from the rumen to meet these needs. It is now necessary to assess the requirements for N by ruminants in terms of ammonia and amino acids of rumen microbes and the amount of digestible 'by pass protein'. The sum of these two sources of

digestible protein represents the metabolisable protein (Leng, 1989).
Dietary supplementation is the most obvious way of manipulating the supply of absorbed amino acids, glucose and glucose precursors (Preston, 1995). Most conventional supplements like fishmeal are expensive and their use in ruminant nutrition competes with monogastric animal and human. Feedstuff purchase and ration formulation should give primary consideration to least cost and maximum biological returns.
Animal waste products originate daily from slaughter house of large animals (abattoirs), or fish processing shop. Reports (Adewoye, 1982; Abubakar, 1988; Polonen *et al*., 1998; Fanimo, 1998) showed that abattoir by products such as rumen contents, blood, meat scraps, bones, hair, feather, hooves, horns, slaughter offal, shrimp wastes, chicken offal meals and fishmeal have been used as livestock feed ingredients for increased growth rate of cockerels, broilers, pigs, dairy calves and sheep in Nigeria. Their collective individual findings underscored the potentialities that waste from animal processing hold for livestock feeding. Despite its obvious availability, proven potential as animal feed and their capability in ameliorating the hitherto dearth of feedstuff, their neglect as evidenced in their erstwhile improper disposal constitutes a serious urban environmental pollutant. With appropriate processing methods, it is worthwhile to evaluate such waste and examine their suitability in livestock ration.
Estimation of degradation characteristics have been successfully employed to evaluate the nutritional attributes of feedstuffs (∑rskov & Mac Donald, 1979; ∑rskov, 1991) particularly for ruminants. This experiment was aimed at assessing the degradability of groundnut cake, Guinea grass, the rumen epithelial scraping of cattle and diets based on RES in West African dwarf sheep

Materials and Methods.
***Sources and Description of Rumen Epithelial Scraping*.**
Samples of RES for this study were collected at the main abattoir in Bodija market, Ibadan, Nigeria. The processing involved initial disemboweling of rumen content. This was then followed by the washing and boiling of empty rumen in hot water for about ten minutes. The inner epithelial layer was then scraped off with a blunt knife. The scraped layer formed the wet RES.
The collected fresh RES was immediately spread on a nylon sac on a clean concrete platform and properly sundried until crisp. The sample was then ground in a Wiley mill to pass through a 0.5mm sieve and then stored until required for chemical analysis or incorporation into the diets.
Concentrate supplements were formulated such that 0% (A), 50% (B), and 100% (C) of GNC were replaced weight for weight with RES in a concentrate diet containing 20% GNC. The formulated diets were then used for the experiment.

The Experimental Site, Animal Feeding and Management
The study was carried out at the International livestock Research Institute (ILRI) Ibadan, Nigeria. The station is located between latitudes $6^0$10" and $9^0$10" North of the equator and longitudes 3^0 and 6^0 of the Greenwich.
Three matured rams with a mean live weight of 26.53 ± 3.32kg each one fitted with a permanent ruminal cannula, were used for the degradability studies. The

sheep were housed in individual pens on a smooth concrete floor with wood

Table 1: Composition and nutrient contents of RES -based diets

Ingredients (g/100g)	A	B	C
Groundnut Cake	20.00	10.0	-
RES	-	10.00	20.00
Cassava Peel	30.00	30.00	30.00
Wheat Offal	34.00	34.00	34.00
Palm kernel cake	10.00	10.00	10.00
Oyster Shell	2.00	2.00	2.00
Bone meal	3.25	3.25	3.25
*Vitamin - Mineral premix	0.25	0.25	0.25
Common salt	0.50	0.50	0.50

**Composition of the Vitamin – Mineral premix*
0.2% Vitamin/ Mineral Premix for Sheep and Goat
(Vitadiz SG) Each 2.5kg contains;

Vitami n A	*10, 000, 000 i.u*	*Manganese*	*50g*
Vitamin D3	*1, 000, 000 i.u.*	*Zinc*	*100g*
Vitamin E	*15,000 i.u.*	*Iron*	*100g*
Calcium	*600mg*	*Iodine*	*1g*
Phosphorus	*400mg*	*Selenium*	*0.2g*
Anti -Oxidant 15g		*Cobalt 0.5g*	

Table 2 : Chemical composition of GNC, Guinea grass and RES -based diets.

Composition (Air -dried Sample)	GNC	Grass	RES	A	B	C
Dry matter	*96.80*	*93.76*	*80.95*	*91.90*	*92.53*	*91.69*
Crude protein	44.75	6.23	44.19	20.25	19.87	19.65
Crude fibre	3.81	41.12	1.52	5.32	5.76	6.12
Ether Extract	9.20	2.34	2.06	4.38	4.27	3.51
Ash	4.89	11.13	3.04	17.67	11.85	16.34
Nitrogen Free Extracts	37.35	39.28	49.19	52.38	58.25	54.38
Ca (%)	0.2	0.32	3.29	1.00	1.06	1.05
P (%)	0.5	0.42	0.82	0.76	0.87	0.80
Gross Energy (Kcal/g)	-	3.56	3.93	2.77	2.74	2.75
Gross Energy (KJ/g)	-	14.90	16.46	11.59	11.46	11.51

72 and 96h. After withdrawal, the samples were washed in cold water until rinsing water became colourless. Duplicate lots from each treatment were used to determine washing losses of soaking them in lukewarm water for about one hour before washing. The bags were dried at 60 ^{0}C for 48h; DM loss from the incubated samples was described by the exponential equation;

$P = a + b\,(l\ \ e^{-ct})$ (Mehrez *et al.,* 1980), where *P = degradation at time t,* a = *water soluble fraction, b = insoluble but degradable fraction, c = rate of degradation of b at time t and PD (Potential degradability) = extent of degradation (a + b) after time t.* The effective degradability values (ED) at rumen outflow rates of 0.02 and 0.05 were estimated electronically (∑rskov & Mac Donald, 1979) using the equation:

$$ED = a + \frac{b \times c}{c + k}$$

where a = water soluble fraction,
b = insoluble but degradable fraction,
c = rate of degradation,
k = rumen out flow rate.

Undegradable DM was also calculated according to the formula of AFRC (1992).

Chemical Analysis

Proximate composition of RES, RES based diets and grass was determined using the AOAC (1984) methods. Mineral content of air dried samples of RES was determined by AOAC (1990) methods. Gross energy was determined with ballistic bomb calorimeter using thermo chemical benzoic acid as standard.

Statistical Analysis

All data were subjected to the analysis of variance (Gomez and Gomez, 1985) to determine the significant differences in the various parameters analyzed while the means were separated by the Duncan multiple range test.

Results and discussion

Degradation characteristics of the crude protein are presented in Table 3. There were significant ($p<0.05$) variations among the water soluble fraction of CP values. The values for CP obtained for both RES and GNC (27.44% and 16.5%) were higher than that obtained for Guinea grass (1.56%). The values of 9.25%, 9.19% and 6.31% were obtained for diets A, B and C respectively. The variations observed in the values decreased significantly ($P<0.05$) with the inclusion of RES in the diet.

The 'a' value of RES (27.44%) obtained in this study was remarkably lower than 71.04% obtained by Isah (2001) for the same ingredient incubated in the rumen of WAD goats. However, storage of animal protein has been known to cause a significant decrease in solubility of protein (Opstvedt, 1974; Mehrez *et al*, 1980). Thus, factors like variations in freshness, or duration of storage of the ingredients, physical characteristics like fineness of the test material, type of animal and the nature of the basal ration might contribute significantly to the observed disparities in 'a' values. Gonzalez *et al.* (1988) reported a similar 'a' values ranging from 14.4% to 27.7% and 22.4% to 44.4% for fishmeal and meat meal respectively. Observed values for GNC (16.5%) in this study compared favourably with 17.4% (Harstad and Prestlokhen, 2000), 18% (Ljokjel *et al,* 2000) and 18.6% (Isah, 2001) obtained for SBM in WAD goat. The lowest 'a' value observed for Guinea grass could be as a result of its relatively higher fibre content compared with RES or GNC

Table 3 : Degradation characteristics and estimated effective degradabilities of crude protein from GNC, RES, grass and RES based -diet.

Parameters	GNC 44.75% CP	RES 44.19% CP	GRASS 6.23% CP	DIET A 20.25% CP	DIET B 19.87% CP	DIET C 19.65% CP
a%	16.50 $_b$	27.44 $_a$	1.56 $_e$	9.25 $_c$	9.19 $_c$	6.31 $_d$
b%	38.69 $_a$	20.50 $_b$	4.44 $_f$	10.88e	11.00 $_d$	12.56 $_c$
c^{h-1}	55.19 $_a$	47.94 $_b$	6.00 $_e$	20.19 $_c$	20.19 $_c$	18.88 $_d$
P%	0.035 $_c$	0.025 $_d$	0.027 $_d$	0.040 $_a$	0.039a	0.037 $_b$
Lag Time	4.90 $_a$	2.60 $_c$	3.13 $_b$	0.47e	0.31 $_f$	2.00 $_d$
U%	44.56 $_d$	52.06 $_c$	94.00 $_a$	79.87 $_b$	79.81 $_b$	81.13 $_b$
ED 0.02	43.88 $_a$	30.38 $_b$	3.50 $_e$	13.44 $_c$	13.38 $_c$	12.31 $_d$
ED 0.05	38.19	19.63	2.13	9.00	8.88	8.06
RSD	1.04	0.17	0.60	0.82	0.70	0.73

Means along the same row with identical subscripts are not significant ($p<0.05$)
Footnote:
a = water soluble fraction; b = insoluble but degradable fraction; c^{h-1} = rate of degradation; LT = Lag time; U = Undegradable fraction; ED = Effective degradability at 0.02 and 0.05 outflow rates; RSD = Relative standard deviation

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The Insoluble but Fermentable CP Fraction 'b'

The 'b' values obtained for GNC, RES and Guinea grass were 38.69%, 20.5% and 4.44% respectively. GNC contained the highest level of degradable CP. The lower 'b' estimate for RES suggested that RES could be resistant to degradation by rumen microbes (Preston, 1995; Prakash *et al*., 1996). Gonzalez *et al*. (1998) reported similar value ranging between 26.5% to 26.8% and 22.4% to 38.6% for fishmeal and meat meal respectively. The 'b' value obtained for GNC was about 2.35 times that of 'a' values. The relative higher degradable portion compared with soluble portion suggested that CP content of GNC though relatively insoluble in water is more highly fermentable in rumen. Degradable estimates for diets A, B and C were 10.88% 11.00% and 12.56% respectively and were significantly improved ($P<0.05$) by the inclusion of RES in the diets.

The Potentially Degradable Fraction 'a + b'

The mean values of the potentially degradable CP fraction of 55.19%, 47.94% and 6.00% respectively, obtained for GNC, RES and Guinea grass were significantly ($P<0.05$) different. The PD values of the test diets A, B and C ranged between 18.88 to 20.19%, higher for diets A and B. The 'b' value constituted about 70% of the PD fraction of GNC while it constituted just 43% of the fraction of PD in RES. This suggested that the 'b' value was responsible for the high PD estimates of GNC while 'a' value contributed more to that of RES.

Rate of Degradation

The mean rate of degradation constant (C^{h-1}) obtained for the test ingredients and rations were 0.035, 0.025, 0.027, 0.040, 0.039 and 0.037 for GNC, RES, grass and diets A, B and C respectively. Least and similar values were obtained ($P<0.05$). The disparity in C^{h-1} values for diets decreased significantly with the inclusion of RES ($P<0.05$). The disparity in C^{h-1} value of the test ingredients was adduced to the difference in fibre content of the degradable portion. Compared with RES, GNC recorded a significantly higher and faster CP degradation rate constant, which suggested higher and faster microbial colonization of the feed ingredient (GNC). Preston and Leng (1987) and Hoover *et al,* (1989) suggested that the rate of degradation was affected by the presence of lipid and other water insoluble substances in the material incubated. Thus, lipid might reduce surface area of the protein that was accessible to microbial protease. Therefore, the slower rate of degradation of CP observed for animal protein could be due to the presence of residual fat and other water insoluble components of low fibre content. This lends credence to the earlier observation that animal protein degrades slowly in the rumen (Preston, 1995; Prakash *et al.,* 1996).

Effective Degradability of CP

The effective degradability (ED) values obtained for CP are shown in Table 3 GNC recorded the highest value (43.88 and 38.19 at 0.02 and 0.05 outflow rates respectively). Least values were obtained for Guinea grass (3.5% and 2.13% at 0.02 and 0.05 outflow rates respectively). The mean ED values obtained for RES (30.38% and 19.36% at 0.02 and 0.05 outflow rates respectively) compared favourably with 18.2%, 31.8% reported for meat meal (Ljokjel *et al*., 2000) but lower than 48.1 and 41.1 60.4 reported for fishmeal (Gonzalez *et al*., 1998) and markedly lower than 71.35-7.45 reported by Isah (2001) for the same ingredient incubated in the rumen of WAD goats. Prakash *et al*. (1996) obtained 49.2% ED value for fishmeal in buffaloes.

The major factor known to influence the ruminal degradation of protein in animal protein based feedstuff is the length of time that such animal product has been stored prior to processing and usage. Mehrez *et al.* (1980) observed that storage of fishmeal for 3 days before processing increased ruminal CP degradation by 14% units. This increase was ascribed to enzymatic and bacteriological proteolysis of fishmeal muscles. Preston and Leng (1987) also affirmed that the rate of degradation of protein was influenced by the presence of lipids or other water insoluble substances in the materials incubated. There appeared to be a high variability in CP degradation values reported for animal products. In their study, Madsen and Hvelplund (1985) and Yoon *et al*. (1996), proved that degradation of fishmeal could reasonably be estimated

Table 4 : Mean crude protein disappearance of feeds and feedstuffs incubated in the rumen of WAD sheep.

Crude Protein Incubation Time	Hours					
	0	12	24	48	72	96
GNC44.75% CP	13.53	15.45	52.6	73.96	85.02	94.99
RES44.19% CP	26.81	38.77	35.2	42.44	45.54	46.90
Grass6.23% CP	2.14	2.50	3.50	4.73	5.38	5.69
Diet A20.25% CP	13.02	13.31	15.90	18.5 2	19.50	19.90
Diet B 19.87% CP	12.40	13.27	15.90	18.52	19.54	19.96
Diet C 19.65% CP	10.02	10.21	13.33	16.63	17.94	18.50

from buffer protein solubility values. This observation applies to RES. Other factors such as difference in lag phase, type and quality of raw materials, drying temperature, storage and other processing conditions could also contribute to the variation observed in value for RES.

Prakash *et al.* (1996) remarked that oilseed cake proteins are more highly rumen degradable than fishmeal in buffaloes. He obtained crude protein values of 93.25%, 82.1% and 49.2% for GNC, Soybean cake and Fishmeal respectively. The ED of CP (at 0.02 outflow rate) in diets was lowered significantly ($P<0.05$) by the inclusion of RES in the diets.

The Undegradable Crude Protein Fraction.

RES recorded significantly higher ($P<0.05$) RUP values (52.06%) compared with GNC (44.56%). Guinea grass contained the most RUP protein (94.0). The RUP values increased ($P<0.05$) with the inclusion of RES in the diets. The obtained RUP values for diets A, B and C were not significant ($P<0.05$). This might be because other ingredients included in the formulation could have contributed significant level of lowly degradable protein in the rumen.

Guinea grass recorded the highest RUP fraction probably due to its higher level of fibre and lignin contents. The RUP fraction of RES was higher than that of GNC. This might probably be because RES being an animal protein source may be more resistant to microbial degradation (Preston 1995; Prakash *et al.,* 1996) in the rumen. Thus, the value of 52.06% obtained for RES was available as 'by pass protein'.

However, this notable higher RUP content of RES must be interpreted with caution vis-à-vis the true protein content of the RUP fraction, since it is the true protein fraction of the RUP that will be digested further in the alimentary canal (abomasum). Particularly, observed higher value could be due to high level of collagen or possibly contamination with hairs during collection and processing.

Disappearance

The values obtained for the disappearance of crude protein in feedstuff, feeds and grass samples are shown in Table 4. There was a general increasing trend in crude protein disappearance of all samples. At zero hour, RES recorded the highest level of disappearance (26.81%). This could be because RES contained the least level of fibre and therefore more soluble. Compared with GNC, RES CP disappeared more slowly over time than that of GNC.

The CP content of GNC recorded the highest level of disappearance. There was an initial slow disappearance of CP in GNC between 0 to 12 hours and then a much more rapid disappearance from 12 to 48 hours.

Preston and Leng (1987) observed that during the lyses of protein in the rumen, the soluble protein was absorbed rapidly unto the bacterial cell prior to lysis. Thus, microbial accessibility to the CP of RES was faster at the initial hours than that in GNC. With time however, crude protein from GNC was subsequently broken down. This might probably be due to increasing attack and degradation of fibre in GNC as the retention time in rumen increased thus making the protein available.

The disappearance of CP of the diets decreased with increased inclusion of RES at different incubation time. This observation suggested that the high initial CP solubility value of RES compared with other feedstuffs in this study and low relative degradable fraction made it a ready source of needed nitrogen for rumen microbes on one hand and also a veritable source of RUP for the animal down the alimentary tract (in the abomasum)

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