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Rate and extent of ruminal degradation of crude protein from selected feedstuffs used in cattle feedlots as measured by the *in sacco* technique

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Ruminal degradation of crude protein from feedstuffs was determined in steers that were fed diets based on either maize (whole, meal or flakes) or on by-products of the milling industry. In addition to the *in sacco* degradation study, fractional outflow rates of Cr-mordanted fish-meal particles were studied at DM intakes between 0.04 and 0.12 kg/kg $W^{0,75}$/d. Predicted crude protein degradation was calculated at rate constants for outflow of 0.04 and 0.06/h respectively. The rate constant for outflow was correlated with level of DM intake (r^2 = 0.87). Lag phase prior to degradation of crude protein of cottonseed oilcake meal differed significantly with diet of the host animal (whole maize vs. maize meal and flaked maize), but the predicted extent of degradation did not differ significantly. The predicted extent of degradation of crude protein was higher for maize meal than for whole or flaked maize. Extent of crude protein degradation of other selected feedstuffs was not significantly different between two replicate periods of measurement and ranged from a low of 29.8% at a rate constant for outflow of 0.06/h for gluten 60 to a high of 87.3% for wheat bran.

Die rumendegradering van grondstofruproteïen is bepaal in osse wie se diëte gebaseer was op óf mielies (heel, gemaal of vlokkies) óf neweprodukte van die maalbedryf. Benewens die *in sacco*-degraderingstudie is die fraksionele uitvloeitempo van Cr-gemerkte vismeelpartikels by DM-innamepeile tussen 0.04 en 0.12 kg/kg $W^{0,75}$/d bestudeer. Die voorspelde ruproteïendegradering is by tempokonstantes vir uitvloei van onderskeidelik 0.04 en 0.06/h bereken. Die tempokonstante vir uitvloei was gekorreleerd met DM-innamepeil (r^2 = 0.87). Die voorspelde degradering van ruproteïen van katoensaadoliekoekmeel is nie betekenisvol beïnvloed deur dieet van die gasheerdier nie, maar die waarde by nultyd het wel betekenisvol verskil (heel mielies vs. mieliemeel en mielievlokkies). Die voorspelde degradering van mieliemeelruproteïen was betekenisvol hoër as dié van heel mielies en mielievlokkies. Die ruproteïendegraderingswaardes van 'n reeks ander grondstowwe het nie betekenisvol verskil tussen twee metingsperiodes nie en het gevarieer van so laag as 29.8% vir gluten 60 by 'n tempokonstante vir uitvloei van 0.06/h tot so hoog as 87.3% vir koringsemels.

Keywords: Feedlot, fractional outflow, *in sacco*, protein degradation, steers.

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The cattle feedlot industry in South Africa used primarily maize as an energy source in the seventies. More recently, a variety of products obtained from the milling industry have replaced maize as the primary energy source. Also grain sorghum is used more often. These feedstuffs usually contain more protein than maize and, because this protein may contain variable proportions of the deep core and upper layer proteins of the kernel, they may have a different ratio of ruminally degraded to undegraded protein. While there has been a systematic effort to categorize popular feedstuffs in South Africa in terms of ruminally degraded protein in general (Cronjé, 1983), and specifically for the dairy cow (Erasmus *et al.*, 1988; 1990a; 1990b), less is known about protein degradation when nylon bags are incubated in the rumens of steers on feedlot diets. Most *in vivo* and *in situ* studies of crude protein degradation have been conducted with sheep fed standardized diets. For purposes of quantification, work with the applicable animal and diets probably is more appropriate, because retention time and rumen substrate may have an effect (Ha & Kennelly, 1984; Erdman *et al.*, 1987; Nocek, 1988). Therefore, in the present study, crude protein degradation of selected feedstuffs was investigated by the *in sacco* technique (Mehrez & Ørskov, 1977) in steers fed typical feedlot diets at appropriate levels of intake.

Procedures

Experiment 1

Aims

The purpose was twofold: (a) to study the passage rate of protein particles from the rumen of steers which receive feedlot diets at different levels with a view to calculating a prediction equation, and (b) to study the effect of differently processed maize as a rumen substrate on crude protein degradation of cottonseed oilcake meal, while crude protein degradation of the maize itself is measured simultaneously.

Animals and diet

Eight rumenally fistulated steers weighing 250—350 kg were fed diets based on either whole maize, maize meal or flaked maize. Cottonseed hulls were used as the source of roughage at a level of 7% for the whole maize and 11% for the maize meal and flaked maize diets. Urea was added to increase dietary crude protein to 12% of DM. Further additions to the diets included minerals, vitamins, 1.3% $CaCO_3$ and 0.5% $NaHCO_3$ as buffers, an ionophore and an antibiotic according to general feedlot practice. The steers were adapted to their diets and feeding levels for three weeks. Feeding levels ranged from 0.04 to 0.12 kg DM/kg $W^{0.75}$/d.

Ruminal outflow

The technique of Udén *et al.* (1980), as modified by Eliman & Ørskov (1981), for outflow of protein particles was used. A detailed description of the procedure was given by Erasmus *et al.* (1986). Briefly, a single dose of 100 g Cr-mordanted fish-meal was introduced into the rumen through the cannula and the ruminal contents were mixed thoroughly. Faeces were collected for 72 h at 6-h intervals and the samples were dried individually at 100°C. The dried samples were analysed for chromium by atomic spectrophotometry (Arthur, 1970).

The excretion of Cr with time was fitted to the Grovum and Williams Model assuming first-order kinetics (Grovum & Williams, 1973; Eliman & Ørskov, 1981). The rate constant for outflow of protein particles from the rumen was assumed to be the slope of a plot between the natural log of the decreasing Cr concentration in the faeces and time, using only values after the peak marker concentration had been reached. The linear regression equations calculated for data of individual animals fitted well (r^2 = 0.86—0.99).

There were five observations on whole maize, three on maize meal and two on flaked maize (cf. Table 2). The rate constant for outflow of Cr-mordanted fish-meal particles obtained by the procedure described above, was regressed on level of intake by least squares procedures. Separate regression equations were calculated for whole maize and processed maize (maize meal and flaked maize) but, as covariance analysis did not detect a significant difference between regression parameters, the data were pooled to calculate one prediction equation.

In situ *degradation*

Degradation of crude protein of cottonseed oilcake meal was studied by incubating nylon bags (140 × 90 mm; 53 μm pore size) filled with *ca.* 5 g air-dry material milled through a 2-mm screen for 0, 1, 2, 4, 6, 8, 12 and 24 h, respectively (Mehrez & Ørskov, 1977). Three ruminally fistulated steers per treatment (whole maize, maize meal and flaked maize) were used in two replications. In addition, crude protein degradation of the three types of maize was determined in steers receiving the corresponding maize-based diet, *i.e.* whole in whole, meal in meal and flaked in flaked. Whole kernels were split in half before incubation to facilitate fermentation, but no further grinding to simulate chewing was employed. Treatment of samples after incubation was as described by Erasmus *et al.* (1988). The percentage crude protein disappearing at each incubation time was calculated from the proportion remaining after incubation. No correction for bacterial attachment was attempted but the bags were thoroughly washed and rinsed (Erasmus *et al.*, 1988).

Rate of degradation was fitted to the equation as suggested by Ørskov & McDonald (1979) where particular parameters give an estimate of the soluble fraction (a)*, the insoluble but potentially degraded fraction (b), and the rate of degradation (c) of (b). The parameters (a), (b) and (c) usually are estimated by an iterative least squares procedure, but because this procedure often gives unrealistic estimates for a + b (which represents the maximum extent of degradation) (Erasmus *et al.*, 1990a; 1990b), the handfit method proposed by Ørskov (1982) was employed. The method by Ørskov & McDonald (1979) also gives an equation whereby the extent of crude protein degradation can be calculated if the rate constant for ruminal outflow is known.

* This procedure does not differentiate between solubilization of protein and small particles that may escape from the nylon bag (Nocek, 1985).

Experiment 2

Aim

Crude protein degradation of selected feedstuffs, especially by-product energy sources, was determined over two periods to increase sensitivity of analyses and to ascertain whether the time period during which the host animal is on feed makes a difference.

Animals and diet

The same ruminally fistulated steers of Experiment 1 were used. They were fed a feedlot diet based on the feedstuffs that were incubated in nylon bags. The ME content of the diet was 12 MJ/kg DM and the crude protein content was 12% of DM. Cottonseed hulls at 3% and lucerne pellets at 2% of total diet were included as roughage sources. The crude fibre content of the diet was 10.8% of DM. The steers were adapted to the diet for three weeks and were given *ad libitum* access to the feed.

In situ *degradation*

Crude protein degradation of the feedstuffs was determined by the *in sacco* method as described under Experiment 1. Three (animal) replications per feedstuff were included and the procedure was repeated four weeks later. Steers were allocated at random to feedstuffs and eight bags were incubated in the rumen of every steer at any one time. The chemical composition (standard Weende method, AOAC, 1980) of each feedstuff is shown in Table 1. Period effects were tested for by one-way analysis of variance and Tukey's test.

Results and Discussion

Chemical analysis of feedstuffs

Values for TDN and ME were calculated from the results of the Weende analysis according to prediction equations of Kearl (1982).

The maize used in Experiment 2 contained more N and fibre than the maize used in Experiment 1; thereby, it was calculated to contain less energy. Maize bran was obtained from two commercial sources and was termed low and high fibre, respectively. Sorghum samples were representative of the non-bird resistant ('sweet') varieties.

Ruminal outflow

The relationship between the rate constant for outflow of Cr-mordanted fish-meal particles and level of intake is shown in Table 2.

The linear regression equation was highly significant (r^2 = 0.87), although individual deviations from the prediction equation were substantial (see $Sy.x$ in Table 2). The fit was somewhat poorer than that obtained with dairy cows by Erasmus *et al.* (1986) (r^2 = 0.97)

The rate constant for outflow was predicted to vary from 0.02 to 0.06/h when DM intake ranged from 0.04 to 0.12 kg/kg $W^{0.75}$/d. The 0.12 kg DM intake was *ad libitum* and corresponds to a daily DM intake of 8.65 kg for a 300-kg steer. The reciprocal of the rate constant represents mean rumen retention time which indicates that at the 0.04 kg level of DM intake, retention time was about 50 h and at *ad libitum* intake (0.12 kg), about 16.7 h.

The rate constants for outflow obtained in the present study correspond with estimates on dairy cows between levels of

Table 1 Chemical composition of feedstuffs on a DM basis (%, except ME)

Feedstuff	DM	N	Crude fibre	Ether extract	Ash	NFE	TDN[1]	ME (MJ/kg)[1]
Cottonseed hulls	89.3	0.73	60.2	1.98	3.65	29.6	34.8	5.11
Cottonseed oilcake meal	93.3	6.78	19.2	1.42	6.34	30.7	62.9	9.52
Fish-meal	92.0	10.9	–	7.52	18.9	5.43	77.0	11.7
Hominy chop	90.7	2.02	9.07	8.77	4.09	65.4	85.3	12.9
Lucerne, pelleted	89.5	2.10	34.3	1.85	11.8	38.9	55.8	8.45
Maize bran, high fibre	90.8	1.45	24.9	6.59	3.05	56.4	72.7	11.0
Maize bran, low fibre	90.6	1.90	15.2	8.67	4.18	60.1	81.4	12.3
Maize germ	90.6	2.19	12.6	14.7	4.49	54.5	86.7	13.1
Maize germ, defatted	91.7	2.12	9.98	3.22	4.05	69.5	79.6	12.0
Maize gluten, 20% CP	87.5	3.93	12.8	2.16	8.26	52.2	70.6	10.7
Maize gluten, 60% CP	93.3	11.1	2.18	1.14	2.55	24.7	75.6	11.4
Maize meal								
Experiment 1	88.9	1.56	2.72	4.21	1.53	81.8	89.2	13.5
Experiment 2	89.1	1.77	4.69	3.98	1.39	78.9	85.7	13.0
Sorghum meal	89.3	1.56	4.75	3.74	1.67	80.1	87.4	13.2
Wheat bran	89.1	2.79	14.5	5.49	6.60	56.0	73,1	11.1

[1] Calculated from Kearl, 1982.

Table 2 Rate constant for outflow of Cr-mordanted fish-meal particles from the rumen of steers as affected by level of intake and method of maize processing

Steer no.	Method of feeding maize	Level of intake (kg DM/kg $W^{0,75}$/d)	Rate constant (/h)
1	whole	0.118	0.063
7	whole	0.091	0.043
8	whole	0.080	0.033
11	whole	0.060	0.029
12	whole	0.080	0.038
6	meal	0.060	0.037
8	meal	0.099	0.049
10	meal	0.042	0.021
11	flakes	0.080	0.047
13	flakes	0.120	0.056

Linear equation: Rate constant = 0.470 level of intake + 0.003

r^2 = 0.87**

$Sy.x$ = 0.005

Rate constant at: 0.080 kg DM = 0.04/h

0.120 kg DM = 0.06/h

Whole and processed maize did not differ significantly ($P \leq 0.05$).

** Highly significant ($P \leq 0.01$).

intake obtained in early and late lactation (range 0.02 to 0.08) (Erdman, 1981; Lindberg, 1982; Erasmus *et al.*, 1986), but our average is somewhat higher than that obtained for sheep receiving mixed diets (range 0.01 to 0.04) (Mansbridge & Ørskov, 1980). Comparison between rate constants for outflow is difficult because of many variables. Variables are e.g. different physical forms of the diet (Coombe *et al.*, 1979), long vs. short particles (Warner, 1981), and concentrate–roughage ratios (Owens *et al.*, 1979). These results indicate that rate constants for outflow of protein must be determined for a specific animal and applicable diet, before crude protein degradation can be predicted effectively.

The method whereby the rate constant is determined also contributes to the variation. In the present context, passage rate of Cr-mordanted fish-meal particles was measured; this does not necessarily predict passage rate of all feedstuffs accurately because the mordant procedure increases the specific gravity of particles (Lindberg, 1982). Also, the Grovum & Williams Model may overestimate passage rate (Pienaar *et al.*, 1983), and logarithmic dilution alone following peak marker concentration may be insufficient to evaluate two-pool systems. Consequently, our estimates of ruminal outflow may have been affected by both the particle type and the mathematical model we used.

Crude protein degradation

The effect of whole maize, maize meal and flaked maize as host animal diet on crude protein degradation of cottonseed oilcake meal is shown in Table 3(a). Extent of degradation was calculated at rate constants for outflow of both 0.04/h and 0.06/h to determine if the difference was significant.

The difference between whole and processed maize diets on parameter (a) for cottonseed oilcake meal was significant. Parameter (a) normally represents the soluble protein fraction. As solubility is not likely to vary, we concluded that the lag phase before fermentation starts was increased when the rumen substrate was processed maize. Processed maize is fermented faster than whole maize (Owens *et al.*, 1986), which may decrease rumen pH (Hoover, 1986) and increase the lag phase of digestion of fibrous material (Varga, 1987). Unfortunately, rumen pH was not measured in the present study and, therefore, this explanation is tentative.

Although parameter (a) was altered, predicted degradation was not altered significantly by host animal diet [Table 3(a)]. This suggests that once ruminal fermentation started, it continued at the same rate and to the same extent in animals fed either whole or processed maize. Consequently, we concluded that the crude protein degradation of feedstuffs is not likely to change much between normal feedlot diets, provided differences in level of intake are considered. Mehrez & Ørskov (1977) suggested that degradation rates are altered less by diet of the host animal for animal proteins than for plant proteins.

Table 3(a) Effect of host animal diet on parameters describing crude protein degradation of cottonseed oilcake meal

	Host animal diet				
Parameter	Flaked maize	Maize meal	Whole maize	MSE[1]	PR>F
a	20.9[a]	19.1[a]	25.8[b]	2.36	0.032
b	47.0	50.7	49.7	8.61	0.825
c	0.080	0.071	0.069	0.023	0.819
* *P* 0.04 (%)	52.2	51.5	57.3	7.01	0.490
* *P* 0.06 (%)	47.8	46.6	52.4	6.63	0.493

Table 3(b) Effect of processing on parameters describing crude protein degradation of maize[2]

Parameter	Flaked maize	Maize meal	Whole maize[3]	MSE[1]	PR>F
a	3.10[a]	15.2[b]	0.70[a]	3.42	0.022
b	32.2[a]	66.8[b]	27.1[a]	5.18	0.0002
c	0.075	0.101	0.061	0.023	0.191
* *P* 0.04 (%)	24.1[a]	63.0[b]	17.1[a]	2.90	0.0001
* *P* 0.06 (%)	21.0[a]	57.1[b]	14.4[a]	2.71	0.0001

[a,b] Values in the same line with different superscripts differ significantly.

* Predicted crude protein degradation at rate constants for outflow of 0.04 and 0.06/h respectively.

[1] Mean standard error.

[2] Host animal diets corresponded with the maize type evaluated *in sacco*.

[3] Whole maize kernels were split once for the *in sacco* study.

Because of cost, plant proteins (plus urea) rather than proteins of animal origin usually are used in the South African feedlot industry. The present results suggest that feedlot nutritionists should not be unduly concerned with the conclusion of Mehrez & Ørskov (1977).

Table 3(b) shows the difference in crude protein degradation between whole maize, maize meal and flaked maize. Crude protein degradation of whole maize should be considered tentative. Whole maize is not thoroughly chewed by cattle. Usually the particles collected from the rumen immediately after a meal range from untouched kernels to kernels split twice to four times (unpublished results). Rumination subsequently reduces particle size, but particles are never broken down to match those of maize meal before passage from the rumen (particles between 2 and 6 mm readily leaves the rumen of cattle according to Ulyatt, 1982). Kernels in this study were split once only before incubation; they produced a value of 14.4% at a rate constant of 0.06/h vs. 57.1% for maize meal in Experiment 1 and 66.6% in Experiment 2 [Table 4(a)]. Predicted crude protein degradation in the rumen of 40—50% for whole maize *in vivo*, is probably more realistic. Aguirre *et al.* (1984) and Streeter *et al.* (1989) reported values between 36 and 53% for whole maize from flow and N disappearance studies.

Parameter (a) was greater in maize meal than in whole or flaked maize [Table 3(b)]. Although an increased lag phase explanation as proposed above probably applied to whole maize, the more likely reason for the low a-value for flaked maize is decreased protein solubility due to heat damage during treatment that denatures proteins and makes them less extensively degraded (Beever & Thomson, 1981; Van der Walt & Meyer, 1988). This hypothesis is supported by the observation that both the degraded portion (b) and the extent of degradation (a + b) were significantly lower with flaked maize than with maize meal, resulting in a lower predicted extent of degradation. Heat damage, however, apparently does not always occur with flaked maize, as Aguirre *et al.* (1984) reported a degradation value of 48%.

Note that crude protein degradation at a rate constant of 0.06/h was not much lower than at a rate constant of 0.04/h. In fact, this difference was not significant (cf. MSE in Table 3). Results are similar with other feedstuffs in Table 4. This contrasts with reports by Tamminga *et al.* (1979) and Erasmus and co-workers (1988; 1990a; 1990b) for *in situ* measurements in dairy cows, where the rate constant for outflow had a substantial effect on extent of degradation, especially with slowly fermenting sources. This difference may be due to differences in the host animal or the feedstuff source. Firkins *et al.* (1986) and Rahnema *et al.* (1987), working with feedlot steers, also found no significant effect of feeding level on protein degradation. These results, therefore, confirm that it makes little difference whether a rate constant of 0.05/h (ARC, 1984) or 0.04 or 0.06/h to accommodate variation in feeding level, is used to calculate extent of crude protein degradation in feedlot diets.

Table 4(a) Parameters describing crude protein degradation of selected feedstuffs and the predicted degradation at rate constants for outflow of 0.04 and 0.06/h

Feedstuff	a	b	c	*P* 0.04 (%)	*P* 0.06 (%)
Cottonseed hulls	35.0	14.5	0.108	45.6	44.3
Fish-meal	30.3	21.4	0.043	41.4	39.2
Gluten 20	64.7	27.0	0.159	86.3	84.3
Gluten 60	15.0	23.6	0.101	31.9	29.8
Hominy chop	36.3	46.5	0.112	70.6	66.6
Lucerne, pelleted	20.0	56.3	0.133	63.3	58.8
Maize bran, high fibre	37.0	44.1	0.150	71.8	68.5
Maize bran, low fibre	38.2	46.7	0.112	72.6	68.6
Maize germ	41.1	45.2	0.096	73.0	68.9
Maize germ, defatted	13.9	49.9	0.123	51.6	47.4
Maize meal	37.2	44.8	0.114	70.4	66.6
Sorghum meal	16.0	69.3	0.075	61.2	54.5
Wheat bran	53.4	42.9	0.227	89.9	87.3
MSE	4.32	5.70	0.046	4.69	4.46

Table 4(b) Effect of period on estimates of crude protein degradation of selected feedstuffs

	Period		
Parameter	1	2	PR>F
a	33.3	34.1	0.570
b	42.6	39.2	0.062
c	0.133	0.119	0.339
P 0.04 (%)	64.9	62.7	0.137
P 0.06 (%)	61.4	59.2	0.135

The difference in predicted crude protein degradation of maize meal between Experiments 1 and 2 [cf. Tables 3(b) and 4(a)] can be explained mainly by parameter (a) (soluble fraction), because the extent of degradation (a + b) was 82% in both cases. The N content of maize meal in Experiment 2 was 1.77% vs. 1.56% in Experiment 1 (Table 1). The higher N content probably was due to a larger soluble fraction which would support differences reported by Erasmus *et al.* (1990a) for maize and high-lysine maize samples. In their study, parameter (a) was 16.9% for maize and 55.1% for high-lysine maize; these values compare to 15.2% and 37.2% for maize in Experiments 1 and 2 respectively.

Comparison between the results of Erasmus *et al.* (1988; 1990a) and those of the present study in predicted degradation at a rate constant for outflow of 0.05/h, reveals very close agreement: fish-meal – 40.3 vs. 40.2%; cottonseed oilcake meal – 54.5 vs. 51.0% (average); gluten 20 – 83.3 vs. 85.2%; gluten 60 – 30.0 vs. 30.8%; hominy chop – 73.0 vs. 68.4%; maize meal – 63.0 vs. 59.9% (Experiment 1), 68.3% (Experiment 2); sorghum – 60.0 vs. 57.6% and wheat bran – 87.0 vs. 88.6%. The close agreement is surprising when the difference in the diets fed is considered. Blackburn & Hobson (1962) and Allison (1970) claimed that rumen substrate should have no effect, but other workers disagree (Ha & Kennelly, 1984; Erdman *et al.*, 1987; Nocek, 1988). Because rumen substrate is unlikely to differ much between feedlot diets, and time on feed of the host animal has a negligible effect [Table 4(b)] (Weakley *et al.*, 1983), our results suggest that if the effect of feeding level is corrected for, variation in rate and extent of degradation of feedstuffs in feedlot diets will result only because of variation between samples and host animals (Mehrez & Ørskov, 1977).

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