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Effects of fish-meal supplementation on the digestion and rumen degradation of ammoniated wheat straw

CM Guedes, A Dias-da-Silva

Universidade de Trás-os-Montes e Alto Douro, Department of Animal Production, Apartado 202, 5001 Vila Real Codex, Portugal

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Summary — A digestibility experiment was designed to investigate the effect of fish-meal (FM) supplementation on digestion of urea-treated wheat straw. Ammoniated straw was offered alone in restricted amounts or with FM at the levels of 2.5, 5.0 and 10.0% of daily air-dried straw. Untreated straw supplemented with 10.0% FM was also offered. Straw was sprayed with a solution of urea to achieve similar levels of total nitrogen in all diets. Five mature rams fitted with rumen cannulae were used in a 5 x 5 latin square design. FM supplementation significantly increased organic matter (OM) and neutral detergent fibre (NDF) digestibility ($P < 0.05$) of urea-treated straw. There was no significant effect of FM level ($P > 0.05$). OM digestibility was enhanced 9 percentage units due to urea treatment (47.2 *vs* 56.3) when the straw was supplemented with 10% fish meal in the diet. The size of the potentially degradable fraction and the rate of degradation measured *in situ* by the nylon bag technique were consistently increased by FM supplementation. No effect on rate constant of dry matter and NDF degradation was observed due to urea treatment. The degradation lag time of NDF was reduced ($P < 0.05$) by FM supplementation.

straw / urea treatment / digestion / rumen / fish meal

Résumé — Effets d'une complémentation avec de la farine de poisson sur la digestion et la dégradation ruminale d'une paille de blé traitée par l'urée. *Un essai a été réalisé, sur des moutons, en vue d'analyser l'effet de la complémentation par la farine de poisson sur la digestion d'une paille de blé traitée par l'urée. Nous avons distribué aux animaux la paille traitée en quantités limitées (48 g kg^{-1} pois vif^{0,75}) soit seule, soit complémentée par 3 niveaux de farine de poisson (2,5, 5,0 et 10,0% de la paille séchée à l'air). Nous avons aussi distribué aux animaux la paille de blé non traitée complémentée par 10,0% de farine de poisson. On a effectué l'aspersion de la paille par une solution d'urée pour que les teneurs en azote des régimes soient les mêmes. Cette étude a été réalisée sur 5 moutons munis de canules dans le rumen, selon un carré latin 5 x 5. On a mesuré la digestibilité* in vivo *et la dégradabilité* in situ *selon la méthode des sachets de nylon. La complémentation par la farine de poisson a augmenté significativement (P < 0,05) la digestion de la matière organique et la digestion des composants pariétaux (NDF) de la paille traitée par l'urée. L'effet du niveau de farine de poisson n'a pas été significatif (P > 0,05). Le traitement à l'urée a augmenté la digestion de la matière organique de la paille de blé*

de 9 unités (47,2 contre 56,3%). La fraction potentiellement dégradable dans le rumen et la vitesse de dégradation ont augmenté lors de la complémentation par la farine de poisson. On n'a observé aucun effet du traitement à l'urée sur la vitesse de dégradation de la matière sèche et des composants pariétaux de la paille. La complémentation par la farine de poisson a réduit ($P < 0,05$) le temps de latence de la dégradation des composants pariétaux. En conclusion, ces résultats montrent que la complémentation de la paille de blé traitée à l'urée par 2,5% de farine de poisson est suffisante pour provoquer une stimulation de la dégradation de la paille de blé dans le rumen.

paille / traitement à l'urée / digestion / rumen / farine de poisson

INTRODUCTION

Ammonia treatment of cereal straws using urea as a source of ammonia, generally results in increased digestibility and intake (Cloete *et al*, 1983; Dias-da-Silva and Sundstøl, 1986; Djajanegara and Doyle, 1989). Research has demonstrated that these effects are very probably due to the additional nitrogen retained by straw following treatment and to physicochemical changes occurring in the cell-wall structure (Dias-da-Silva and Guedes, 1990).

Most of the nitrogen retained by ammoniated straw is in the water-soluble form (Dryden and Kempton, 1983; Dias-da-Silva and Sundstøl, 1986). This suggests that it is available for microbial growth. Indeed, in most cases, it can be expected that the amount of non-protein nitrogen retained in the straw provides levels of rumen-degradable nitrogen that are optimal for microbial activity. However, a number of studies with cereal straws supplemented with non-protein nitrogen, both *in vitro* and *in vivo*, have shown that fibre digestibility can be enhanced when additional nitrogen from protein sources is present in the rumen (Coombe, 1985; Thomsen, 1985; McAllan and Griffith, 1987; McAllan *et al*, 1988; Stritzler *et al*, 1992). These studies have demonstrated that responses to protein supplementation varied with the kind as well as the amount of protein.

It is generally thought that protein supplements that are slowly degraded in the rumen are more effective in promoting fibrolytic activity of rumen microbes than those rapidly degraded. This is the reason why fish meal has been widely used in these kind of studies. However, little information is available about the levels of fish meal that could be optimal for rumen degradability of ammoniated straws.

The experiments reported here were designed to study the effects of fish-meal levels on the extent of *in vivo* digestion and on the rate and extent of *in situ* digestion of wheat straw treated with urea.

MATERIALS AND METHODS

Straw

Untreated wheat straw (*Triticum aestivum* cv unknown) and urea-treated straw were used. Treatment with urea was performed in a stack as described by Dias-da-Silva and Sundstøl (1986). The amount of urea applied was 55 g kg^{-1} straw. After 60 d the stack was uncovered, the bales were air-dried and stored under cover. At the beginning of the study the average dry matter (DM) content of the straw treated with urea was approximately 87%. The straw was shredded through a bale grinder with a 40-mm screen prior to feeding. The chemical composition of the straw and fish meal (FM) used is given in table I.

Digestibility study

Five male sheep (54 kg live-weight) fitted with 50-mm rumen cannulae were individually housed in metabolism cages. They were randomly

Table I. Chemical composition of straw and fish meal used (%).

	DM	*Ash*	*Nitrogen*	*NDF*
		%DM		
Untreated straw ($n = 5$)*	88.1	6.5	0.50	79.7
Urea-treated straw ($n = 5$)	87.8	6.3	1.75	75.6
Fish meal ($n = 4$)	92.8	23.8	9.65	–

*In parentheses: number of composite samples.

assigned to the following 5 diets in a 5 x 5 latin square design: UTS0 urea-treated straw; UTS25 urea-treated straw + 25 g FM kg^{-1} air-dried straw; UTS50 urea-treated straw + 50 g FM kg^{-1} air-dried straw; UTS100 urea-treated straw + 100 g FM kg^{-1} air-dried straw; and NTS100 untreated straw + 100 g FM kg^{-1} air-dried straw.

The diets were offered in restricted amounts (48 g kg^{-1} live weight$^{0.75}$). In a few cases the diet offered was not eaten in totality and refusals were then recorded. The straw in diets UTS0, UTS25, UTS50 and NTS100 was sprayed with a solution of urea (40g urea l^{-1}) to give a final nitrogen (N) concentration of 25.4 g kg^{-1} as was expected in diet UTS100. All animals were offered 20 g of a mineral–vitamin mixture daily which provided per kg mixture: 140 g Ca, 140 g P, 47 g Na, 25 g Mg, 5 400 mg Zn, 3 600 mg Mn, 720 mg Cu, 680 mg Fe, 45 mg I, 27 mg Co, 2.7 mg Se, 180 000 IU vitamin A, 90 000 IU vitamin D3 and 135 mg vitamin E as declared by the manufacturer. Animal feeding and management were as described by Dias-da-Silva and Sundstøl (1986).

Incubation study

During the last week of the digestibility experiment, ground (4 mm) samples of treated and untreated straw were incubated in the rumen to estimate DM and neutral detergent fibre (NDF) degradation using the nylon-bag technique (Ørskov *et al*, 1980). A sample of approximately 2 g DM was placed in each bag. Dry matter loss (DML) and N disappearance of fish meal were also determined by the same procedure with the sheep fed on diet UTS100. Only 3 sheep on each diet were used for these measurements. The procedure of incubation described by Dias-da-Silva and Guedes (1990) was followed without weights to anchor the bags. Samples were incubated for 3, 9, 18, 24, 36, 48, 60, 72, 96 and 120 h. Each incubation was repeated once per sheep. In total, there were 6 replicates for each straw sample (3 sheep x 2 times x 1 bag).

Rumen liquor was sampled at 0, 1, 2, 4, 6, 8 and 12 h after the diet was given at 08.00 h on 2 non-consecutive days during the incubation period. The liquor was immediately used for pH measurement and then frozen at –20°C for subsequent analysis for ammonia nitrogen.

Chemical analysis

Samples of feedstuffs, refusals and faeces were dried in a forced-air drying oven at 60°C for 48 h and the DM content was calculated. Ground samples (1 mm) were analyzed for ash, kjeldahl nitrogen (Association of Official Analytical Chemists, 1990) and NDF as described by Robertson and van Soest (1981). Nitrogen in urea-treated straw was determined in samples dried at 35°C for 24 h and then ground (1 mm). Rumen liquor was alkalized by NaOH addition and ammonia determined by steam distillation followed by titration.

At the end of the incubation time, the bags were removed and washed in tepid water in a washing machine with a 30 min cycle. Washed bags were dried in a forced-air drying oven at 65°C for 48 h, weighed and the DML recorded. The residues were then extracted with neutral detergent solution to determine the NDF content. In the case of FM, the contents of the bags were removed and analyzed for nitrogen. The 2 residues for each time period of each sheep were pooled for this purpose.

Statistical analysis

The digestibility data were subjected to analysis of variance (Steel and Torrie, 1982) and the significance of differences between treatment means was determined using the least significant difference procedures when the overall *F*-test was significant ($P < 0.05$).

The disappearance of DM from the nylon bags was fitted to the exponential equation $p = a + b(1 - e^{-ct})$ proposed by Ørskov and McDonald (1979) where p = the actual degradation after time t, a = the intercept of the degradation curve at time zero, b = the fraction that will be degraded when given sufficient time for digestion in the rumen, c = the rate constant for the degradation of fraction b and t = the time of incubation. The constants a, b, and c were estimated by an iterative least squares procedure and best-fit values were chosen using the smallest sums of squares after at least 15 iterations.

RESULTS

Supplementation with FM consistently increased organic matter (OM) and NDF digestibility of urea-treated straw (table II). In all cases but one the differences were significant ($P < 0.05$) over unsupplemented straw. There was no effect of level of FM supplementation ($P > 0.05$). At the same level of FM supplementation (10%) the OM digestibility of untreated straw was 9 percentage units lower than that of treated straw ($P < 0.001$).

Table II. Effects of FM supplementation on dry matter (DM) and organic matter (OM) digestibility of total diets and on OM and neutral detergent fibre (NDF) digestibility of wheat straw by sheep.

Diets	*Total diet*		*Straw*	
	DM	*OM*	*OM**	*NDF*
UTS0	48.5^{a}	52.0^{a}	52.0^{b}	57.6^{b}
UTS25	54.1^{b}	57.1^{b}	56.3^{c}	62.9^{c}
UTS50	56.0^{b}	57.9^{bc}	56.4^{c}	64.4^{c}
UTS100	55.8^{b}	59.5^{c}	56.3^{c}	61.8^{bc}
NTS100	47.3^{a}	50.9^{a}	47.2^{a}	50.4^{a}
SEM	0.80	0.55	1.18	1.25

SEM: standard error of means; * OM digestibility of FM was assumed to be 0.90 (INRA,1988); mean values in the same column with different superscripts are significantly different ($P < 0.05$).

The equation used to describe straw degradation incubated in nylon bags fitted the data well since the residual standard deviation was low (average value of 15 curves: DM 2.06 and NDF 2.89). Although the size of the potentially degradable fraction (a + b; tables III and IV) consistently increased due to FM supplementation, the differences over unsupplemented straw were only significant ($P < 0.05$) for treatments UTS50 (DM and NDF) and UTS100 (NDF). The rate of DM degradation of urea-treated straw (table III) was not affected by FM supplementation but the rate of NDF degradation was higher ($P < 0.05$) for treatment UTS100. However, the rate constants for straw supplemented with FM were consistently higher than for urea-treated straw alone. The degradation time-lag of NDF was reduced ($P < 0.05$) by supplementation. The extent of DM and NDF degradation of untreated straw supplemented with FM was lower ($P < 0.001$) than that of urea-treated straw supplemented with the same level of FM. No difference was observed between untreated and treated straw on rate of DM and NDF degradation due to FM supplementation ($P > 0.05$).

Rumen diurnal ammonia concentration and rumen diurnal pH values are presented in figures 1 and 2, respectively. The pattern of variation of ammonia levels in the rumen was not influenced by diet type. The levels increased sharply after feeding, peaking at about 1–2 h post feeding for all diets and then fell progressively reaching minimum values around 80 mg $N\text{-}NH_3$ l^{-1} rumen liquor. Differences between diets were only significant ($P < 0.05$) 6 h after feeding, diets supplemented with 10% FM having ammonia levels higher than a diet supplemented with 5% FM.

Although the pattern of diurnal variation of pH was similar for all diets, variation between diets at each sampling time was much higher than that recorded for $N\text{-}NH_3$. When animals were fed on untreated straw

Table III. Effects of FM supplementation on DM degradation of wheat straw in the rumen of sheep as determined by the method and the model proposed by Ørskov and McDonald (1979).

Treatment	a	b	a + b	c(%h^{-1})	*Incubated straw*
UTS0	8.2^{ab}	60.6^{ab}	68.8^{b}	2.33^{a}	urea treated
UTS25	7.5^{a}	64.3^{bc}	71.8^{abc}	2.53^{a}	urea treated
UTS50	9.1^{bc}	66.9^{c}	76.0^{c}	2.80^{a}	urea treated
UTS100	10.6^{c}	61.3^{b}	71.9^{bc}	2.75^{a}	urea treated
NTS100	7.1^{a}	56.6^{a}	63.7^{a}	2.87^{a}	untreated
SEM	0.41	1.25	1.26	0.15	
N degradability of FM	24.5	66.6	91.1	1.27	

SEM: standard error of means; mean values in the same column with different superscripts are significantly different ($P < 0.05$).

Table IV. Effects of FM supplementation on NDF degradation of wheat straw in the rumen of sheep as determined by the method and the model proposed by Ørskov and McDonald (1979).

Treatment	a	b	a + b	t L(*h*)	c(%h^{-1})	*Incubated straw*
UTS0	-11.6^{a}	80.4^{ab}	68.8^{a}	6.7^{c}	2.33^{a}	urea treated
UTS25	-9.2^{ab}	79.9^{ab}	70.7^{ab}	5.0^{b}	2.47^{a}	urea treated
UTS50	-4.8^{c}	82.6^{b}	77.8^{c}	2.2^{a}	2.73^{ab}	urea treated
UTS100	-5.0^{c}	82.0^{b}	77.0^{bc}	1.8^{a}	3.25^{b}	urea treated
NTS100	-7.1^{bc}	71.7^{a}	64.6^{a}	3.9^{b}	2.77^{ab}	untreated
SEM	0.94	2.81	2.05	0.36	0.20	

SEM: standard error of means; *t L* = lag time, calculated from the equation $p = a + b\,(1 - e^{-ct})$ for $p = 0$; mean values in the same column with different superscripts are significantly different ($P < 0.05$).

supplemented with 10% FM and urea solution, their rumen pH values were always significantly ($P < 0.05$) higher than when they were fed on treated straw supplemented with the same level of FM. Feeding other diets resulted generally in intermediate values (fig 2).

DISCUSSION

The level of enhancement in OM digestibility of urea-treated straw due to FM supplementation observed in this study was *ca* 4.3 percentage units. The response observed was mainly a consequence of a higher fibre digestion (table II). Similar results were obtained by Coombe (1985) in sheep fed a basal diet of oat straw supplemented with protein meals compared with supplementation with urea alone. McAllan and Griffith (1987) and McAllan *et al* (1988) in steers given diets consisting of approximately equal proportions of a concentrate mixture and NaOH-treated straw generally found significant increases in structural

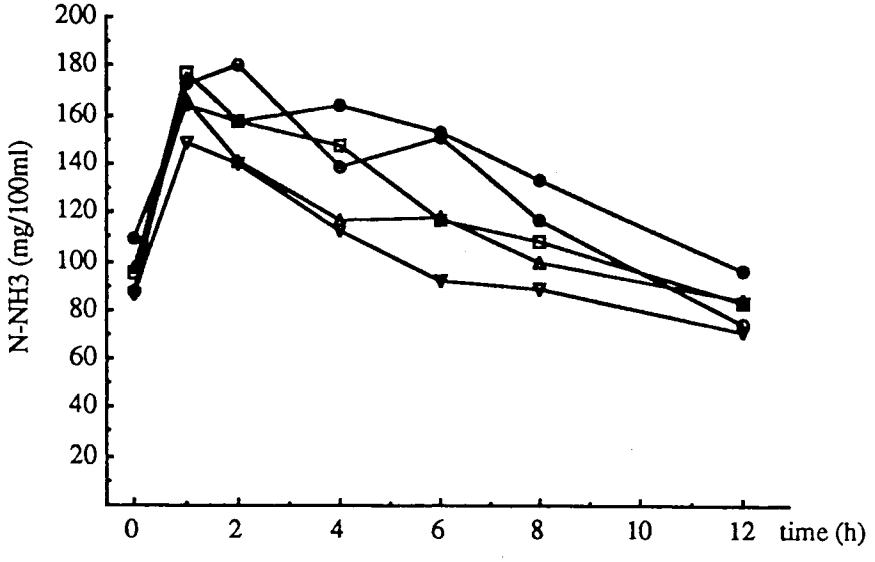

Fig 1. Rumen diurnal N-NH_3 variation. UTS: urea-treated straws; NTS: untreated straw; 0, 25, 50, 100: levels of FM (g kg^{-1} air-dried straw). □ UTS0; Δ UTS25; ∇ UTS50; O UTS100 and ● NTS100.

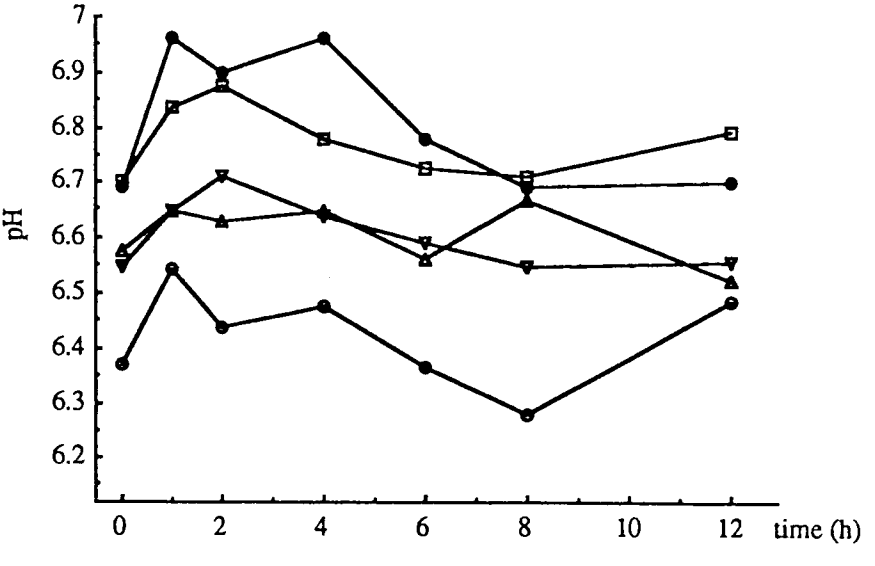

Fig 2. Rumen diurnal pH variation. UTS: urea-treated straw; NTS: untreated straw; 0, 25, 50, 100: levels of FM (g kg^{-1} air-dried straw). □UTS0; Δ UTS25; ∇ UTS50; O UTS100 and ● NTS100.

carbohydrates digestibility when protein supplements were fed instead of urea and casein. In a study with barley straw, Stritzler *et al* (1992) reported a significant increase of 3.1 percentage units in OM digestibility *in sacco* due to supplementation with 6.2% of FM.

On the whole the results of the nylon bag studies confirm those obtained in the digestibility experiment. Although the effect of FM supplementation on rate of digestion of treated straw was not statistically significant in all treatments but one, it is clear that the figures for supplemented straw were consistently higher than for urea-treated straw alone. The finding that the rate of digestion of urea-treated straws is quite similar to the rate of digestion of untreated straw is contrary to recent observations by Ibrahim *et al* (1989). These authors found that urea treatment of 5 cultivars of rice straw increased the potentially degradable fraction, and also that the rate of degradation of the rice straw was significantly enhanced. On the other hand, Ørskov *et al* (1988) did not find any consistent effect on rate of DM degradation of straw from 5 cultivars of barley and wheat due to ammonia treatment. Further information is required before we can generalize on the effect of ammonia-treatment on the rate of digestion of cereal straws.

Since levels of ammonia-nitrogen in the rumen were similar in all the diets and were almost always above the level of 85 mg l^{-1}, which has been suggested as critical for maximum microbial growth (Buttery, 1977), it is likely that the mechanism by which FM supplements supported greater fibre digestion was not by increasing rumen ammonia pool size. It is generally thought that the particular efficacy of FM over other protein sources is due to its low degradability in the rumen. This would allow a slow release of peptides or amino acids, which could be beneficial to the activity of fibrolytic bacteria (Huque and Thomsen, 1984; Thomsen and Johnsen, 1984). However, studies carried out by McAllan and Griffith (1987) with 2 batches of FM designated as having normal degradability (0.33) and low degradability (0.11) did not show any difference in the extent of fibre digestion. Moreover McAllan *et al* (1988) using different protein supplements ranging in degradability from 0.51 to 1.0 observed that these supplements stimulated fibre digestion to different extents, but this was not related to differences in degradability. In our study the degradation constants (Ørskov and McDonald, 1979) of the FM used were 24.5, 66.6 and 0.013 h^{-1} for the rapidly soluble fraction (a), the potentially degradable fraction (b) and the rate constant (c) for the degradation of fraction (b), respectively. Assuming a fractional outflow rate of protein particles of 0.02 h^{-1}, the effective degradability of the FM would be 0.51.

The finding that the degradation time-lag of NDF was consistently lower in the FM-supplemented diets, suggests that FM exerts its effects by stimulating the microbial population intimately associated with the feed particles, which is in agreement with results presented recently by Stritzler *et al* (1992). The decrease in the time-lag can contribute to alleviate physical restrictions on intake of ruminants fed straw-based diets.

This study suggests that a level of FM supplementation as low as 2.5% can be beneficial for stimulating straw degradation in the rumen. However, the higher levels of slowly degradable protein supplements in the rumen have proven to be necessary to increase the intake of straw-based diets (Abidin and Kempton, 1981; Perdok and Leng, 1986). This effect has been attributed to a more favourable balance of absorbed nutrients (Preston and Leng, 1987) rather than to an effect on the rate or extent of fibre degradation in the rumen.

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