

RELATIONSHIPS BETWEEN NUTRIENT COMPOSITION AND FEED DIGESTIBILITY DETERMINED WITH ENZYME AND NYLON BAG (*IN SITU*) TECHNIQUES IN FEED SOURCES

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Summary

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This study was conducted to determine the effect of crude protein, crude fibre and nitrogen free extracts nutrients as predictors of the digestibility of feeds and to investigate the relations between enzyme and *in situ* methods.

The investigation was carried out with 35 concentrate feed samples – oat (5), barley (5), wheat (5), maize (5) soybean meal (5), cottonseed meal (5), sunflower meal (5), and 10 hay samples: alfalfa hay (5) and grass hay (5). All the 45 samples were collected from different regions in Turkey. Enzyme and *in situ* (nylon bag technique) techniques were used to determine the digestibility and degradability of the feeds. By the enzyme technique, the following correlations were estimated: between crude protein levels and organic matter digestibility for forages and protein sources – at $r = -0.99$ ($P < 0.01$) and $r = 0.87$ ($P < 0.05$) respectively, between crude fibre levels and organic matter digestibility – at $r = 0.999$ ($P < 0.01$) and $r = -0.925$ ($P < 0.01$), respectively; In the nylon bag technique there were correlations: between crude protein levels and organic matter degradability for forages, protein sources and grains – $r = 0.990$ ($P < 0.01$), $r = 0.978$ ($P < 0.01$) and $r = -0.088$ ($P > 0.05$), respectively; between crude fibre levels and organic matter degradability for forages, protein sources and grains – at $r = -0.990$ ($P < 0.01$), $r = -0.971$ ($P < 0.01$), and $r = 0.130$ ($P > 0.05$), respectively.

The results of study indicated the strong relationships between feed components and organic matter digestibility/degradability. Besides, high correlations were determined between enzyme and nylon bag techniques.

Key words: digestibility, enzyme technique, nylon bag technique

INTRODUCTION

The evaluation of quality of feeds is important for the prediction of animal performance. *In vivo* measurements are expensive and accompanied by difficulties associated with the procedures. For these reasons, different corresponding techniques have been developed. The energy value of feeds can be determined by enzymatic methods which do not require

rumen fluid. The use of nylon bag *in situ* technique gives an estimation of the kinetics of degradation in the rumen.

The digestibility of feeds is generally related to separate chemical components or fractions of the feed. Observed digestibility and metabolizable energy values will however be lower than the potential maximum due to the escape of potentially

digestible feed from the rumen (Minson, 1976). The digestibility and metabolizable energy value of feedstuffs are influenced by many chemical factors. The loss of potentially digestible material depends on animal species, physical form of the feed, and the ability of the feed and animal supply of elements required by the rumen microflora for maximum digestion. This loss is however relatively small so it is unlikely to invalidate any conclusions regarding the effect of chemical composition on the digestibility and metabolizable energy value of feedstuffs (Minson, 1982). For many purposes quick and cheap laboratory methods of predicting digestibility are required. *In vitro* methods (Tilley & Terry, 1963; Brown *et al.*, 2002) usually predict *in vivo* digestibility with a lower error than any chemical method.

The objective of the present work was to determine the relationship between these two methods and to investigate the effect of crude protein, crude fibre and nitrogen free extract levels in the feeds on their organic matter digestibility/degradability.

MATERIALS AND METHODS

Feed samples and chemical analysis

The investigation was carried out with 35 concentrate feed samples – oat (5), barley (5), wheat (5), maize (5) soybean meal (5), cottonseed meal (5), sunflower meal (5) and 10 hay samples – alfalfa hay (5) and grass hay (5), collected from different regions in Turkey (Table 1).

Feed samples and *in situ* samples were analyzed for dry matter (DM), crude protein (CP), ether extracts (EE) and ash following the procedures described by AOAC (1990). Crude fibre (CF) analysis

was performed as described by Crampton & Maynard (1983).

In vitro enzymatic technique

In this study, the enzymes cellulase (*Trichoderma viride*, Sigma C-9422), hemicellulases (*Aspergillus niger*, Sigma H-2125) amylase (extracted porcine pancreas, Sigma A-3176) and pepsin (Merck, 7190-2000 FIP-U/g) were used. All procedures were carried out according to D'Orleans *et al.*, (1980), Aufrère (1982) and Sauvart *et al.* (1987). The results were expressed as the digestibility of 24 h incubation.

In situ nylon bag technique

Three ruminally fistulated sheep (two years old and with body weight of average 60 kg), maintained on a 900 g good quality alfalfa hay and 300 g concentrate diet according to their requirements were used. Water and trace mineralized salt were available at all times. Dacron bags (7 cm x 11 cm; 40 µm pore size) that contained approximately 5 g of feed samples (2.5 mm screen) were placed in the rumen of each sheep at incubation times 0, 24 and 48 h. After incubation, all nylon bags were washed with tap water until the water was clear. Bags were then dried at 60 °C for 48 h in a forced-air oven, desiccated, weighed, and stored. In the enzyme technique, the incubation time was 24 h. The estimates of organic matter missing from rumen followed the model described by Ørskow *et al.* (1980): $y = a + b [1 - e^{-(c \times t)}]$, y being the percentage which disappears in time t ; a – the soluble or easily degradable fraction; b – the fraction potentially degradable and c – the fractional rate of fraction b . The effective degradability (De) from organic matter was calculated for a speed of ruminal emptying of 0.06 h^{-1} , according to Ørskow *et al.* (1980):

Table 1. Chemical composition of the feeds (DM, %)

Samples	n	Dry matter	Ash	Organic matter	Crude protein	Crude fibre	Ether extracts	Nitrogen free extract
Oat	5	91.33±1.42	3.89±0.74	96.11±0.73	10.39±3.64	11.52±1.75	5.42±1.41	68.78±4.15
Barley	5	90.32±0.68	2.57±0.40	97.43±0.40	11.22±0.99	4.70±1.53	2.88±1.06	78.63±1.33
Maize	5	89.71±0.35	1.73±0.36	98.27±0.39	10.38±2.97	5.67±1.51	3.96±1.02	78.26±0.95
Wheat	5	90.03±0.97	4.49±0.96	95.50±1.10	9.97±1.51	3.20±1.22	2.37±0.94	79.96±1.54
Alfalfa	5	92.71±0.70	10.50±1.61	89.50±1.59	19.97±1.18	23.13±1.22	5.09±1.20	41.31±1.41
Grass Hay	5	92.27±2.46	9.86±1.15	90.14±0.72	12.96±1.99	30.64±0.56	2.93±1.30	43.60±1.20
Soybean meal	5	90.68±1.17	6.28±1.85	93.71±1.18	52.75±2.10	8.13±1.11	4.43±1.23	28.40±2.40
Cottonseed meal	5	94.36±1.41	5.28±1.52	94.72±1.25	30.79±1.32	22.02±1.55	6.34±1.41	35.65±1.22
Sunflower meal	5	93.31±0.89	6.36±0.96	93.64±1.02	24.94±1.20	23.60±0.94	3.20±0.85	41.90±1.63

$De = a + (b \cdot c)/c + k$; k being the fractional outflow rate from the rumen.

Statistical analysis

The computer programme SPSS for Windows (Release 6.0, Spss inc., 1993) was used for the regression procedures. The coefficients of correlation (r) between the estimates were calculated. Data of simple regression procedures were analysed according to the model:

$$Y = B_0 + B_1X$$

where Y was the dependent variable (organic matter digestibility/degradability), B_0 and B_1 were regression coefficients, X was the independent variable: CP, CF, nitrogen free extract (NFE) and nylon bag organic matter digestibility (OMD).

RESULTS

The correlations between crude protein, crude fibre or nitrogen free extract levels and organic matter digestibility/degradability are presented in Table 2.

Correlations between nylon bag technique and enzyme technique for forages, protein sources and grains were manifested by the correlation coefficients $r = -0.990$ ($P < 0.01$), $r = 0.710$ ($P < 0.01$) and $r = 0.710$ ($P < 0.01$) respectively (Table 3).

DISCUSSION

The correlations between crude protein and organic matter digestibility, were positive for protein sources, but not positive for forages in the enzyme technique (Table 2). This may be due to different feed sources and enzymes used in studies which could change the research results (Brown *et al.*, 2002). Stern *et al.* (1977) reported that in the enzyme technique,

enzyme activities in medium might be insufficient compared to those in ruminal medium.

Organic matter degradability was positively correlated with the level of crude protein in the feed dry matter in protein sources (Table 2). Crude protein levels and organic matter degradability were correlating positively for forages using the nylon bag technique (Table 2). Minson (1982) reported that the quantity of digestible crude protein in each 100 g of feed dry matter was linearly related to the quantity of protein in the feed dry matter. These results were in accordance with those of Sullivan (1964). However, other authors (Lindberg *et al.*, 1984; Nocek, 1988; Michalet-Doreau & Cerneau, 1991) reported that dry matter and organic matter degradability may vary due to different microbial activity in the nylon bag.

In this study, a significant relationship ($P < 0.05$) was found between fibre and nitrogen free extract contents and digestibilities of feeds, except for protein sources (enzyme technique) and in grains by both techniques. Fibre is the most common variable used to predict energy content of feeds (Nichols & Dixon, 1984). Lavrencic *et al.* (1998) verified that, with ryegrass and tall fescue hays incubated in the rumen, there was a close association between the chemical composition and the time of maximum degradation rate, suggesting that the structural arrangement and types of linkages within and between the components regulate the fermentation process (Rodrigues *et al.*, 2002).

In this study, a high correlation (negative for forage, positive for protein and grain sources) was found between nylon bag and enzyme techniques (Table 3). These results are similar to those of Xiong & Feng (1989). Lopez *et al.* (1998) reported that the differences in the chemical

Table 2. Relation between crude protein (CP), crude fiber (CF), nitrogen free extract (NFE) contents and organic matter digestibility/degradability (Y, OMD) of feedstuffs determined by both techniques

Feeds	<i>In vitro</i> enzyme technique			Nylon bag (<i>in situ</i>) technique		
	Simple regression	Correlation coefficient	P	Simple regression	Correlation coefficient	P
Forages (n=10)	Y(OMD)= 56.033-6.39 CP	-0.999	P<0.01	Y(OMD)= 53.37+0.17 CP	0.990	P<0.01
	Y(OMD)= 29.484+0.596 CF	0.999	P<0.01	Y(OMD)= 64.61-0.16 CF	-0.990	P<0.01
	Y(OMD)=-37.161+1.947 NFE	0.997	P<0.01	Y(OMD)= 82.90-0.53 NFE	-0.998	P<0.01
Protein sources (n=15)	Y(OMD)= 49.92+0.37 CP	0.870	P<0.05	Y(OMD)= 55.15+0.24 CP	0.978	P<0.01
	Y(OMD)= 71.58-0.62 CF	-0.925	P<0.01	Y(OMD)= 70.99-0.38 CF	-0.971	P<0.01
	Y(OMD)= 82.81-0.64 NFE	-0.702	P>0.05	Y(OMD)=80.71-0.47 NFE	-0.881	P<0.01
Grain feeds (n=20)	Y(OMD)= 73.97-1.31 CP	-0.120	P>0.05	Y(OMD)= 70.03-0.58 CP	-0.088	P>0.05
	Y(OMD)= 58.94+0.14 CF	0.080	P>0.05	Y(OMD)= 62.73+0.14 CF	0.130	P>0.05
	Y(OMD)= 71.48-0.15 NFE	-0.120	P>0.05	Y(OMD)= 73.06-0.12 NFE	-0.160	P>0.05

Table 3. Relations between the enzyme technique for determination of organic matter digestibility (OMD) and nylon bag (*in situ*) technique for determination of organic matter degradability (nylon bag OMD) as analytical methods for feedstuffs

Feeds	Simple regression	Correlation coefficient	P
Forages (n=10)	Y(OMD) = 263.421-3.62 nylon bag OMD	-0.990	P<0.01
Protein sources (n=15)	Y(OMD) = - 43.21+1.617 nylon bag OMD	0.710	P<0.01
Grain feeds (n=20)	Y(OMD) = -43.135+1.62 nylon bag OMD	0.710	P<0.01

composition and in the cell wall structure between grasses and legumes may influence the results obtained with each method, and therefore some methods could be inadequate for some hays depending on their botanical composition. Therefore, it seems that no single method probably will work for all situations and therefore it is necessary to establish the reasons of these discrepancies and the suitability of each procedure in each particular case.

CONCLUSIONS

Significant correlations between feed digestibilities, and feed components (crude protein, crude fiber, nitrogen free extract) with enzyme and nylon bag techniques were found out. It was shown that crude protein was a good predictor for protein sources but not for forage and grain feeds (energy sources); crude fibre and nitrogen free extract were good predictors for forage but not for protein sources and grain feeds, and grain feeds were not effective in the determining of prediction of quality of feeds. In this study, the enzyme and nylon bag techniques were highly correlated.

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