Journal of Animal and Veterinary Advances 6 (1): 68-75, 2007

Effect of Protein Level, Main Protein and non Forage Fiber Source on Digestibility, N Balance and Energy Value of Sheep Rations

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Abstract: Two *in vivo* digestion trials were conducted, by using a latin square 4×4 experimental design with castrated rams, to evaluate the effects of diet's CP level, N degradability and Non-Forage Fiber Source (NFFS) on nutrient digestibility, N balance and energy value of sheep rations. In each trial, rams were fed at maintenance level four isocaloric-isonitrogenous and isofibrous rations, differing in main protein and/or NFFS source. At the first trial mean CP/ME ratio of the diets was 17 g MJ^{-1} ME and at the second trial 13 g MJ^{-1} ME. At both trials, the 1st ration contained Cotton Seed Cake (CSC) and Wheat Bran (WB), the 2nd CSC and Corn Gluten Feed (CGF), the 3rd Corn Gluten Meal (CGM) and WB and the 4th CGM and CGF. Data of both trials were analyzed in common as 2×2×2 factorial experimental design. Low N degradability (CGM) had positive effect on DM, OM, CP, NDF and ADF digestibility of the ration, whilst high N degradability (CSC) resulted in higher EE digestibility. Main protein source (CSC versus CGM) did not affect energy value and N balance of the diets. Those results suggest that an increase in Rumen Undegradable Protein (RUP) content does not negatively affect digestibility nor nutritive value of sheep rations. CGF significantly elevated CF digestibility, in comparison with WB. On the other hand WB increased EE and ADF digestibility of the rations. NFFS did not affect N balance nor energy metabolizability (q) of the diets. Rations having high CP/ME ratio had higher digestibility of CP in comparison with those having low CP/ME ratio; the opposite was true for EE, CF, NDF and ADF digestibilities. CP level×N degradability interaction negatively affected energy value of the rations that had high CP level and high N degradability. Former suggest that when CP content is high then N degradability should be low otherwise ME of the ration is negatively affected. CP digestibility and coefficient q of rations containing WB and having high N degradability (N degradability×lowest suggesting that the combination of CSC and WB affect negatively CP digestibility and energy value of the ration. This could be explained, probably, by a reduced microbial CP synthesis, due to inadequate fermentable metabolizable energy of these rations, or lower RUP digestibility or both.

Key words: Sheep; CP level, N degradability, non-forage fiber, digestibility, energy value

INTRODUCTION

Usually the main factors that affect rations' nutrient digestibility and energy value are separately examined. Thus there are numerous studies dealing with CP level of the ration on nutritive value of ruminant rations (Hatfield *et al*., 1998; Haddad *et al*., 2001). This factor expressed as g kg^{-1} DM of the diet or as a percentage of ME or NE is a useful tool for monogastric animals, but unfortunately on ruminants is useful only as a start point. Another main factor that has been extensively studied is N degradability relaying to its important role on ruminants' productivity (Mabjeesh *et al*., 1998; Landau *et al*., 2005; Milis *et al*., 2005a). Also, N degradability plays the major role on the amount of energy that is wasted for blood NH_3 transformation into urea and environmental pollution with NH_3 both from urine and feacal origin (Miller and Baig, 2002). Interest on nutritional value of Corn Gluten Meal (CGM) has been elevated latest, because it is a feed with high concentration in Rumen Undegradable Protein (RUP) comparable to that of animal by-products (Milis *et al*., 2005b). Cotton Seed Cake (CSC) is a high degradable protein source (Wadhwa *et al*., 1993; Ahmed and Abdalla, 2005) because in the origin feed (whole cotton seed) the soluble protein fraction is three times higher than the fractions of the insoluble proteins (Wadhwa *et al*., 1993; Arieli, 1998). Nevertheless it seems that CSC reduces CP digestibility in comparison with protein sources high in RUP content (Sultan *et al*., 1995; Liamadis *et al*., 2003). Possibly CSC can not be the

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main protein source for high productive lactating ruminants, but, in opposite, this is not valid on fattening ruminants (Weixian *et al*., 1994), suggesting that the level of CSC inclusion in the diet is crucial.

The higher production of ruminants due to the genetic improvement lead to the feeding of extremely high concentrate rations (30: 70 forage to concentrate ratio). This makes necessary to use Non Forage Fiber Sources (NFFS) in order to partly replace forage fiber in purpose to avoid metabolic disorders. It is now known that the fiber content of a diet is not sufficient in expressing its capability to maintain proper rumen function, but the effective fiber content is much more appropriate. NFFS are multipurpose feeds that contribute to the covering of protein, energy and fibrous requirements. Emphasis on nutritive evaluation of NFFS has been focused mostly on lactating cows' diets regarding the estimation of the effective NDF (eNDF) and consequently of substitution rate. Corn Gluten Feed (CGF) is a NFFS high in eNDF content (Armentano and Pereira, 1997; Allen and Grant, 2000; Milis *et al*., 2005a). Also, the soluble and degradable fraction of DM and CP of CGF are very high (Carvalho *et al*., 2005).

There are no found at the literature trials that involve all these factors in the same time. So, the interactions' effect of these main factors on nutrient digestibility, N balance and energy value of ruminant rations are almost unknown, whilst interaction of main factors most of the times affect nutritive value of ruminant rations (Arroquy *et al*., 2004; Milis *et al*., 2005b). Our objectives were to evaluate the main effects of CP level, N degradability (CGM versus CSC) and different NFFS (CGF versus wheat bran; WB) and their interactions' effect on nutrient digestibility, N balance and energy value of sheep rations.

MATERIALS AND METHODS

Experimental design and procedure

Trial 1: An *in vivo* digestibility trial was conducted with four castrated rams, 19-23 months of age and 59-63 kg live body weight, by using four rations in a 4×4 latin square design. The formulation, chemical composition and nutritive value of the diets are presented in Table 1. The ration (A) contained CSC and WB (H-CW), the (B) CSC and CGF (H-CF), the (C) CGM and WB (H-GW) and (D) CGM and CGF (H-GF). The four diets were isocaloric, isonitrogenous and isofibrous and were formulated to meet maintenance energy requirements according to values suggested by Jarrige (1978). Rams were placed into metabolism crates 10 days before trial begins (preliminary period); during this period rams were fed with ration H-CW (control), whilst measuring body weight every 2 days (3 times) in order to adjust the quantity of feed needed for maintenance. Experimental diets were fed in two equal amounts daily at 08:00 and 17:00 at a rate of 0.8 kg d^{-1}, as TMR. Each of the four periods consisted of 14 days adaptation period and 8 days collection period. Any animal at any treatment left no refusals. Water was freely accessible through individual drinkers. Feces and urine were collected and weighted at approximately 08:00 each day, composted by treatment and ram. Samples were stored at a temperature of 2-3 °C until all samples for that collection period had been taken. Rations' samples were taken for laboratory analysis by grab sampling as the feed allowances were being weighted.

Table 1: Formulation, chemical analysis and nutritive value of experimental rations in trial 1

	Rations[1]			
Item	A (H-CW)	B (H-CF)	C (H-GW)	D (H-GF)
Composition (g kg^{-1})				
Corn grain 380	400	380	400	
Alfalfa hay 270	300	320	300	
Wheat straw	-	-	10	60
Cotton seed cake	160	160	-	-
Corn gluten meal 60%	-	-	100	100
Wheat bran	150	-	150	-
Corn gluten feed	-	100	-	100
Salt 10 10	10	10		
Dicalcium phosphate	15	15	15	15
Meriden 001[2]	15	15	15	15
Sum	1000	1000	1000	1000
Chemical analysis				
Dry matter (DM) (g kg^{-1})	872	873	869	871
Organic matter (g kg^{-1} DM)	961	965	965	968
Crude protein (g kg^{-1} DM)	179	185	190	190
Ether extract (g kg^{-1} DM)	41	40	37	37
Crude fiber (g kg^{-1} DM)	160	166	162	168
NDF (g kg^{-1} DM)	344	339	335	316
ADF (g kg^{-1} DM)	184	189	178	177
Gross energy (MJ kg^{-1} DM)	18.3	18.3	18.4	18.4
Nutritive value [3]				
Metabolizable energy (MJ kg^{-1} DM)	10.9	10.9	11.0	11.0
Fermentable metabolizable energy (MJ kg^{-1} DM)	9.8	10.1	10.4	10.7
Metabolizable protein (g kg^{-1} DM)	91	95	113	115
Effective rumen degradable protein (g kg^{-1} DM)	141	150	129	130
Digestible undegradable protein (g kg^{-1} DM)[4]	35	36	53	53

[1]H = high CP content, CW = cotton seed cake+wheat bran, CF = cotton seed cake+corn gluten feed, GW = corn gluten meal+wheat bran, GF = corn gluten meal+corn gluten feed, [2]Meriden 001 = premix of vitamins and trace elements, [3]MAFF (1990), [4]DUP was calculated for a rumen outflow rate of 0.02 h^{-1}

Trial 2: The same procedure was used with differences focused in rams' age (23-27 months), live body weight (64-67 kg) and in CP level of the diets. The CP content of the diets was lower in this trial in purpose to strictly meet energy and protein requirements. The same rams were used in both trials. Rations were fed at a rate of 0.9 kg d^{-1}, corresponded to maintenance requirements in energy. The formulation, chemical composition and nutritive value of the four diets (L-CW; L-CF; L-GW and L-GF for A, B, C and D respectively) are shown in Table 2.

Chemical analysis: Feed and composite fecal samples were ground to pass through a 1mm screen. DM was determined by drying in an oven at 55 °C for 48 h. Ash was determined by ignition in a muffle furnace at 550 °C for 4 h. CP was measured as Kjeldahl N×6.25 (AOAC, 1990). Ether Extract (EE) was measured using the Soxhlet instrument. Gross Energy (GE) was measured by using an adiabatic bomb calorimeter (Parr Instrument Co, 1970). Crude Fiber (CF) was determined by the Weende procedure (AOAC, 1990). NDF and ADF were analyzed as described by Robertson and Van Soest, (1981) with sodium sulfite, decalin and amylase omitted from the procedure; residual ash was not subtracted from either NDF or ADF values). N Free Extract (NFE) was calculated by the difference DM-(Ash+EE+CP+CF). Urinary N content (UN%), was measured by Kjeldahl method (AOAC, 1990). For all methods, measurements were made in triplicate and standards were included in each run of each method. Urinary Energy (UE) was calculated by Streets *et al.* (1964) equation: UE (kcal g^{-1}) = 0.027+0.119 (UN%). Gaseous energy (G), is broadly proportional to the apparent digestibility of the diet (Blaxter and Clapperton, 1965) and, at maintenance level of nutrition, can be predicted by the expression: $100\ G/I = 3.67 + 6.22\ (I-F)/I$, where G is the energy lost as methane; I the intake of energy as the enthalpy of combustion of the diet; and F the fecal energy. Digestible Energy (DE) was calculated by the difference: DE = GE-F and Metabolizable Energy (ME) was calculated by the difference: ME = DE-(UE+G).

Table 2: Formulation, Chemical Analysis and Nutritive Value of experimental rations in trial 2

Item	Rations[1] A (L-CW)	B (L-CF)	C (L-GW)	D (L-GF)
Composition (g kg^{-1})				
Corn grain	490	500	500	500
Alfalfa hay	250	240	230	230
Wheat straw	60	80	100	120
Cotton seed cake	80	80	-	-
Corn gluten meal 60%	-	-	50	50
Wheat bran	80	-	80	-
Corn gluten feed	-	60	-	60
Salt	10	10	10	10
Dicalcium phosphate	15	15	15	15
Meriden 001[2]	15	15	15	15
Sum	1000	1000	1000	1000
Chemical analysis				
Dry matter (DM) (g kg^{-1})	870	872	871	872
Organic matter (g kg^{-1} DM)	959	961	961	963
Crude protein (g kg^{-1} DM)	142	144	143	145
Ether extract (g kg^{-1} DM)	35	35	37	34
Crude fiber (g kg^{-1} DM)	160	163	159	164
NDF (g kg^{-1} DM)	356	343	332	327
ADF (g kg^{-1} DM)	193	190	179	182
Gross energy (MJ kg^{-1} DM)	18.1	18.1	18.2	18.2
3. Nutritive value[3]				
Metabolizable energy (MJ kg^{-1} DM)	10.9	11.0	11.0	11.0
Fermentable metabolizable energy (MJ kg^{-1} DM)	10.0	10.2	10.2	10.4
Metabolizable protein (g kg^{-1} DM)	92	91	103	102
Effective rumen degradable protein (g kg^{-1} DM)	105	108	93	93
Digestible undegradable protein (g kg^{-1} DM)[4]	35	34	43	42

[1]L = low CP content, CW = cotton seed cake+wheat bran, CF = cotton seed cake+corn gluten feed, GW = corn gluten meal+wheat bran, GF = corn gluten meal+corn gluten feed, [2]Meriden 001 = premix of vitamins and trace elements, [3]MAFF (1990), [4]DUP was calculated for a rumen outflow rate of 0.02 h^{-1}

Statistical analysis: Digestibility of nutrients, energy value and N balance coefficients were analyzed statistically using S. Plus (2001). Data of both trials were analyzed in common by the use of a 2×2×2 factorial design with dependent variables being ram, CP level, protein source (degradability) and non-forage fiber source. Period within each trial did not had significant effect and the period between the two trials was not expected to have any impact (confounding between protein level and period) thus was not included in the model. Significance was declared at $p<0.05$. Multi comparisons were conducted by using LSD.

RESULTS

Effect of CP level on nutrient digestibility, N balance and energy value of sheep diets: Results about main factors effects on nutrient digestibility, N balance and energy value are shown in Table 3. Mean digestibilities of EE, CF, NDF and ADF of rations having low CP level were higher ($p<0.05$) compared to those with high CP level. The opposite was found for CP digestibility ($p<0.003$). CP level did not affect DM, OM and NFE digestibility of the rations. Also, N retention measured as (NR/NI, %) and (NR/ND, %) was not affected as though as DE/GE, ME/GE and ME/DE ($p>0.05$).

Effect of N degradability on nutrient digestibility, N balance and energy value of sheep diets: Low N degradability (CGM) positively affected (Table 3) the digestibility of DM, OM, CP, NDF and ADF of the rations ($p<0.05$). CSC in comparison with CGM increased EE ($p = 0.001$) digestibility. These effects are valid irrespective the CP level and the NFFS used.

N degradability did not affect significantly N retention measured as (NR/NI, %; $p = 0.908$) or

Table 3: Mean values of nutrient digestibility, N balance and energy value of sheep rations as affected by protein level of the diet, main protein source (differing in rumen undegradable protein) and non-forage fiber source

	Protein level		Main protein source		Non forage fiber source			^{1}p-value		
Item	H	L	CSC	CGM	WB	CGF	S.E.M.	1	2	3
Dry matter (%)	75.3	74.8	74.2a	75.9b	74.6	75.5	0.426	0.430	0.011	0.143
Organic matter (%)	78.4	79.1	78.0a	79.5b	78.4	79.1	0.435	0.320	0.010	0.183
Crude protein (%)	77.3a	71.0b	70.7a	77.6b	73.1	75.2	1.078	0.003	0.001	0.534
Ether extract (%)	62.9a	87.9b	78.7a	72.1b	78.1a	72.7b	1.225	0.001	0.001	0.004
Crude fiber (%)	34.0a	53.0b	44.2	42.9	40.4a	46.7b	1.344	0.001	0.504	0.003
NFE (N free extract) (%)	87.0	86.9	86.8	87.1	86.6	87.3	0.719	0.866	0.803	0.489
NDF (%)	51.7a	57.4b	52.8a	56.3b	56.1	53.0	0.913	0.001	0.001	0.085
ADF (%)	41.1a	53.4b	44.5a	50.1b	51.2a	43.3b	1.499	0.001	0.001	0.042
Retained N (% intake)	21.3	20.9	21.3	20.9	20.5	21.8	2.765	0.924	0.908	0.745
Retained N (% digested)	27.4	28.8	29.6	26.6	27.3	28.9	3.834	0.804	0.587	0.758
(DE/GE) (%)	76.4	77.1	75.9	77.5	76.3	77.1	0.531	0.694	0.675	0.177
(ME/GE) (%)	60.5	62.8	60.8	62.5	61.1	62.2	0.714	0.113	0.372	0.213
(ME/DE) (%)	79.2	81.5	80.1	80.6	80.1	80.7	0.853	0.225	0.774	0.674

H = high protein level; L = low protein level; CSC = cotton seed cake; CGM= corn gluten meal; WB = wheat bran; CGF= corn gluten feed, ^{ab}Mean values in the same row with different superscript significantly differ, ^{1}Statistical comparison: 1 = high CP level versus low CP level, 2 = high N degradability versus low protein degradability, 3 = wheat bran versus corn gluten

Table 4: Mean values of nutrient digestibility, N balance and energy value of sheep rations as affected by the interactions of protein level of the diet, main protein source (differing in rumen undegradable protein) and non-forage fiber source

	CP level high				CP level low					^{1}p-value			
	N degradability High		N degradability Low		N degradability High		N degradability Low						
Item	WB	CGF	WB	CGF	WB	CGF	WB	CGF	S.E.M	1	2	3	4
Dry matter (%)	73.4	74.8	76.5	76.5	73.9	74.7	74.5	76.1	0.426	0.745	0.658	0.250	0.331
Organic matter (%)	76.5	77.7	79.7	79.6	78.9	78.8	78.6	80.2	0.435	0.878	0.655	0.203	0.364
Crude protein (%)	72.2	73.5	83.5	79.9	62.9	74.1	73.6	73.3	1.078	0.070	0.185	0.051	0.739
Ether extract (%)	66.2^{a}	69.6^{a}	72.3^{a}	43.6^{b}	89.3^{a}	89.5^{a}	84.7^{a}	87.9^{a}	1.225	0.001	0.001	0.060	0.001
Crude fiber (%)	32.6	39.5	25.9	37.9	51.0	53.5	51.9	55.7	1.344	0.408	0.111	0.146	0.626
NFE (N free extract) (%)	85.2	88.9	87.6	86.2	87.0	86.0	86.4	88.0	0.719	0.531	0.694	0.675	0.071
NDF (%)	49.8	47.8	61.5	47.7	56.8	56.6	56.2	59.9	0.913	0.760	0.005	0.008	0.081
ADF (%)	47.2	30.2	53.2	33.9	52.1	48.3	52.2	60.9	1.499	0.009	0.003	0.236	0.934
Retained N (% intake)	23.1	12.8	30.4	18.9	19.5	29.9	8.9	25.4	2.765	0.754	0.005	0.082	0.654
Retained N (% digested)	32.5	17.7	36.2	23.3	27.9	40.3	12.5	34.4	3.834	0.608	0.009	0.173	0.728
(DE/GE) (%)	74.4	75.7	77.7	77.6	76.6	76.9	76.6	78.2	0.531	0.975	0.750	0.109	0.252
(ME/GE) (%)	58.5	59.1	62.3	62.1	62.3	63.3	61.2	64.3	0.714	0.041	0.113	0.042	0.081
(ME/DE) (%)	78.6	78.1	80.2	80.0	81.4	82.3	80.1	82.2	0.853	0.575	0.154	0.084	0.111

WB = wheat bran; CGF = corn gluten feed. ^{a,b} Mean values in the same row with different superscript significantly differ (p = as shown at column 4). ^{1}Statistical comparison: 1 = N degradability x non-forage fiber source interaction, 2 = CP level x non-forage fiber source interaction, 3 = CP level×N degradability interaction, 4 = CP level×N degradability x non forage fiber source interaction.

(NR/ND,%; p = 0.587). The same result was revealed on DE/GE (p = 0.675), ME/GE (p = 0.372) and ME/DE (p = 0.774).

Effect of non-forage fiber source on nutrient digestibility, N balance and energy value of sheep diets: CGF significantly increased the digestibility of CF (p = 0.003) compared to WB (Table 3). On the other hand, WB increased EE and ADF digestibilities. Nevertheless, these differences did not affect the energy value of the diets, expressed as DE/GE (p = 0.177), ME/GE (p = 0.213) and ME/DE (P = 0.674). The results on energy value are consistent with similar coefficients of N retention irrespective of the non-forage fiber source.

Interactions effect on nutrient digestibility, N balance and energy value of sheep diets

N degradability×NFFS interaction: Results about the interactions' effects of the main factors on nutrient digestibility, N balance and energy value are shown in Table 4. CP digestibility of CW rations was the lowest ($p<0.1$). Metabolizability of energy of GF rations was higher compared to CW.

CP level×N degradability interaction: The rations with low CP level and high N degradability had impaired CP digestibility in comparison with rations having high CP level and low N degradability. Moreover, the combination of high CP level and high N degradability negatively affected ME/GE ($p<0.05$).

CP level×NFFS interaction: Rations that had high CP level and contained CGF had impaired EE digestibility as so as NDF and ADF digestibility due to lower cellulose digestibility. N retention was negatively affected in rations that had high CP level and contained CGF and those with low CP level and WB. CP level×NFFS interaction did not affect energy value of the rations.

CP level×N degradability×NFFS interaction: Triplicate interaction was revealed on EE digestibility of the H-GF ration ($p<0.05$).

DISCUSSION

Substitution of CSC by CGM positively affected DM (0.742 versus 0.759), OM (0.780 versus 0.795), CP (0.707 versus 0.776), NDF (0.527 versus 0.563) and ADF (0.445 versus 0.501) digestibilities. The higher digestible undegradable protein/metabolizable protein (DUP/MP) ratio of CGM compared to CSC rations (0.440 versus 0.376) resulted in higher CP digestibility. It is interesting that the 7% difference in DUP/MP ratio resulted in equivalent difference in CP digestibility. This is explicable because the energy was limiting in both CGM and CSC rations (effective rumen degradable protein/fermentable metabolizable energy; ERDP/FME: 10.6 versus 12.6, respectively) whilst FME content was the same, suggesting that the microbial CP (MCP) yield was almost equal. Positive effect of low N degradability on DM, OM and CP digestibility of the whole ration has been reported previously (Mabjeesh *et al*., 1998). The effect of low N degradability on diets' N digestibility is not constant. In most cases positive or no effects have been reported. More important is the RUP digestibility of the protein source, which depends on the nature of the protein source providing the RUP. The higher CP digestibility in our study must have been obtained both by the low N degradability and the high RUP digestibility of CGM. The most reliable result indicating the positive effect of low N degradability on CP digestibility is the observed positive effect of heat treatment of the same feed on total tract CP digestibility (Mabjeesh *et al*., 1998). By this approach the nature of the protein source is isolated. The positive effect of low N degradability on fiber fraction digestibility was not expected. The lower N degradability probably led to synchrony between N and fiber fermentation which improved rations' digestibility (Stern *et al*., 1994; Melaku *et al*., 2005). It has been reported higher fiber degradation in animals supplemented with moderately soluble protein compared to those supplemented with rapidly degradable protein (Jetana *et al*., 1998). Probably, N recycling may be a mechanism that allows RUP to contribute to the ruminal N pool (Wickersham *et al*., 2004). It can be speculated that ruminal ammonia concentrations were sufficient to sustain microbial growth and fiber fermentation for both treatments (ERDP/FME = 9). On the other hand, CSC positively affected ($p = 0.001$) diet's EE digestibility (0.787 versus 0.721). This could be attributed to higher content in EE of CSC compared to CGM. Probably EE digestibility is related to lipid content of the diet (Ahmed and Abdalla, 2005). NFE is not affected by the protein source of the ration (Chan *et al*., 1997).

The DUP content of CGM rations (34.6 versus 47.4 g kg^{-1} DM for CSC and CGM rations, respectively) does not seem to significantly affect ($p = 0.675$) digestibility of energy (0.759 versus 0.775). Also, the differential DUP/MP did not affect NR/ND (0.296 versus 0.266; $p = 0.587$) nor NR/NI (0.213 versus 0.209; $p = 0.908$). The above mentioned results accompanied by similar ($p = 0.372$) metabolizability of energy (0.608 versus 0.625) and similar ($p = 0.774$) ME/DE ratio (0.801 versus 0.806, for rations containing CSC or CGM, respectively) suggests that nutritive value of sheep diets is not negatively affected by the low N degradability.

Similar N balance must be related to normal microbial CP synthesis of rams used at these trials. Nevertheless, the measure of N balance in mature rams, with low capacity for protein accretion, at maintenance level of nutrition, is of limited value for the evaluation of ruminant rations (Milis *et al*., 2005b).

CF digestibility of rations containing CGF (0.467) was significantly higher ($p = 0.003$) compared to those containing WB (0.404). The opposite result ($p<0.05$) was obtained for ADF digestibility (0.512 versus 0.433). It has been reported that WB has positive effect on ADF digestibility of the ration (Melaku *et al*., 2005). CGF is characterized by a high digestibility of the fiber fraction (Jaster *et al*., 1984; Bernard and McNeill, 1991a; Bernard *et al*., 1991b; Allen and Grant, 2000), significantly higher compared to WB (Milis *et al*., 2005b). Jaster *et al*. (1984) have proposed that processing methods used in wet milling of corn may result in delignification making hemicellulose more digestible in the rumen.

The CP digestibility was not different between CGF and WB diets (0.731 versus 0.752). This result is constituent with equal ERDP/FME (11.6) and DUP/MP (0.40) of these diets. The above mentioned result is in line with unaffected DE/GE (0.763 versus 0.771), ME/GE (0.611 versus 0.622) and ME/DE (0.801 versus 0.807, for WB and CGF containing rations, respectively). Results of the present trial indicate that CGF does not negatively affects the nutrients digestibility or the energy value of sheep rations and probably increases the digestibility of the fiber fraction of the diet due to its high eNDF content (Swain and Armentano, 1994; Zhu and Stokes, 1997). This effect is valid ($p<0.05$) regardless of the CP level and the N degradability of the ration (Table 3).

Mean coefficients of digestibility of EE (0.629 versus 0.879), CF (0.340 versus 0.530), NDF (0.517 versus 0.574) and ADF (0.411 versus 0.534) in the rations having low CP level were higher ($p<0.05$) compared to those having high CP level. The diets in trial 2 (low CP level) had higher content in corn grain compared to corresponding rations in trial 1. It was expected that the rations in trial 2 to have lower fiber fraction digestibility due to supposing depression of ruminal pH (Mould and Orskov, 1983). Anyhow it seems that the synchronization between N and fiber degradation is more important than the CP level, concerning the fiber fraction digestibility of the diet. Other researchers have reported that CP level does not affect NDF and ADF digestibility in isoenergetic rations (Haddad *et al*., 2001). Low CP content increases total tract retention time ($p<0.05$) which mostly relays on slower particulate passage rate of the forages (Caton *et al*., 1988; Hatfield *et al*., 1998). This theory could explain the higher fiber fraction digestibility in the rations having low CP level in our study, since the rations had, approximately, 30: 70 forage to concentrate ratio and a slight increase of rumen retention time of the forage fiber would lead to higher fiber fraction digestibility (Wickersham *et al*., 2004). Probably there is an optimum ERDP/FME ratio for the fiber fraction digestibility (Fig. 1). DM digestibility was not affected which is in line with other reports (Hatfield *et al*., 1998). The opposite was found on CP digestibility (Table 3). It has been reported impaired CP digestibility in rations having too low CP content (Haddad *et al*., 2001). Nevertheless, these researchers did not find differences on CP digestibility in rations which CP ranged between 12 and 18%. In our study the difference was revealed in rations differing about 42 g CP kg^{-1} DM (186 versus 144 g CP kg^{-1} DM). MP content of rations in trial 1 was higher (Table 1 and 2) compared to corresponding rations in trial 2 (103 versus 96 g kg^{-1} DM). This probably lead to underestimation of CP digestibility of rations in trial 2 due to the higher proportion of endogenous N excretion compared to corresponding rations in trial 1. Previous theory can explain the half difference on CP digestibility between the rations in trial 1 and 2. The other half is explained by the higher DUP/MP ratio of the rations in trial 1 (0.43 versus 0.39). Nevertheless, it can be speculated that high CP content negatively affected EE digestibility.

CP digestibility of CW rations (0.675; 0.738; 0.785; 0.766 for CW, CF, GW and GF rations, respectively) was the lowest ($p<0.1$) suggesting that the combination of CSC and WB negatively affects ration's CP digestibility. This could be explained, probably, by a reduced microbial CP synthesis, due to inadequate FME of this ration, or lower RUP digestibility or both. Moreover, CGF inclusion in CF rations increased CP digestibility, compared to CW, even though DUP/MP ratio was low, at both rations, due

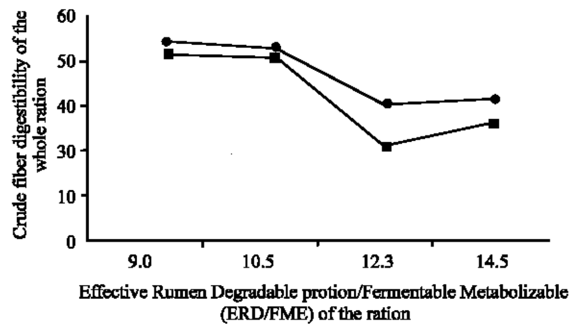

Fig. 1: Crude fiber digestibility of the whole ration depending on non-forage fiber source (■ Wheat bran; ● Corn gluten feed) and ERDP/FME ratio

to high FME content of CGF. Metabolizability of energy of GF rations was higher compared to CW, indicating that when N degradability is high and FME is limiting then there is a metabolic cost associated with formation of urea (Parker *et al*., 1995). At this instance a NFFS high in FME content like CGF should be used.

CP level x NFFS interaction negatively affected NDF and ADF digestibilities of rations with high CP level that contained CGF. This interaction also negatively affected N retention. CGF is a high degradable protein source (Carvalho *et al*., 2005). When ruminally degraded CP is excessive this results in inefficient N use, excess N recycling and possible excretion of N excess to that incorporated into microbial protein because of limitations in ruminal digestible OM (Gadberry *et al*., 2005). The most important interaction was CP level×N degradability. This interaction significantly affected CP digestibility and ME/GE. The rations that had high CP level and low N degradability exceed from all other on CP digestibility. Also, the rations that had low CP level and high N degradability had the lowest CP digestibility. ME/GE and ME/DE were inferior in the rations that had high CP level and high N degradability. It must be noticed that the negative effects on CP digestibility and energy value were observed in the rations that had a DUP/MP ratio below 0.4. This suggests that (when ERDP/FME ratio is equal or higher to suggested value; at this instance = 9) at least 40% of MP needs should be covered by feed origin amino acids. Former result suggests that a minimum in DUP/MP ratio, of the whole ration, is essential to avoid decrease on energy value of sheep rations. The importance of DUP/MP ratio is more evident when CP level of the ration is high.

CONCLUSION

The results of this study have showed that an, approximately, 8-10% increase in diet's DUP/MP ratio did not have undesirable effect on apparent digestibility of nutrients, N retention and energy value of sheep rations, irrespective of the CP level and non-forage fiber source. It seems that a DUP/MP ratio below 0.4 negatively affects CP digestibility and probably energy value of sheep rations. Replacement of WB by CGF may have positive effect on fiber fraction digestibility of the ration, regardless of the CP level and N degradability. When effective fiber of different NFFS is to be compared this has to be done between rations that have equal ERDP/FME ratio (dependent variable). Mean CP digestibility of rations having high CP level are likely to be higher in comparison with rations having low CP level, whilst the opposite seems to be true for EE, CF, NDF and ADF digestibilities. High ERDP/FME content of the diet reduces CP digestibility and energy value of the ration.

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