Effect of the type of non-fibre carbohydrates in grass silage-based diets on *in sacco* ruminal degradability and protein value of lupin (*Lupinus angustifolius* L. cv. Sonet) seeds ground to different particle sizes

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ABSTRACT: The study was conducted to find out the effects of the type of non-fibre carbohydrates (NFC) included in grass silage-based diets on *in sacco* degradability of dry matter (DM), crude protein (CP) and neutral detergent fibre (NDF) of lupin (*Lupinus angustifolius* L. cv. Sonet) seeds ground to different particle sizes. Measurements of degradability were carried out as a 3 × 2 × 3 × 3 experimental design of treatments with carbohydrates included in diets as primary sources of NFC (starch vs. pectin vs. sugars), particle size of ground seeds (1.0 vs. 5.7 mm), period of the trial (1 vs. 2 vs. 3) and cannulated cow (1 vs. 2 vs. 3). The variables of *in sacco* kinetics were calculated according to the equations of Ørskov and McDonald (1979) for degradation data corrected for the particle loss. The protein value of lupin seeds for ruminants was expressed as the amount of protein digested in the small intestine, and it was estimated based on the obtained characteristics of degradability. Under our experimental conditions, the type of NFC included in the grass silage-based diets and the particle size of seeds affected the course and extent of rumen degradation of lupin seed nutrients. Higher values of DM and CP degradation were estimated after 8 and 16 h of incubation ($P < 0.05$). The higher rate of degradation of insoluble but potentially degradable fractions ($P < 0.05$) and higher effective degradability ($P < 0.05$) were found out in the rumen of cows receiving diets containing sucrose compared with cows receiving diets containing starch. In comparison with starch and pectin, sucrose decreased the amount of protein digested in the small intestine ($P < 0.05$). The results suggested that the type of NFC determined ruminal microbial activity. An enlargement of the lupin seed particle size from 1.0 to 5.7 mm decreased the rumen degradability of lupin seed nutrients ($P < 0.05$) and increased the amount of protein digested in the small intestine ($P < 0.05$). The results indicated that feeding standards should consider the influence of the grinding level of lupin seeds in order to assess their nutritional value.

Keywords: cattle; rumen degradability; grass silage; non-fibre carbohydrates; lupin seeds; particle size

The synchronized provision of dietary nitrogenous and energetic compounds to the rumen at the appropriate time and in appropriate amounts increases the efficiency of microbial mass synthesis (Hall and Weimer, 2007) and can be used to minimize pollution emissions (Reynolds and Kristensen, 2008). Grass silage is widely used in dairy cow feeding. Diets based on grass silage are characterized by a high level of rapidly degraded crude protein in the rumen and for the successful nutritional synchrony, the supply of corresponding fermented carbohydrates is of great importance for microbial

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metabolism (Givens and Rulquin, 2004; Hall and Huntington, 2008). It is likely that the nutritional synchrony also affects feedstuff degradation in the rumen, but the information describing these relationships is limited. Results from an *in sacco* study showed that the rumen degradability of barley grain nutrients was influenced by the type of non-fibre carbohydrates included in grass silage-based diets (Niwińska, 2009). There is no information about the effects of plant protein supplements like lupin seeds on *in sacco* degradability, although the ruminal degradation of feed protein is an essential factor for assessing the feed protein value according to feed evaluation systems for ruminants (Jarrige, 1989; NRC, 2001).

In recent years, the interest in legume seeds as a source of protein in ruminant feeding has increased mainly due to the positive ecological functions in sustainable crop rotations of these plants (Veneklaas et al., 2003; Jensen et al., 2004). Among the legume seeds, lupin has the highest protein content of 295–482 g/kg dry matter (Sujak et al., 2006). Coarsely ground lupin seeds can successfully replace soybean meal in high-producing dairy cows (Froidmont and Bartiaux-Thill, 2004). However, feeding standards should consider the influence of the grinding level of lupin seeds on the accurate assessment of their nutritional value (Froidmont et al., 2008).

Taking into consideration the promotion of sustainable crop rotations with lupins (Jensen et al., 2004), the objective of this study was to investigate the effect of the type of non-fibre carbohydrates (starch, pectin or sucrose) added to silage grass-based diets on differences in the rumen degradability of nutrients from narrow-leaved lupin seeds ground to different particle sizes and on the amount of protein digested in the small intestine.

Table 1. The chemical composition of lupin seeds, barley grain, unmolassed dried beet pulp, molasses and grass silage

Item	Lupin seeds	Barley grain	Unmolassed sugar beet pulp	Molasses	Grass silage
Dry matter[1] (g/kg fresh weight)	891	880	884	770	264
Chemical composition (g/kg DM)					
Crude protein	319	114	98	115	117
Ether extract	49	20	7	20	33
NDF	273	218	412	1	605
ADF	219	62	260	1	387
ADL	10	12	28		42
Ash	35	24	34	123	84
Starch		510	8		
Pectin[2]		57	290		
Sugars			151	629	
Sucrose			76[3]	220[4]	
Lactic acid					48
Acetic acid					42
Propionic acid					1
Butyric acid					3
pH					4.1
Nonprotein N (g/kg total N)					520

Calculated according to the data presented in: [1]chemical composition from laboratory analysis (n = 3), [2] feed tables (NRC, 2001), [3]Zhu et al. (1993), [4]Teclu et al. (2009)

MATERIAL AND METHODS

Samples

The seeds of narrow-leaved lupin *(Lupinus angustifolius* L.) cv. Sonet originating from the company Poznan Plant Breeding Ltd. based in Tulce near Poznan were used in the experiment. The lupin was cultivated according to VCU methodology (1998).

Experimental design

The ruminal *in sacco* degradability measurements of lupin seed dry matter (DM), crude protein (CP) and neutral detergent fibre (NDF) were carried out as a 3 × 2 × 3 × 3 experimental design of treatments with carbohydrates included in the concentrate portion of grass silage-based diets (starch in STA diet vs. pectin in PEC diet vs. sucrose in SUC diet), particle size of ground seeds (1.0 vs. 5.7 mm), cannulated cow (1 vs. 2 vs. 3) and period of the trial (1 vs. 2 vs. 3).

Animals and feeding

The experimental animals were treated according to guidelines approved by the Polish National Ethics Committee for the Use of Animals in Biomedical Experiments.

Three non-lactating Polish Holstein-Friesian cows (700 ± 27 kg of body weight) fitted with a 10 cm ruminal cannula (Ankom Technology, Fairport, USA) were used in the experiment. Experimental diets were composed of grass silage (55% of diet dry matter) and concentrate. The grass silage was prepared from the first cut of mixed grass varieties (*Festuca pratensis* × *Poa pratensis* × *Phleum pratense* × *Dactylis glomerata*) harvested on June 9–12, 2006 at the full-head stage of maturity. Silage was prepared in a silo with Labacsil® Bakterie and Labacsil® Acid additives (Sano Co., Sękowo, Poland). Concentrate portions were formulated to obtain starch from barley (STA diet), pectin from unmolassed dried beet pulp (PEC diet) and sucrose from molasses (SUC diet) as the primary source of highly digestible carbohydrates. The chemical composition of lupin seeds, barley, unmolassed beet pulp, molasses and grass silage is shown in Table 1. The nutritional value of diets and protein and energy requirements of cannulated cows were determined according to the French feeding system for ruminants (Jarrige, 1989). The ingredient composition and the nutrient content of experimental diets are presented in Table 2. In restricted daily ration the cows received an average of 5.8 kg of DM, 500 g of protein digested in the small intestine originating from the rumen-undegraded dietary protein and protein synthesized from the rumen-degraded dietary protein (PDI) and 5.5 units of net energy for milk production (UFL). The feed was offered in two meals at 08:00 and 16:00 h (in equal portions) and the concentrates were offered on top of grass silage. Cows were housed in individual tie stalls and had free access to water and block of salt throughout the trial. The experiment was conducted from October to December 2006 in a 12-week trial with three *in sacco* degradability study periods. Each period lasted 28 days: days 1 to 17 were for the adaptation of cows to dietary treatments, and days 18 to 28 were used for *in sacco* degradability studies.

Processing method

Samples of lupin seeds were grounded using a laboratory cutting mill (Pulverisette 15, Fritsch GmbH, Oberstein, Germany) with a pore size of 1.0 and 5.7 mm.

In sacco degradability studies

The rumen degradability of lupin seeds was determined by the polyester bag technique as described by Michalet-Doreau et al. (1987). The ratio of the sample size to the bag surface area was 20 mg/cm^2. The polyester bags with a pore size of 50 ± 15 µm (Ankom Technology, Fairport, USA), containing 3 g of an air-dried seed sample were incubated in the rumen for 2, 4, 8, 16, 24, and 48 h in 2, 2, 3, 3, 4, and 6 replicates, respectively. To obtain the 0 h-value bags with a sample of each particle size (in 2 replications) were twice washed in a washing machine (Polar PFL 805, Warsaw, Poland) for 10 min in cold water; this washing procedure was also applied to the bags after the rumen incubation. Bags after washing were dried at 60°C for 48 h and weighed. The residues were ground to pass a 1 mm screen and were used for chemical analysis.

Table 2. The ingredient and nutrient composition of experimental diets

Item	Diet		
	STA	PEC	SUC
Ingredients (g/kg DM)			
Grass silage	546	545	555
Barley grain	248	45	73
Wheat grain	93		
Unmolassed sugar beet pulp	23	272	69
Wheat bran	22	22	45
Molasses		16	141
Rapeseed cake (cold pressed)	23	47	71
Soybean meal	23	33	24
Vitamin-mineral mix	15	15	15
Sodium bicarbonate	6	6	6
Chemical composition (g/kg DM)			
CP	145	147	154
NDF	313	364	305
ADF	177	230	190
ADL	150	155	155
CP-rumen degraded	113	111	126
NFC[1]	335	278	309
Starch	181	30	45
Pectin	33	89	35
Sugars	35	66	116
Sucrose	2	23	32
Nutritional value			
PDIN[2] (g/kg DM)	81	81	85
PDIE[3] (g/kg DM)	91	94	89
Net energy for milk production (Mcal/kg DM)	1.6	1.6	1.6
Fermentable Organic Matter (g/kg DM)	652	625	614
N-rumen degraded protein (g/kg Fermentable Organic Matter)	26	26	30

[1]estimated according to Cozzi at al. (2002)
[2]digestible protein in the small intestine supplied by microbial protein from rumen-degradable protein (Jarrige, 1989)
[3]digestible protein in the small intestine supplied by microbial protein from rumen-fermented organic matter (Jarrige, 1989)

Water solubility

The water solubility (WS) of DM and CP was estimated in the filter test according to the procedure described by Ramos-Morales et al. (2010).

Chemical analysis

DM was determined gravimetrically after drying for 4 h at 103°C (ISO 6496:1999) and ash was determined after combustion for 3 h at 550°C (ISO

5984:2002). CP was determined using the Kjeldahl method (ISO 5983-1:2005). Crude fat was determined gravimetrically after 6 h extraction with petroleum ether (ISO 6492:1999). NDF (with amylase treatment), acid detergent fibre (ADF) and acid detergent lignin (ADL) excluding residual ash were determined according to the methods of Van Soest et al. (1991). Starch was determined using a polarimetric method (ISO 6493:2000) and sugar according to the Luff Schoorl EEC official method (79/786/EEC, Annex II).

Calculations

The composition of the experimental diet was determined according to the Jarrige (1989) recommendation using INRAtion software (ver. 3.3, INRA, 1988–2004). Dry matter intake (DMI) was established using the following equation (NRC, 2001):

$$\text{DMI (kg)} = NE_M \times (NE_D \text{ concentration of diet})^{-1}$$

where:

NE_M = net energy of lactation required for cow maintenance (Mcal)

NE_D = concentration of net energy of lactation in the diet (Mcal/kg DM)

The content of non-fibre carbohydrates (NFC) was calculated according to Cozzi et al. (2002) as 1000 – (CP + fat + ash + NDF) and expressed as g/kg DM. The content of pectin was calculated according to data in feed tables (NRC, 2001).

The particle loss (PL) was calculated as a difference between the fraction lost through the pores during washing (0 h-value) and the WS fraction. The individual degradability values were corrected for particle loss during washing according to the equation of Weisbjerg et al. (1990):

$$cPt = Mdt - PL - [Mdt - (PL + WS)]/[1 - (PL + WS)]$$

where:

cPt = corrected degradation at time t

Mdt = measured degradation at time t

PL = particle loss

WS = water solubility

The variables of *in sacco* kinetics of DM, CP and NDF were calculated by fitting the corrected degradation data to the model of Ørskov and McDonald (1979):

$$P = a + b\,[1 - e^{-(c \times t)}]$$

where:

t = time of incubation

P = degradation after t hours

a = rapidly soluble fraction

b = potentially degradable fraction

c = rate constant for degradation of b

e = base of natural logarithm

The corrected effective degradability (ED) was calculated using a rumen passage rate of 0.06/h (Vérité et al., 1987) according to the formula of Ørskov and McDonald (1979):

$$ED = a + (b \times c)/c + k$$

where:

ED = effective degradability corrected for the particle loss

k = outflow rate

a, b, c = as described above

The nutritional value of seed protein in the PDI system was expressed as protein digested in the small intestine depending on the amount of N from the seeds according to the following equations (Jarrige, 1989):

$$PDIN = PDIA + PDIMN$$

$$PDIA = 1.11 \times CP \times (1 - deg) \times dsi$$

$$PDIMN = 0.64 \times CP \times (deg - 0.1)$$

where:

PDIN = potential protein digested in the small intestine depending on the amount of N from the seeds, when energy is available for microbial synthesis in the rumen

PDIA = rumen-undegraded protein of seed origin digested in the small intestine

deg = the coefficient of rumen protein degradability, which was estimated using the ED determined in the experiment ($deg = ED \times 10^{-2}$)

dsi = the coefficient of intestinal digestion of rumen-undegraded protein (*dsi* = 0.93) estimated for *Lupinus angustifolius* seeds (Niwińska, 2001)

PDIMN = protein of microbial origin truly digested in the small intestine and potentially synthesized in the rumen from seed protein, when energy and other nutrients are not limiting. The nutritional value of seed protein for ruminants is expressed in g/kg of seed crude protein

Statistical analysis

The Mixed Procedure of SAS (SAS Institute, Inc., Cary, USA) with REML estimation method was used to analyse degradability data with cow as a random variable. Least-squares means were reported. Differences were declared significant at $P < 0.05$. The variables of *in sacco* kinetics were estimated using the Non-Linear Model (NLIN) of SAS Procedure with Marquardt's method. The statistical estimation did not confirm the effects of period and cannulated cow and the results are not presented. The differences in variables of *in sacco* kinetics and in the nutritional value of lupin seed protein characteristics were tested by *t*-tests and were declared significant at $P < 0.05$.

RESULTS

Results of the chemical analysis of lupin seeds, barley grain, unmolassed dried beet pulp, molasses

Table 3. Effects of diet composition and particle size of seeds on measured degradation of lupin dry matter, crude protein and neutral detergent fibre during incubation periods

	Incubation periods (h)	Diet[1]				Particle size (mm)			Mean	SEM
		STA	PEC	SUC	effect[2]	1.0	5.7	effect		
Dry matter degradation (%)	2	37	35	36	ns	45	27	*	36	12
	4	40	40	43	ns	51	31	*	41	8
	8	52[b]	58[a]	57[a]	*	65	46	*	55	13
	16	76[b]	78[a]	77[a]	*	82	72	*	77	19
	24	85	86	82	ns	90	79	*	84	27
	48	97	95	96	ns	98	94	*	96	12
Crude protein degradation (%)	2	49[b]	49[b]	50[a]	*	63	36	*	50	6
	4	49[b]	51[b]	55[a]	*	66	38	*	52	5
	8	65[b]	66[b]	69[a]	*	85	48	*	66	7
	16	76	77	77	ns	92	61	*	77	8
	24	92	92	92	ns	96	87	*	92	5
	48	99	99	99	ns	99	99	ns	99	7
Neutral detergent fibre degradation (%)	2	21	23	25	ns	31	15	*	23	19
	4	29	32	32	ns	34	28	*	31	14
	8	36[b]	41[a]	40[a]	*	44	33	*	39	33
	16	42[b]	49[a]	45[ab]	*	42	49	*	46	68
	24	77[a]	72[b]	73[b]	*	79	68	*	74	134
	48	93	89	92	ns	95	88	*	91	60

[1]diets: STA = containing starch, PEC = pectin and SUC = sucrose

SEM = standard error of mean

[2]significant difference found for * at $P < 0.05$, ns = non-significant at $P > 0.05$

[a,b,c]means in a row within diet with different superscript letters are significantly different

and grass silage (Table 1) were comparable to those presented in the Tables of Chemical Composition and Nutritive Value of Feedstuffs (Bach, 2005). The estimated CP content in lupin seeds was similar to that reported by Sujak et al. (2006).

The experimental diets fed to cannulated cows were characterized by similar contents of CP (148 ± 5 g), rumen degraded CP (116 ± 8 g) and net energy for milk production (1.6 ± 0.1 Mcal) in kg DM (Table 2). The diets contained a similar level of non-fibre carbohydrates in kg DM (307 ± 28 g), which varied in their composition: STA diet contained the highest level of starch (54% on average), PEC diet contained the highest amount of pectin (32%) and SUC diet contained the highest level of sugars (38%). Sugars in the SUC diet were mainly composed of sucrose (30% of sugars).

The degradation of seed DM, CP and NDF during incubation in the rumen was affected by the composition of the diet given to cannulated cows and by the particle size of ground lupin seeds (Table 3). Higher ($P < 0.05$) degradation of DM and NDF after 8 and 16 h of incubation and lower ($P < 0.05$) degradation of NDF after 24 h of incubation were found out for

Table 4. The effect of diet composition and the particle size of lupin seeds on the variables of *in sacco* kinetics corrected for particle loss of seed dry matter, crude protein and neutral detergent fibre

Item	Diet[1]				Particle size (mm)			Mean	SEM
	STA	PEC	SUC	effect[2]	1.0	5.7	effect		
Dry matter									
a	26	25	26	ns	32	19	*	26	0.51
b	63	65	63	ns	55	73	*	64	0.65
c	0.085[b]	0.077[b]	0.094[a]	*	0.084	0.088	ns	0.085	0.002
ED	54[c]	55[b]	57[a]	*	58	52	*	55	0.16
Water solubility (g/kg DM)					221	112	*	167	16.5
Particle loss (g/kg DM)					141	95	*	118	10.5
Crude protein									
a	40	39	42	ns	50	31	*	40	0.99
b	55	54	54	ns	36	73	*	54	1.33
c	0.137[b]	0.152[a]	0.167[a]	*	0.172	0.132	*	0.152	0.008
ED	63[b]	63[b]	66[a]	*	74	54	*	64	0.31
Water solubility (g/kg CP)					265	145	*	205	19.0
Particle loss (g/kg CP)					126	86	*	106	9.4
Neutral detergent fibre									
a	24	25	25	ns	28	21	*	25	0.44
b	70[a]	63[b]	67[ab]	*	62	72	*	67	0.74
c	0.121[a]	0.106[b]	0.110[b]	*	0.124	0.099	*	0.112	0.005
ED	43	44	44	ns	45	43	*	44	0.26
Particle loss (g/kg NDF)					121	84	*	103	8.5

[1]diets: STA = starch, PEC = pectin and SUC = sucrose

SEM = standard error of mean

[2]Significant difference found for * at $P < 0.05$, ns = non-significant at $P > 0.05$

a = rapidly degradable fraction corrected for particle loss (%),

b = insoluble but potentially degradable fraction corrected for particle loss (%),

c = rate of degradation of b fraction (h^{-1})

ED = effective degradability corrected for particle loss (%)

[a,b,c]means in a row within diet with different superscript letters are significantly different

lupin seeds incubated in the rumen of cows receiving SUC diets compared with those incubated in the rumen of cows receiving STA diet. Higher ($P < 0.05$) degradation of CP in the rumen of cows receiving SUC diet in comparison with that in the rumen of cows receiving STA and PEC diets was estimated after 2, 4, and 8 h of incubation. Higher ($P < 0.05$) degradation of DM, CP and NDF was determined for lupin seeds ground to a particle size of 1.0 mm in comparison with that found out for seeds ground to a particle size of 5.7 mm for each period of incubation, except for CP after 48 h of incubation, when the estimated degradation was similar irrespective of the particle size (99% on average).

The variables of *in sacco* kinetics of DM, CP and NDF of lupin seeds, corrected for the particle loss, were affected by the composition of the diet administered to cannulated cows and by the particle size of ground lupin seeds (Table 4). The highest value of ED estimated for DM degradation was found out in the rumen of cows receiving SUC diet, the intermediate value in the rumen of cows fed PEC diet, and the lowest value in the rumen of cows fed STA diet ($P < 0.05$). The degradation of CP in the rumen of cows fed SUC diet was characterized by the higher value of variable c ($P < 0.05$) and by the higher value of ED ($P < 0.05$) compared with the values determined for ruminal degradation in cows receiving PEC and STA diets. Similar values of ED were determined for degradation of lupin seed CP in the rumen of cows fed PEC and STA diets ($P > 0.05$). The degradation of NDF in the rumen of cows receiving STA diet was characterized by a higher value of variables b and c ($P < 0.05$) in comparison with that estimated in the rumen of cows fed PEC and SUC diets ($P > 0.05$). The content of the rapidly degradable fraction a of DM, CP, and NDF was not influenced by diet composition ($P > 0.05$). Higher values of variable b and lower values of variable a and ED were found out for seeds ground to a particle size of 5.7 mm in comparison with seeds ground to a particle size of 1.0 mm ($P < 0.05$).

The nutritional value of lupin seed crude protein was differentiated by the diet composition and by the particle size of ground lupin seeds (Table 5). The crude protein of lupin seeds degraded in the rumen of cows receiving SUC diet was characterized by higher ($P < 0.05$) content of RDP and PDIMN and lower ($P < 0.05$) content of RUP, PDIA and PDIN compared with that estimated for crude protein digested in the rumen of cows receiving STA and PEC diets. Similar relationships were found out when comparing the degradation of crude protein of lupin seeds ground to a particle size of 1.0 mm

Table 5. The effect of diet composition and the particle size on the protein fraction seeds-N-dependant digested in the alimentary tract of ruminants (in g/kg CP)

Item	Diet[1]				Particle size (mm)			Mean	SEM
	STA	PEC	SUC	effect[2]	1.0	5.7	effect		
RDP	630[b]	630[b]	660[a]	*	740	540	*	640	3.10
PDIMN	339[b]	339[b]	358[a]	*	410	282	*	346	0.05
RUP	370[a]	370[a]	340[b]	*	260	475	*	360	30.40
PDIA	382[a]	382[a]	351[b]	*	268	485	*	372	28.20
PDIN	721[a]	721[a]	709[b]	*	678	756	*	717	21.20

[1]diets: STA = starch, PEC = pectin and SUC = sucrose

SEM = standard error of mean

[2]Significant difference found for *at $P < 0.05$, ns = non-significant at $P > 0.05$

RDP = rumen-degraded protein

PDIMN = protein truly digested in the small intestine of microbial origin potentially synthesized from seed rumen degraded protein, when energy and other nutrients are not limiting

RUP = rumen-undegraded protein

PDIA = rumen-undegraded protein of seed origin truly digested in the small intestine

PDIN = PDIA + PDIMN, protein truly digested in the small intestine seeds-N-dependant when energy is available for microbial synthesis in the rumen

[a,b,c]means in a row within diet with different superscript letters are significantly different

to the degradation of crude protein of lupin seeds ground to a particle size of 5.7 mm.

DISCUSSION

The results of the study indicated that the type of non-fibre carbohydrates included in grass silage-based diets affected the course and extent of rumen degradation of lupin seed nutrients. Lupin seed DM and CP are more susceptible to the degradation process in the rumen environment of cows receiving diets containing sucrose compared with susceptibility in the rumen of cows receiving diets containing starch, as indicated by higher degradation values estimated after 8 h and 16 h of incubation ($P < 0.05$), higher rate of degradation of insoluble but potentially degradable fractions ($P < 0.05$), and higher effective degradability ($P < 0.05$) corrected for the particle loss. The results obtained as well as the findings of Hall et al. (2010) indicated that the type of non-fibre carbohydrates can interact with the soluble dietary N fraction to give different directions of response for ruminal fermentation. The crude protein of experimental diets contained 34% of the non-protein N fraction derived from grass silage. The soluble non-protein N fraction of grass silage is characterized by a high rate of degradation which is about 250%/h (Volden et al., 2002). The degradation of lupin seed DM and CP seems to reflects the utilization of nitrogen substances regarding the energy availability for microbial metabolism from non-fibre carbohydrates and is attributed to the faster degradation of sugars compared to starch in early fermentation hours. The rate of disappearance for sucrose was estimated to be 1200–1404%/h (Weisbjerg et al., 1998) and the starch of barley degrades in the rumen at a rate from 14 to 24%/h (Offner et al., 2003). As shown by Ribeiro et al. (2005) in continuous culture fermenters, the synchronism between the availability of energy from sucrose and soluble protein from lucerne hay caused a change in the bacterial activity. The authors gathered that the increase of sucrose from 0% to 8% improved the efficiency of microbial protein synthesis from 36.7 to 56.4 g per kg of organic matter truly digested. Results of *in vivo* experiments confirm this dependence. The replacement of maize starch with sucrose in the silage-based diet contributed to a reduction in the ruminal concentration of ammonia and excretion of urinary urea-N and urinary total N (Broderick et al., 2008). This dependence is in line with the results reported by Poláková et al. (2010), who found out that the increased supplementation of sugars from 25% to 44% of NFC in place of starch in rations administered to high-yielding dairy cows decreased the urea concentration in blood serum from 4.7 to 3.9 mmol/l as a result of better utilization of nitrogen substances in the rumen. It seems that the more intensive degradation of lupin seed DM and CP takes place in the rumen conditions resulting from the better synchronization of energy availability for microbial metabolism, which is associated with the introduction of dietary sucrose in place of starch.

Our results indicated that the diet composition affected the course of degradation of lupin seed NDF. The lower content of the insoluble but potentially degradable fraction and the higher degradation rate of this fraction ($P < 0.05$) were characterized by degradation of seed NDF during incubation in the rumen of cows receiving diets with sucrose or pectin compared with that found out during incubation in the rumen of cows receiving diets with starch. Similarly, a reduction of susceptibility to digestion of lucerne hay NDF as the effect of dietary sucrose supplementation was observed in continuous culture fermenters by Ribeiro et al. (2005). The influence of the type of non-fibre carbohydrates included in the rations on the course of NDF degradation was reported by Alamouti et al. (2009), who found out that the degradation of lucerne NDF in the early hours post feeding in sheep was affected by a significant increase in rumen pH as a result of partial replacement of pectin by starch in the diets.

The type of carbohydrates included in grass silage-based diets influenced the amount and fractions of protein digested in the small intestine depending on the amount of N from the seeds. The estimated higher amount of protein digested in the small intestine was due to lower susceptibility to degradation in the rumen of cows receiving rations with starch or pectin compared with susceptibility to degradation in the rumen of cows receiving the ration with sucrose. The differences in the susceptibility to degradation have largely been generated from the synchronization of N availability from the soluble non-protein N fraction of grass silage and energy availability from starch or pectin compared with energy availability from sucrose for the rumen microbial ecosystem.

The results demonstrated that the increase of lupin seed particle size from 1.0 mm to 5.7 mm slowed down the rumen degradability of lupin seed nutrients and increased the amount of protein truly digested in the small intestine. The reduction in susceptibility to degradation can be explained by the lower area/mass ratio, unfavourable nutrient solubilization and microbial access to substrates. Our results are in line with the results of Kibelolaud et al. (1991), who found that the intestinally digestible protein of lupin seeds can be significantly increased by lowering the intensity of mechanical treatment applied to the seeds. Similarly, recent research by Froidmont et al. (2008) indicated that the increase of particle size from 0.5 to 2.0–4.2 mm improved the content of digestible protein in the small intestine of bulls by more than 40%. The results of our study and the results cited above indicate that the extent of mechanical processing of lupin seeds appears to be the main factor determining the ruminal degradation of nutrients and the nutritional values of lupin seed protein.

CONCLUSION

Results from the *in sacco* study indicated that both the type of non-fibre carbohydrates included in grass silage-based diets and the physical properties of seeds influenced the rumen degradability of lupin seed nutrients and the amounts of protein digested in the small intestine depending on the amount of N from the seeds.

Replacing starch with sucrose in the diets results in higher degradation of lupin seed DM and CP after 8 h and 16 h of incubation, higher rate of degradation of insoluble but potentially degradable fractions, and the higher effective degradability corrected for the particle loss estimated for those nutrients also results in the lower content of protein truly digested in the small intestine. It seems that the energy availability from carbohydrates affected the degradation of lupin seed nutrients as a consequence of the increased ruminal microbial activity.

Enlarging the particle size from 1.0 to 5.7 mm decreases the ruminal degradation of lupin seed nutrients and increases the amount of protein digested in the small intestine. The results show that feeding standards should consider the influence of the grinding level of lupin seeds in order to assess their nutritional value accurately.

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