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Research Article

THE DETERMINATION OF METABOLIZABLE PROTEIN OF UNTREATED AND TREATED CANOLA MEAL WITH UREA AND MICROWAVE USING NYLON BAGS TECHNIQUE

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ABSTRACT

This study was carried out to determine the nutritive value of canola meal treated and untreated with 0.5% urea and microwave using nylon bags technique in Gizel sheep. Two fistulaed Gizel sheep with average BW 45±2 kg were used in a complete randomized design. The treatments were as following; A: canola meal, B: canola meal treated with 0.5% urea, C: canola meal treated with microwave. The ruminal dry matter and crude protein disappearance were measured at 0, 2, 4, 6, 8, 12, 16, 36 and 48 h. At 48 hours of incubation, ruminal degradability of canola meal treated with microwave was more than other treatments and this was indicative of the significant effect of processing in the increase in protein degradation and metabolizable protein in feed. Canola meal treated with microwave (167.16 g/kg DM) and canola meal (137.31 g/kg DM) had the highest and lowest effective ruminal degradable protein, respectively. The subjects in this experiment, the metabolizable protein of canola meal treated with 0.5% urea (357.163 g/kg DM) accounted for the highest value than the other treatments. Results show that increased metabolizable protein by processing meals with urea and microwave.

Keywords: *Canola meal, In situ, Metabolizable protein, Microwave, Urea*

INTRODUCTION

Canola is a modified variety of rapeseed whose oil contains less than 2% erucic acid and its meal contains 16 to 30 micromoles of glucosinolate per gram. The crude protein of canola meal is 32 to 38%. It also contains a suitable and valuable combination of amino acids whose ruminal degradability of protein is about 67% (Boila and Ingalls, 1992). Of course, the amount of the crude protein content of canola meal in our country ranges from 35- 37% (Hashemi, 1991). Since canola meal protein gets degraded faster in the rumen than other protein supplements, therefore; processing of canola meal to enhance rumen bypass protein and to reduce its degradable part in the rumen has gained attention in recent years.

In processing to increase bypass protein of the canola meal, the physical and chemical methods, or a combination of these two methods have been employed. Various methods have been proposed to reduce the degradability and anti-nutritional factors of the meal. These methods involve the thermal processes (roasting and autoclaving), chemical (acid and formaldehyde) and radiation (Sadeghi and Shawrang, 2006).

Rapeseed meal with low glucosinolate, up to 34%, was used in the diets of male fattening calves and no reduction was observed in their growth (Brunschwig *et al.,* 1994). Reports indicated that the new varieties of canola meal can be used up to 25% concentrate in fattening calves (Paquay *et al.,* 2003). Given that canola meal has a good potential to be used in the livestock industry; however, it is often limited the diet due to containing anti-nutritional factors and variations in nutritional value. The reduction of the shell and indigestible polysaccharides of the meals is the primary goal for improving the quality of these nutrients through processing (Krokhina *et al.,* 1989). Glucosinolate along with their hydrolysis enzymes, i.e. thioglucosidase, exist in all parts of the cannula (Brunschwig *et al.,* 1994).

The reaction of glucosinolate hydrolysis in the body is obtained in the study of Pusutai (1989). The present study was conducted to identify the degradability rate and determine the digestion coefficients

of canola meal and increase and optimize its use in animal feed and prevent wasting them and polluting the environment.

MATERIALS AND METHODS

Canola Meal Collection

Examples of meals were obtained randomly sampled from oil extracted reputable manufacturers and canola meal productive companies, Iran. The experimental treatments were; A: canola meal, B: canola meal treated with 0.5% urea, C: canola meal treated with microwave, that were prepared in the laboratory. Besides, 3 parts of solution and 1 part of canola meal were mixed in plastic containers and were kept in room temperature and away from sunlight for 60 days; samples were taken out of the containers and dried in the sunlight and milled in a 2 mm size to be used in other phases of the experiment. Animals used in this experiment were fed at maintenance level. The animals were fed with a mixture of 60% forage and 40% concentrate diet (Ørskov and McDonald, 1979).

Chemical Composition

Feedstuffs dry matter (DM, method ID 934.01), ash (method ID 942.05), ether extract (EE, method ID 920.30), and crude protein (CP, method ID 984.13) were determined by procedures of AOAC (AOAC, 1990). The neutral detergent insoluble fiber (NDF) and acid detergent fiber (ADF) concentrations were determined using the methods of Van Soest *et al.,* (1991), without sodium sulphite. Neutral detergent insoluble fiber was analyzed without amylase with ash included (Moghaddam, 2015).

Measured In Situ

To estimate the degradability of the nylon bag technique, the feed samples were milled with a special mill and 2-mm sieve (Moghaddam *et al.*,2012). 5 grams of each nutrient were poured into bags made of synthetic polyester fiber as 6 × 12 cm and pore diameter of 50 mm. Two fistulated sheep with average BW 45±2.5 kg were used in a complete randomized design. Incubation times were 0, 2, 4, 6, 8, 12, 16, 36 and 48 h. After each incubation time, the bags were removed and rinsed with cold water until the water is completely cleared out. After washing, bags were incubated for 24 h at a temperature of 65 °C to evaporate and for 24 h at 105 °C in oven (Moghaddam *et al.*,2012). Degradation parameters (soluble, insoluble, and fixed rate of degradation) were calculated with Naway. For matched degradation data used from $P=a+b(1-e^{-ct})$ that a=The degradation of soluble fraction (%), b=The degradation rate of insoluble fraction (%), c=The constant degradation rate (%/h), t=The incubation time (h), e=The constant factor (2.718) and P=The degradation rate at the time t. Effective degradability was calculated at $ED=[a+(b\times c)]\div(c+k)$ that k is passage rate which were considered in this study 0.02 (Moghaddam, 2015).

Statistical Analysis

The obtained data from in situ study was analyzed according to a completely randomized design with 4 replicates by the GLM procedure (SAS, 2002). The treatment means were compared by the Duncan test.

RESULTS AND DISCUSSION

Results

The chemical composition of treatments is presented in Table 1. The data show that treatment B had the most (91.67%) and treatment A had the least (90.82%) amount of dry matter ($P>0.05$). Regarding the percentage of crude protein treatment B (45.66%) and treatment A (39.56%) had the highest and the lowest amount of crude protein ($P<0.05$). According to table 1, there were significant differences in crude protein, acid detergent fiber, and neutral detergent fiber in tested feed ($P<0.05$).

According to the results reported in Tables 2 at different times of incubation, treatments B and A are the highest and lowest DM disappearance values, respectively. Also, according to the results obtained at 0 h of incubation, treatment B (16.62%) had the lowest and treatment C (20.76%) had the highest rate of dry matter disappearance that there were significant differences between treatments B and C

(P<0.05). The data shows that canola meal processed with microwave had initially higher value of degradability of dry matter than the other treatments. However, after 36 hours of ruminal incubation, the rate of degradability of dry matter of canola meal processed with 0.5% urea was higher than the other treatments. Degradation of all treatments has increased trend in during incubation in the rumen of sheep. Treatments C (20.76%) and B (16.62%) had the highest and lowest (a) coefficient value for DM, respectively, that due to the high solubility of urea, these results are predictable and justifiable. Treatments A and B (34.77%) and C (28.49%), had the highest and lowest fermentable material (coefficient b), respectively. The results reported in this study revealed that the coefficients a and b indicated significant differences between treatments which were due to the treatment effects (P<0.05).
Means of the data presented in Tables 3 show that in zero-hour of incubation, treatments B (15.29%) and A (4.87%) had the highest and lowest rumen CP disappearance (P<0.05). Results of dry matter and crud protein degradation show processing with urea and microwave increase the degradability and metabolizable protein in the canola meal. Crud protein degradability coefficients of the treatments presented in table 3 show that coefficient (a) had the highest and lowest values for treatments B (15.29%) and A (4.87%), respectively (P<0.05). These results were predictable due to the high solubility of urea. Treatments A (39.2 %) and B (28.16%) had the highest and lowest coefficient (b) that were significantly different (P<0.05). This could be due to their high levels of crude protein which the cause is microbial growth and increment the protein degradation.
The data presented in Table 1 shows the metabolizable protein components of the experimented feed. The results obtained for the quick degradation protein indicated that the processing of canola meal increased the amount of QDP. Effective ruminal degradable protein in canola meal processed with microwave with 167.17 g/kg DM and canola meal with 137.31 g/kg DM had the most and the least ERDP, respectively. Besides, the data obtained revealed that canola meal enriched with 0.5% urea with 357.163 g/kg DM of metabolizable protein had higher metabolizable protein than the other treatments.

Discussion

At 48 h, canola meal processed by 0.5% urea had the highest dry matter degradation and show a significant difference with treatments A and C (P<0.05). The results showed that enriching canola meal with 0.5% urea decreased the quickly degradable part (a) of the dry matter. During all hours of incubation, the crude protein of the treatment had different rates of degradability (P<0.05). The comparison of the treatments indicated that the processed canola meals had the highest rate of the disappearance of the crude protein. Tashakorri Bar Abadi *et al.*, (2008) concluded that the nitrogenous parts of the feed are degraded by different kinetics in the rumen. Also, the ruminal degradability of the nitrogenous parts was significantly affected by the type of the meal and protein level (P<0.001).
According to the data provided in Table 5, the canola meal enriched with 0.5% urea and the canola meal had the most and least values of coefficient a, respectively. This can be due to two reasons. First, the amount of crude ash in rapeseed meal enriched with 0.5% urea is high and its organic matter is low. Second, the total rate of anti-nutritional materials and their degradability rate in canola meal is more than the other treatments. This can prevent the activity of protein-degrading microorganisms and prevent the degradation of protein (Moghaddam *et al.*, 2012). Sadeghi *et al.*, (2006) reported coefficient a of the crude protein of rapeseed meal 25.51% which is more than the findings of the present study. Shawrang *et al.*, (2008) reported coefficient b of processed rapeseed meal as 72.1% which was higher than the value of the meals processed with microwave in the present study.
These differences can be imputed to the differences in the varieties used, the sampling conditions, the microbial contamination, the method of washing the bags, and the different methods of processing. In the present study, the potential degradability (a+b) of protein of rapeseed meal processed with 0.5% urea was 43.45% which indicated the low degradability rate of crude protein in rumen. According to Table 5, the processing of canola meal with urea and microwave caused a considerable increase in the values of parts a and b.

Table 1: The chemical composition (% DM)* and the parameters estimated from the metabolizable protein (g/kg DM) of feeds

Treatments	DM	CP	OM	NDF	ADF	ash	ADIN	QDP	SDP	ERDP	DUP	UDP	MP
A	90.82 ^{a}	39.56 ^{c}	92.486 ^{a}	29.25 ^{c}	20.75 ^{c}	7.513 ^{ab}	3.88 ^{a}	19.273 ^{c}	118.63 ^{b}	137.31 ^{b}	231.77 ^{b}	257.76 ^{b}	319.65 ^{c}
B	91.67 ^{a}	45.66 ^{a}	92.026 ^{b}	45.19 ^{a}	33.29 ^{a}	7.87 ^{a}	3.36 ^{b}	69.78 ^{a}	108.26 ^{c}	166.667 ^{a}	250.5 ^{a}	278.55 ^{a}	357.163 ^{a}
C	91.17 ^{a}	42.78 ^{b}	92.296 ^{a}	34.53 ^{b}	31.37 ^{b}	7.3 ^{b}	2.95 ^{c}	37.66 ^{b}	137.034 ^{a}	167.17 ^{a}	228.54 ^{b}	253.037 ^{b}	335.53 ^{b}
SEM	0.354	0.313	0.114	0.373	0.356	0.1308	0.04	1.1156	2.068	2.43	1.99	2.215	2.686

*DM=dry matter, CP=crude protein, OM=organic matter, NDF=neutral detergent fiber, ADF=acid detergent fiber, ADIN=acid detergent insoluble nitrogen, ERDP=Effective ruminal degradable protein , DUP=Digestible undegradable protein , MP=Metabolizable protein. A: canola meal, B: canola meal treated with 0.5% urea, C: canola meal treated with microwave.

^{a,b,c}Within a column, means without a common superscript letter differ (P< 0.05).

**Standard error means of the difference amount three treatments means.

Table 2: Means of dry matter degradation and dry matter degradability coefficients of feeds by incubation at different times in the in situ method (% DM)

Treatment	Incubation times (h)									Degradation coefficients				
	0	2	4	6	8	12	16	36	48	A	B	c	ED	RSD
A	20.25 ^{a}	22.19 ^{b}	24.0267 ^{b}	25.75 ^{b}	27.39 ^{b}	30.38 ^{c}	33.057 ^{c}	42.65 ^{c}	46.26 ^{b}	20.25 ^{a}	34.77 ^{a}	0.0287 ^{b}	40.73 ^{a}	0.8867 ^{c}
B	16.62 ^{b}	19.75 ^{c}	22.59 ^{b}	25.18 ^{c}	27.54 ^{b}	31.64 ^{b}	35.033 ^{b}	45.01 ^{a}	47.76 ^{a}	16.62 ^{b}	34.77 ^{a}	0.0472 ^{a}	41.066 ^{a}	1.576 ^{b}
C	20.76 ^{a}	23.38 ^{a}	25.76 ^{a}	27.92 ^{a}	29.87 ^{a}	33.27 ^{a}	36.07 ^{a}	44.2 ^{b}	46.41 ^{b}	20.76 ^{a}	28.49 ^{b}	0.0483 ^{a}	40.9 ^{a}	2.1033 ^{a}
SEM**	0.283	0.223	0.191	0.175	0.168	0.164	0.166	0.193	0.248	0.2724	0.536	0.00138	0.189	0.166

a=Dry matter solution at zero time (%), b=Fermentable material (%), c=Constant degradability coefficients at time t (%/h),
ED=Effective degradation (The passage of time r=0.02),
RSD= Residual standard deviation.
A: canola meal,
B: canola meal treated with 0.5% urea,
C: canola meal treated with microwave.

^{a,b,c}Within a column, means without a common superscript letter differ (P< 0.05).

**Standard error means of the difference amount three treatments means.

Table 3: Means of crude protein degradation and crude protein degradability coefficients of feeds by incubation at different times in the in situ method (% DM)

Treatment	Incubation times (h)									Degradation coefficients				
	0	2	4	6	8	12	16	36	48	A	b	C	ED	RSD
A	4.87 ^{c}	9.67 ^{c}	13.88 ^{c}	17.57 ^{c}	20.817 ^{c}	26.16 ^{b}	30.28 ^{b}	40.33 ^{c}	42.37 ^{b}	4.87 ^{c}	39.2 ^{a}	0.06527 ^{c}	34.9 ^{b}	2.63 ^{a}
B	15.29 ^{a}	21.57 ^{a}	26.44 ^{a}	30.21 ^{a}	33.14 ^{a}	37.17 ^{a}	39.63 ^{a}	43.11 ^{b}	43.37 ^{b}	15.29 ^{a}	28.16 ^{b}	0.12647 ^{a}	39.57 ^{a}	2.61 ^{a}
C	8.81 ^{b}	15.69 ^{b}	21.35 ^{b}	25.98 ^{b}	29.8 ^{b}	35.5 ^{a}	39.35 ^{a}	46.23 ^{a}	47.01 ^{a}	8.81 ^{b}	38.55 ^{a}	0.09823 ^{b}	40.83 ^{a}	2.69 ^{a}
SEM**	0.29	0.43	0.61	0.715	1	0.662	0.636	0.315	0.0349	0.29	0.562	0.0073	1.2	0.16

a=Crude protein solution at zero time (%),

b=Fermentable material (%),

c=Constant degradability coefficients at time t (%/h),

ED=Effective degradation (The passage of time r=0.02),

RSD= Residual standard deviation.A: canola meal,

B: canola meal treated with 0.5% urea,

C: canola meal treated with microwave.

a,b,c Within a column, means without a common superscript letter differ (P< 0.05).

This is due to the increase of crude protein and decrease of anti-nutritional materials and cell wall through processing of canola meal. It indicates the efficiency and the improvement of performance and nutritional value of feed due to processing.

Since the proportion of rumen ammonia nitrogen and urea nitrogen transfer rates correlate negatively with the rumen, therefore the change in the proportion of dietary nitrogen digested in the rumen can alter the rumen and urea nitrogen recycle and this effect can be extensively increased by processing canola meal. It can also increase ruminal digestion and subsequent nitrogen recycle and urea nitrogen microbial degradation to rumen (Kiran and Mutsvangwa, 2007). With urea, microorganisms can readily get the needed nitrogen from urea and the feed protein is preserved (Ahmadi *et al.,* 2013). It has been reported that the low nutritional value of this product is due to the presence of anti-nutritional factors. The high rate of quick degradation protein in the meals enriched with 0.5% urea was due to the existence of urea which is highly soluble. The values of slow degradation protein showed that the differences between the results of the treatments was statistically significant ($P<0.05$). Processing with microwave decreased the quick degradation part (a), increased the slow degradation part (b), decreased fixed degradation rate (c), and finally decreased the effective degradability of crude protein in different speeds. In the study done by Sadeghi and Shawrang (2006), processing with microwave had positive effects on the reduction of the degradability of rapesees meal in rumen. The reduction of the degradability of protein was pertained to the penetration of the short waves in the inner structures of the meal and creation of steady heat as a result of the increase in the movement and collision of bipolar molecules. The heat resulting from the processing by microwave causes the formation of protein gel through making structural changes in proteins, increasing the hydrophobicity of the surface of protein due to the separation of hydric bonds and other weak non-covalent bonds, and making changes in the position of amino acids and then increasing hydrophobicity of the surface of protein (Folawiyo and Apenten, 1997). It leads to the reduction of the availability of active chemical groups of protein molecules and solubility and consequently the reduction of degradability of the effective protein in rumen. According to the reports of Sadeghi and Shawrang (2006), the processing of rapeseed meal with microwave with the power of 800 W for 2, 4, and 6 minutes, decreased the effective degradability of protein in the speed of 5% per hour in the rumen of castrated cow from 68.3% in the unprocessed meal to 51.5%, 45.6%, and 42%, respectively.

The effective degradable protein in rumen indicates the total amount of nitrogen in rumen. The microorganisms of rumen use this nitrogen to grow up. The higher the level of feed consumption, the less is the value of ERDP because of the increase in the speed of the feed passing through the rumen (Moghaddam *et al.,* 2012). Considering that the effective degradable protein in rumen is created from quick and slow degradation proteins, therefore; the high amount of effective degradable protein in rumen of meals enriched with 0.5% urea is explainable compared to the control treatment.

The microorganisms in the rumen of ruminants are able to degrade the protein and use the nitrogen for making microbial proteins. If it is fed along with a light source of carbohydrates, it will increase the production of microbial in the ruminants (Ahmadi *et al.,* 2013). Considering that the ration of ammonia nitrogen in rumen has a negative correlation with the rate of urea nitrogen transfer. Therefore, changes in the ratio of urea or diet nitrogen being digested in rumen change the recycle of urea nitrogen in rumen. This can cause changes in the starch digestibility in rumen and consequently increase the recycle of urea nitrogen to rumen as well as the degradation of microbial nitrogen (Moghaddam *et al.,* 2012). The urea entering the rumen is quickly hydrolyzed to ammoniac by bacterial urea and therefore the density of ammoniac of rumen can increase considerably. The results obtained showed that there were significant statistical differences between metabolizable protein of the treatments of this experiment ($P<0.05$). In general, the amount the metabolizable protein of the feed is influenced by factors such as the rate of crude protein and the rate of degradability of protein in rumen. This can be due to processing canola meal with urea. In addition, the difference in the amount of the metabolizable protein of the meals processed with urea and processed with microwave can be concerned with the difference in the amounts of metabolizable protein and the degradability characteristics of the matter which caused the increase in the amount of metabolizable protein in enriched meals with urea compared to the meals enriched with microwave (Moghaddam, 2015).

Conclusion

It can be concluded that canola meal produced in the country can be easily used to 15% of the dry matter consumed by fattened animals. Also, this feed has nutritional value and suitable amount of metabolizable protein and processing with microwave and urea can improve its nutritional value and metabolizable protein. Using microwave is an appropriate strategy for increasing efficiency of using crude protein. The results of effective degradability and digestibility of canola meal with the power of 800 W for 1.5 minutes allowed the protein to pass through. Being quick and cheap, processing with microwave is a useful method for changing the degradability of protein of oilseed meals. Regarding the findings of this research, it is clear that canola meal, canola meal processed with 0.5% urea, and canola meal processed with microwave have high digestive potential. If there is more information about these, they can be used as alternative feed for ruminants.

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