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A comparison of camelina meal and soybean meal degradation during incubation with rumen fluid as tested in vitro

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Summary: Camelina meal is a new by-product that remains after oil extraction for biodiesel production and might be considered as an alternative protein source to soybean meal in animal nutrition. The objective of the present study was to determine the chemical composition and in vitro degradation of camelina meal compared to soybean meal. Feed samples were collected from a commercial feed mill. To estimate ruminal digestion, in vitro nutrients disappearance of camelina meal and soybean meal was determined using ruminal fluid that was collected at a local slaughterhouse. In vitro disappearance at 4, 12 and 24 h of incubation showed significant differences between camelina meal and soybean meal. Soybean meal showed higher DM and NDF degradation compared to camelina meal. On the opposite, CP degradation of camelina meal exactly to increase. In conclusion, no differences were observed between nutrients and feed x time interaction of CP degradability of CM and soybean meal. Thereby, it's concluded that, the CM might be used in ruminant feeds with soybean meal which is common used in rations.

Key words: Ankom technology, camelina meal, incubation, nutrient degradation.

Ketencik küspesi ile soya küspesinin in vitro rumen yıkımlanabilirliklerinin karşılaştırılması

Özet: Ketencik küspesi, biyoyakıt üretimi sırasında yağın ekstraksiyonuyla arta kalan bir yan üründür ve hayvan beslemede soya küspesine alternatif bir protein kaynağı olarak düşünülebilmektedir. Bu çalışma, ketencik küspesinin besin madde bileşiminin ve in vitro yıkımlanabilirliğinin soya küspesi ile karşılaştırılmasını amaçlamaktadır. Araştırmada kullanılan yem örnekleri ticari bir yem fabrikasından temin edilmiştir ve ardından besin madde bileşimleri belirlenmiştir. Yem hammaddelerinin rumendeki yıkımlanabilirliklerinin belirlenmesi, yerel mezbahadan alına rumen sıvısının in vitro ortamda kullanılması suretiyle gerçekleştirilmiştir. İnkubasyonun 4., 12. ve 24. saatlerinde gruplar arasında önemli farklılıklar tespit edilmiştir. Kuru madde ve NDF yıkımlanabilirliği soya küspesinde yüksek değere sahip iken, HP yıkımlanabilirliği ketencik küspesinde daha fazla bulunmuştur. Sonuç olarak ketencik küspesinin ve soya küspesinin besin madde bileşimi ve yem x zaman etkileşimi açısından HP sindirilebilirlik düzeyleri arasında önemli bir farklılığın tespit edilmemiştir. Dolayısıyla, ketencik küspesinin rasyonlarda yaygın olarak yer alan soya küspesi ile birlikte protein kaynağı olarak kullanılabileceği düşünülmektedir.

Anahtar sözcükler: Ankom teknolojisi, besin madde yıkımlanabilirliği, inkübasyon, ketencik küspesi.

Introduction

Camelina *sativa* is an oilseed crop that has gained increasing popularity as a biofuel source. *C. sativa*, also known as false flax or gold-of-pleasure (13), is a flowering plant in the family *Brassicaceae* which includes mustard, rapeseed and cabbage (25). Camelina meal (CM), the by-product of camelina oil extraction, is a protein source for livestock despite its lower crude protein content compared to soybean meal (17). CM has the potential to be used as a protein source in ruminant diets, as it has a greater CP content and an RUP proportion similar to canola meal that is used more in ruminant diets (7). The oil content of the camelina seed ranges from 37 to 41% and presents a high content of n-3 fatty acids (22). The utilization of by-products from oil extraction is a pivotal factor for sustainable biodiesel production from camelina sativa and this could reduce both feed and biodiesel costs while promoting environmental sustainability. Additionally, CM includes glucosinolates that are antinutritional factors and impair the activity of thyroid gland resulting in decreasing feed intake and productivity by down regulating digestibility (15). However, ruminants are more tolerant to glucosinolates compared to monogastric animals (30). Therefore, the evaluation of CM as a potential ingredient in livestock rations is a critical factor to further increase the inclusion levels in animal diets and in turn the economic value of the plant (11). Although scattered European reports and reviews of the potential of feeding CM to cattle are available, little information exists in scientific publications quantifying the feed-value and digestibility of CM (1).

For this purpose, we hypothesized that the CM might have similar degradation rate as well as RUP value by reducing the CP degradation characteristics with soybean meal in the rumen. Therefore, we conducted an in vitro trial aimed at evaluating the degradation of nutrients, including CP, DM and NDF in CM and soybean meal, which might have consequences for the degradation rate of these feedstuffs for ruminants.

Material and Methods

Camelina meal and soybean meal: Winter *C. sativa* grown during the winter season that harvested in 8 months in Russia was used in this experiment. Camelina variety used in human nutrition for providing essential fatty acid, after oil extraction it is used in animal nutrition in Turkey and CM and also soybean meal provided by the commercial feed mill in Turkey. They were firstly screened for chemical composition in this experiment.

Investigation of nutrient degradation using an in vitro incubation technique: To evaluate the degradation of DM, CP and NDF, representative samples of CM and soybean meal, were used as substrates for incubation by using Daisy II incubator Ankom Technology method (3) and the modification of Yilmaz (31). The total incubation time was 24 h. Samples of 0.5 g of both substrates were ground to pass a 1 mm screen. Later on, the samples were transferred into the filter bag F57 with a pore size of 40 µm and closed with heat sealer and incubated in the rumen fluid. Before transfering the samples, bags were placed into acetone for 3 to 5 minutes and dried at 25°C. A bag without sample was prepared and incubated also for correction. Buffer solution A (KH_2PO_4, 10.0 g/L; $MgSO_4.7H_2O$, 0.5 g/L; NaCl, 0.5 g/L; $CaCl_2.2H_2O$, 0.1 g/L and urea, 0.5 g/L) and solution B (Na_2CO_3, 15 g/L and $Na_2S.9H_2O$, 1.0 g/L) were prepared freshly at 39 °C and 6.8 pH condition. The incubator consisted of 4 cylinder jars with a capacity of 2000 mL. Each cylinder jar contained 1600 mL buffer (proportion of A to B is 5:1), 400 mL rumen fluid and 20 filter bags. Rumen fluid was obtained from a feedlot cattle during the slaughter process. The cattle was fed a diet consisting of straw and concentrate with a ratio of 0.21:0.79, respectively. Rumen fluid was immediately transported to our laboratory and strained trough 4 layers of gauze. Empty bags and sealed sample bags were placed into the cylinders for incubation. After jars were flushed with CO_2 and lids were closed immediately, all cylinders were placed into a prewarmed incubator for 24 h. After 4, 12 and 24 h incubation 40 sample bags of each ingredient were removed from cylinder jars and rinsed under the tap water to stop microbial activity and fermentation processes, subsequently were dried at 105°C for 3 h.

Nutrient analysis: Feedstuffs of the experimental substrates were ground to a 2 mm particle size for DM, CP, EE and ash analysis in CM and soybean meal, as well as in vitro tested samples, according to the methods of the Association of Official Analytical Chemists (4). Samples were analyzed for DM by oven-drying at 105°C for 3 h, for ash by combustion of samples over night at 580°C and CP was determined by the Kjeldahl method. The CF, ADF and NDF contents of feeds were determined according to Van Soest *et al*. (29). The formula recommended by Turkish Standards Institute (28) was used to calculate the metabolisable energy.

Calculations and statistical analysis: In vitro true DM disappearance (IVTD), in vitro NDF disappearance (dNDF) and in vitro CP degradation (dCP) were calculated with following model.

$$IVTD\ (\%DM) = 100 - [(W3 - (W1 \times C1)) \times 100\ ]\ (W2 \times \%\ DM_{Feed})$$

$$dNDF\ (\%\ DM) = 100 \times [(W2 \times \%NDF_{Feed}) - (W3 - (W1 \times C1))]/(W2 \times \%DM_{Feed}\ )$$

$$dCP\ (\%DM) = 100 \times [(W2 \times \%CP_{Feed}) - (W3 - (W1 \times C1))]/(W2 \times \%DM_{Feed}\ ),$$

where W_1 is weight of filter bag, W2 is weight of sample, W3 is final weight (Filter bag + sample), NDF_{Feed} is % of NDF contain in feed (%DM), DM_{Feed} is % of dry matter contain in feed, CP_{Feed} is % of CP contain in feed (%DM) and C1 is correction of factor (blank filter bag NDF value).

General statistical evaluation of data was conducted as a completely randomized design, with a factorial arrangement of 2×3, taking into consideration main effects of feedstuff (soybean meal and camelina meal) and time of incubation (4, 12 and 24 hours). The SAS programme (SAS Institute Inc., Cary, NC, version 9.2) (24) was used for data analysing. Differences were determined using the Tukey HSD test. The differences were considered statistically significant at $P < 0.05$ and indicated by superscripts (26).

Results

The chemical analyses of the soybean meal and CM used in the present in vitro experiment are shown in Table 1. CM contains similar ether extract content as soybean meal (14.9 g/kg vs. 16.5 g/kg) due to the exclusion of the solvent extraction step in the production process for CM and soybean meal. However, the levels of CP (369.7 g/kg vs. 482.0 g/kg) were lower in CM than soybean meal. On the opposite, level of NDF was higher in CM than soybean meal. Thus, lower NFC-contents were determined in CM, as well as energy content. (Table 1).

In vitro rumen DM, CP and NDF degradation of CM and soybean meal at various incubation time points is presented in Table 2. After 4 h of incubation disappearance of DM and NDF were lower ($P<0.0001$) in

Table 1. Nutrient composition of soybean and camelina meal, g/kg.
Tablo 1. Soya küspesi ve ketencik küspesinin besin madde bileşimi, g/kg.

Chemical Composition	Soybean Meal	Camelina Meal
DM	896.00	885.90
OM	940.00	946.10
CP	482.00	369.70
EE	16.50	14.90
CF	52.50	110.70
Ash	60.00	53.90
ADF	ND	183.80
NDF	79.60	282.90
NFC	361.90	278.60
ME, MJ/kg	11.67	10.40

ND: not detected

Table 2. In vitro DM, CP and NDF degradation of soybean meal and Camelina meal, %.
Tablo 2. Soya küspesi ve ketencik küspesinin in vitro KM, HP ve NDF sindirilebilirlikleri,%.

Treatments	Feed	Time	DM	CP	NDF
T-1	Soybean meal	4	37.41±0.26^{d}	40.78±2.11	30.78±6.53^{b}
T-2	Camelina meal	4	23.47±0.30^{e}	53.89±0.19	24.30±2.96^{b}
T-3	Soybean meal	12	46.02±0.55^{c}	36.89±1.32	30.42±3.58^{b}
T-4	Camelina meal	12	39.30±0.70^{d}	53.67±1.20	16.44±0.51^{b}
T-5	Soybean meal	24	56.82±0.77^{a}	36.65±2.60	57.60±2.66^{a}
T-6	Camelina meal	24	51.59±0.24^{b}	50.74±1.21	24.48±1.83^{b}
Main effects					
Soybean meal			46.75±1.51^{a}	38.11±1.23^{a}	39.60±4.52^{a}
Camelina meal			38.12±2.15^{b}	52.76±0.67^{b}	21.74±1.55^{b}
4h			30.44±1.61^{c}	47.34±2.66	27.54±3.54^{b}
12h			42.66±0.88^{b}	45.28±3.28	23.43±3.13^{b}
24h			54.21±0.72^{a}	43.69±2.97	41.04±6.43^{a}
P values					
Feed			<0.0001	<0.0001	<0.0001
Time			<0.0001	0.1088	<0.0003
Feed × Time			<0.0001	0.5184	<0.0041

†Values are expressed as means ±standard error
a-e; Means within the same column without common superscripts are significantly different ($P < 0.05$). n=4, *n=10
a-e; Aynı sütunda farklı harf taşıyan ortalama değerler arasındaki fark istatistik bakımdan önemlidir ($P < 0.05$)

CM compared with soybean meal. The difference became even greater at 12 and 24 h of incubation (decrease from 46.02 to 39.30 and from 56.82 to 51.59 in DM; from 30.42 to 16.44% and from 57.60 to 24.48% in NDF, respectively). Additionally, main effect of IVTD and dNDF showed significant (P<0.0001) down regulation effect (18.46% and 45.10%), while in CM higher disappearance rate of CP (38.44%) was shown compared to soybean meal (P<0.0001). No significant (P=0.109) effect between CM and soybean meal for the dCP at various incubation time was observed, however tended to increase at 4, 12. and 24 h of incubation (32.15; 45.49 and 38.45%, respectively).

Discussion and Conclusion

For livestock, soybean meal is irrevocable feed material, because of a high quality protein sources due to its balance of amino acid contents and availability with including high crude protein content (10). Thereby, many researchers investigate many feedstuffs as an alternative to soybean meal for particularly formulation costs. At present, CM was chosen to explore their DM, CP and NDF degradation in rumen fluid using the *in vitro* system ANKOM that makes nutrients disappearance study easy and efficient. To provide the most suitable in vitro method that is used for determination of feedstuff degradation in ruminants, have been studied (8; 11; 14).

To our best knowledge, this is the first time that degradability of CM and soybean meal was compared in an in vitro rumen system. Therefore, digestibility of CM in ruminants should be investigated further with ruminants offered CM diet to determine if it is available. Our study provided that CM had less degradation rate for DM and NDF in the rumen, while the degradation of CP was not altered.

Chemical composition of the CM and soybean meal were within the range of those previously reported by the NRC (17) and by other authors (5; 27).

In ruminants, fiber digestion can occur in the rumen resulting with a dynamic process that is affected by chemical nature of feedstuff fiber and by rate of fiber digestion (17). In situ, Cappellozza *et al*., (6) found no significant effects between CM and soybean meal on disappearance rate or effective ruminal degradability of hay DM and NDF in cows fed CM and soybean meal, however, they didn't indicated that the digestibility of CM. With the partial hydrolysis of hemicelluloses in CM, the remaining NDF in CM was probably less degradable, including high amount of hemicelluloses, than in soybean meal, which may explain the degradation kinetics for CM. In this experiment, another reason for lower IVTD and dNDF in CM might be expected that ruminal pH would be lower in bags including CM than bags including soybean meal, since bags were shown to be micro-environment that pH and microbial activity associated with not only ruminal conditions but also more with the incubated with feedstuffs (18; 23), requires further investigations.

Conversely, the main effect of dCP was higher in CM than soybean meal, while CM tended to down regulation at various rumen incubation time. Such mechanical process because of obtaining meal, involving heat treatment, attributed to a decreasing in the solubility of proteins and often resulted in higher RUP content in feeds (19; 32). CM includes high antinutritional factors such as non-starch polysaccharides, glucosinolates, and phenolic compounds (23). These antinutritional factors react with protein and form various complexes, resulting in reduced CP digestibility in monogastrics (2; 20). On the other side, these factors exactly don't affect CP degradation negatively in ruminants, due to their ruminal microorganisms. The antinutritional factors possibly could be dissolved, afterwards disappear from incubation bags, but the binding activity remains to show their effects in the intestine (9), which means that the CM has the potential to further promote the N digestibility. On the other hand high EE content that may protect of degradation, did not affect negatively its' protein degradation (34). Thereby, CM had higher dCP comparison with soybean meal.

There is lack of evidence concerning the effect of CM on ruminal DM and NDF degradation as well as dCP can be explained by the fact that just maintained in vitro method in the present study. One reason might be the relationship of oil extraction way in feedstuffs and the conditions during the degradability trial such as bag characteristics, incubation condition in the rumen. The possible effect that there is no in vitro investigation that will support the degradation characteristics of CM can not be excluded.

In conclusion, nutritive value generally was a higher for soybean meal than for CM. Whereas CP and energy content is lower in CM, NDF content is higher in CM that can be partially substituted to soybean meal in ruminant diets. IVTD and dNDF was significantly reduced in CM. Moreover, results from this experiment indicated significant increment that the degradation of CP in CM compared to soybean meal. Similarity in nutrient composition and higher DM degradability of CM with soybean meal may affect the costs of formulation, in particular, due to use an alternative protein source in ruminant diets. Further research is warranted to understand the concurrent decrease DM degradation and increase degradation of CP as well as determination of actual nutrients digestibility in ruminants.

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