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In situ Evaluation of Ruminal Degradability and Intestinal Digestibility of Sunflower Meal Compared to Soybean Meal

Research Article

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ABSTRACT

The aim of this study was to compare the nutritional value of sunflower meal (SFM) and soybean meal (SBM) protein by determining both its ruminal degradability and intestinal digestibility. Three non lactating Jersey cows fitted with a rumen and T-type duodenal cannulas were used to estimate rumen degradability and intestinal digestibility of SFM and SBM dry matter (DM) and crude protein (CP). Samples of SFM were collected from seven sunflower processing plants (SFM1 to SFM7). Six different samples of SBM were collected from three main suppliers (SBM1 to SBM6). Both protein feeds were incubated in the rumen of the cows for 0, 2, 4, 8, 16, 24 and 48 h in 6 replications. The rapidly degradable fraction of DM averaged 24.8% for SFM which was lower (P<0.01) than that observed for SBM (29.2%). The effective DM degradability of SFM (56.2 %/h), at mean rumen outflow rate of 0.06/h, was lower (P<0.01) compared with SBM samples (67.3 %/h). The washable fraction *a* of CP was higher (P<0.01) for SFM samples (26.3%) in comparison to all batches of SBM (16.5%). The effective degradability of SFM CP (67.7 %/h) at rumen passage rate of 0.06/h was higher (P<0.01) than in SBM (63.0 %/h). The DM intestinal digestibility of SFM samples (42.6%) measured by the mobile bag technique was lower (P<0.01) compared to SBM samples (71.9%). The intestinal digestibility of SFM CP (89.9%) was also lower (P<0.01) than in SBM (94.6%). Results of this study indicate that SBM samples were more resistant to ruminal degradation than SFM. The data suggest that changing the toasting parameters can decrease degradability of SFM to improve protein quality.

KEY WORDS intestinal digestibility, protein nutritive value, rumen degradability, soybean meal, sunflower meal.

INTRODUCTION

For rational use of the relatively expensive protein supplements, it is important to know their exact nutritional value including estimates of the ruminal degradability and intestinal digestibility. One of the most important and cheapest protein sources for ruminants in Eastern Europe is the sunflower meal (SFM). Its inclusion is usually limited by its high rumen degradability especially when fed to high producing dairy cows or young ruminants in allometric growth phase (Veresegyhazy and Fekete, 1990). Apart from that, SFM has several advantages in comparison to other protein supplements including the lower price and higher content of sulfur containing amino acids such as methionine, cystine and cysteine. The actual nutritional composition of SFM varies greatly among individual experiments and this variation could be partially explained by the different types of SFM samples and *in situ* methodology, ratio of bag surface area to sample size and sample particle size used to characterize it (Wadwa *et al*. 1998; Habib *et al*. 2013). On the other hand, soybean meal (SBM) is the most popular protein supplement used for ruminant nutrition worldwide.

Its protein characteristics for ruminants also varies greatly depending on the variety, type of processing (solvent/mechanical extraction, extrusion, roasting, etc.), and any additional heat treatment post-processing, which in raw condition has a relatively high degree of ruminal degradation (Gonzalez *et al*. 2002).

However, it appears that ruminal degradability of SFM, neither in our country nor in other countries in Eastern Europe, has not been compared to SBM, known as a "golden standard" for the quality of a dietary protein supplements. Additionally, there are limited data available regarding *in vivo* intestinal digestibility of SFM protein. Therefore, this experiment was undertaken to determine the rumen degradability and intestinal digestibility of SFM compared with commonly fed SBM using the *in situ* and mobile bag techniques.

MATERIALS AND METHODS

Animals and samples

All procedures involving animals in this experiment were consistent with Bulgarian animal welfare legislation and in compliance with the Bulgarian Food Safety Agency regulations (registration license No 126).

Three non-lactating Jersey cows with an average body weight of 436 ± 18 kg, fitted with a rumen (made from polycarbonate material with internal diameter=12 cm) and T-shape duodenal cannula (polycarbonate material with internal diameter=4.4 cm) were used in the experiment.

During the adaptation (10 d) and experimental (15 d) periods, cows were fed at maintenance level a ration containing 800 g/kg roughages (63.6% alfalfa hay and 16.4% barley straw) and 200 g/kg concentrate (30.5% ground corn grain, 26.5% ground barley grain, 23.0% wheat bran, 17.0% SFM and 3% mineral and vitamin premix). Cows were housed in the large ruminant facility of Trakia University's Research Center, Faculty of Veterinary Medicine, and were fed at approximately 8.00 h and 16.00 h. Feeding was *ad libitum* targeting 5% refusals.

Sunflower meal samples were collected from the seven largest sunflower processing plants in Bulgaria (SFM1 to SFM7, numbers indicate the individual plant/batch), all members of the Oilseed Oil Producers Association of Bulgaria.

Six different samples of soybean meal were collected from three main suppliers – four batches were imported feeds (two of them from Brazil and two from Argentina) and two batches were locally produced Bulgarian soybean meal (SBM1 to SBM6, numbers indicate the individual supplier/batch). The individual batches from each supplier were collected in an interval of at least 20 days.

In situ rumen incubation

Samples (approximately 2.5 g) of each batch of 2-mm ground SFM and SBM were placed in 5 cm × 10 cm polyester dacron bags and sealed by double sewing the fabric with pore size 45 µm polyester thread (SEFAR® PET 1500, 9410 Heiden, Switzerland). Bags were incubated in the rumen of the cows for 0, 2, 4, 8, 16, 24 and 48 h in duplicates (i.e., a total of 6 bags per incubation time-point). Bags were placed in the rumen in reverse order so that all bags were removed at the same time. Immediately after removal from the rumen, bags were carefully washed by hand under running tap water until the water remained clear. Then, the bags were dried at 65 ˚C for 48 h and dry matter (DM) content of the residue was determined by drying at 105 ˚C for 2 h in a mechanical convection oven.

In situ small intestinal digestibility of ruminal undegraded fraction

Intestinal digestibility of SFM and SBM (DM) and crude protein (CP) were determined by mobile bag technique following the procedure of Woods *et al*. (2003c). Polyester bags, made by double sewing of a nylon fabric with pore size 16 µm (4×8 cm, SEFAR® PET 1500), were filled with 1 g of SFM and SBM previously incubated for 16 h in the rumen as described above. Samples were soaked for 1 h in 1 N HCl solution (pH 2.4) and thereafter incubated in HCl/pepsin solution (pH 2.4) for 2 h at 40 ˚C. Bags were then inserted into the proximal duodenum of the cows via the duodenal cannula, approximately 2 h after the morning feeding (14 bags per cow per day with 30 min interval between the individual bags' insertions). The bags were recovered by rinsing fecal matter with cold water through a large sieve (12 mm openings). Bags were washed, dried (at 65 ˚C for 48 h and then at 105 ˚C for 2 h) and weighed for DM and CP analysis of the residues.

Chemical analysis

Sunflower and SBM samples were ground to pass through a 2-mm screen. Dried samples were analyzed by wet chemistry methods for ether extract (EE, method 2003.05; AOAC, 2006), ash (method 942.05; AOAC, 2000), and minerals (method 985.01; AOAC, 2000) (Table 1). Sunflower and soybean meal samples and bag residues were analyzed for N (Kjeltec™ 8400 Analyzer Unit, FOSS, DK-3400 Hillerod, Denmark) and CP was calculated as N × 6.25.

Calculations and statistical analysis

Ruminal DM and CP degradation data were fitted to the exponential equation of Orskov and McDonald (1979), using the Marquardt algorithm for non-linear regression procedure (SPSS, 2016).

$d = a + b(1-e^{(-ct)})$

Where:
d: degradability (%) at time *t*.
a: soluble and rapidly degradable fraction of DM or CP.
b: potentially degradable fraction.
c: rate of degradation of fraction *b*.
t: incubation time (h).

Effective degradability (ED) of DM and CP were calculated using the following equation (AFRC, 1993):

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

Where:
a, *b*, and *c*: specified above.
Kp: passage rate, assumed at 0.05, 0.06, and 0.08 h^{-1}.

Data were analyzed for the fixed effect of protein source using the GLIMMIX procedure of SAS (2002). Significance was declared at $P < 0.05$. Means are expressed as least squares means.

RESULTS AND DISCUSSION

Chemical composition of SFM and SBM

The average CP content of SFM was 369 (SD=23.5) g/kg, ranging from 337 g/kg DM to 397 g/kg, whereas CP of SBM varied from 459 to 498 g/kg DM and averaged 480 g/kg (SD=12.9) (Table 1). The CP content of both SBM and SFM is comparable with published values (Todorov *et al*. 2007). A slightly higher variation of the CP content was observed for SFM samples. This was mainly due to different varieties of sunflower seeds and quality of raw material used at every individual processing company. However, current values indicate that all sunflower processing plants applied similar extracting technology and all SFM were produced after an average degree of dehulling of the seeds.

Rumen degradability of DM

The rapidly degradable fraction of DM and potentially degradable fraction *b* were lower for SFM (P<0.01) compared to SBM (Table 2). The rate of degradation of fraction *b* (kp) was greater (P<0.01) for SFM compared with SBM samples. Concentrations of soluble DM fraction *a* of SFM reported by Woods *et al*. (2003a), 28.4 ± 4.72% and Marghazani *et al*. (2013), 27.1 ± 1.35% are consistent with our results. The mean values for rapidly degradable fraction of DM for SBM are also in accordance with literature data previously reported (Kleinschmit *et al*. 2007; Lee *et al*. 2016). Values of potentially degradable DM, fraction *b*, observed in this study are higher than values previously reported for SFM (Alcaide *et al*. 2003; Kamalak *et al*. 2005; Gao *et al*. 2015). Gonzalez *et al*. (2002) and Todorov *et al*. (2016) reported similar results for the fraction *b* of SBM of approximately 70% of the DM. Others have reported lower values for potentially degradable fraction *b*, which ranged from 55.8% (Mondal *et al*. 2008) to 59.2% (Maxin *et al*. 2013). According to Kamalak *et al*. (2005), variation in protein degradability was mainly associated with differences in the methods used for sample preparation and processing, and in the bags' porosity used for *in situ* incubation. Another source of variation is the animal species (cattle *vs*. sheep) used in *in situ* experiments (*Orskov et al*. 1983).

The average value of effective DM degradability of SFM at mean rumen outflow rate of 0.06/h was lower (P<0.01) compared to SBM samples (Table 2). One of the possible explanations is the higher fiber content of sunflower meal, which is degraded slowly in the rumen compared with other nutrients.

However, effective DM degradability of sunflower meals are consistent with the results reported by others (Woods *et al*. 2003a; Mondal *et al*. 2008; Gao *et al*. 2015), whereas the degradability of SBM was lower than the reported by Woods *et al*. (2003a) and Lee *et al*. (2016) (78.3 %/h and 75.9 %/h, respectively).

Rumen degradability of CP

The washable fraction *a* of CP was higher (P<0.01) for SFM samples in comparison with SBM in the present study (Table 3). Similar results for SFM were reported by Gao *et al*. (2015) 27.4%, whereas Woods *et al*. (2003b) and Mondal *et al*. (2008) reported higher concentrations of 38.0% to 39.0%, respectively. On the other hand, the values for fraction *a* for SBM measured in the present experiment are in agreement with previously reported results where this fraction was found to average 12.9% with a standard error (SD) of ± 5.69% (Woods *et al*. 2003b).

The estimated effective degradability of CP in SFM at a rumen passage rate of 0.06/h was higher (P<0.01) than in SBM, which is consistent with previous reports (Todorov *et al*. 2016). Other authors reported considerably higher effective degradability (ED) of SFM CP (75.0 to 90.0%; Alcaide *et al*. 2003; Chrenkova *et al*. 2010; Ganbari *et al*. 2015).

However, there were only small differences in the degradability rate of SBM in present experiment which is in agreement with values reported by the NRC (2001). Rates of SBM degradation in our trial are also in agreement with those reported in Nordic countries (Volden, 2011). Differences in the manufacturing processes, particularly excessive heat treatment (Ljokjel *et al*. 2000), are likely the main reason for some of the observed differences in SFM and SBM protein degradability.

Table 1 Dry matter (DM) content and chemical composition (g/kg DM or as indicated) of commercial sunflower and soybean meals used in the present study

Parameters	DM (g/kg)	Crude protein	Ether extract	Ash	Ca	P
Sunflower meal						
Average	916	369	16.9	79.1	5.94	13.3
Minimum	911	337	12.4	67	4.16	10.3
Maximum	929	397	22.3	87.7	9.14	17.1
SD	6.91	23.5	2.91	6.24	1.72	2.19
CV, %	7.52	63.7	17.2	7.88	29.0	16.5
Soybean meal						
Average	918	480	19.9	75.9	5.18	8.17
Minimum	923	498	26.8	81.2	8.43	9.53
Maximum	907	459	16.7	69.2	3.87	7.52
SD	5.23	12.9	33.6	3.89	1.50	0.66
CV, %	5.71	26.8	16.9	5.12	28.9	8.05

SD: standard deviation and CV: coefficient of variation.

Table 2 Ruminal degradation parameters and effective degradability of dry matter of commercial sunflower meal (SFM) and soybean meal (SBM) samples (%, or as specified)

Parameter	SFM	SBM	SE	P-value
a	24.8^{b}	29.2^{a}	0.48	< 0.01
b	55.3^{b}	71.6^{a}	0.61	< 0.01
c/h	0.081^{a}	0.068^{b}	0.0038	< 0.01
kp= 0.045/h^{-1}	59.9^{b}	72.4^{a}	0.54	< 0.01
kp= 0.06/h^{-1}	56.2^{b}	67.3^{a}	0.55	< 0.01
kp= 0.08/h^{-1}	52.2^{b}	62.2^{a}	0.53	< 0.01

a, b, and c/h: soluble, potentially degradable fraction and rate of degradation of fraction *b*, respectively and kp: passage rate from the rumen.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SE: standard error.
Number of observations used in the statistical model= 78.

Table 3 Ruminal degradation parameters and effective degradability of crude protein of commercial sunflower meal (SFM) and soybean meal (SBM) samples (%, or as specified)

Parameter	SFM	SBM	SE	P-value
a	26.3^{a}	16.5^{b}	0.60	< 0.01
b	72.4^{b}	85.3^{a}	0.79	< 0.01
c/h	0.083^{a}	0.072^{b}	0.0039	0.021
kp= 0.045/h^{-1}	72.6^{a}	69.1^{b}	0.59	< 0.01
kp= 0.06/h^{-1}	67.7^{a}	63.0^{b}	0.67	< 0.01
kp= 0.08/h^{-1}	62.6^{a}	56.8^{b}	0.72	< 0.01

a, b, and c/h: soluble, potentially degradable fraction and rate of degradation of fraction *b*, respectively and kp: passage rate from the rumen.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SE: standard error.
Number of observations used in the statistical model= 78.

According to NRC (2001), the proportion of rumen undegraded protein (RUP) in SFM is 2 to 2.5 times lower than SBM. The results of our present study indicate that RUP of SFM was about 15% lower than in SBM.

Additionally, heat or chemical treatments have been shown to decrease ruminal degradability of SFM (Mohammadabadi *et al*. 2008; Diaz-Royon *et al*. 2016) suggesting that feeding value of SFM protein could be substantially increased for ruminants.

However, it will be difficult to obtain low level of ruminal degradation, similar to SBM, because indigestible portion of protein in the acid detergent insoluble nitrogen is higher for solvent extracted SFM compared to SBM (NRC, 2001).

Intestinal digestibility of DM and CP

The average DM intestinal digestibility of SFM samples was lower (P<0.01) compared to SBM (Table 4).

The intestinal digestibility of SFM CP (89.9%) was also lower (P<0.01) than in SBM (94.6%). Our results are in agreement with previous reports who determined intestinal digestibility of SFM CP using the *in vitro* three-step incubation procedure (Weisbjerg *et al*. 1996; Woods *et al*. 2003c; Gao *et al*. 2015). However, the mean intestinal digestibility of CP for SBM in the present experiment is slightly lower than 97.8% reported by Boucher *et al*. (2009). All SBM samples for this study were obtained from manufacturing plants that used similar solvent-extraction processing.

Table 4 Dry matter (DM) and crude protein intestinal digestibility (% of DM) of sunflower meal (SFM) and soybean meal (SBM) samples following a 16-h rumen incubation

Parameter	SFM	SBM	SE	P-value
Intestinal digestibility of RUDM	42.6[b]	71.9[a]	0.60	< 0.01
Intestinal digestibility of RUCP	89.9[b]	94.6[a]	0.46	< 0.01

RUDM: ruminally-undegraded dry matter and RUCP: ruminally-undegraded crude protein.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SE: standard error.

However, the observed deviation may be attributed to small operational differences such as processing time, temperature, pressure, particle size, etc., applied at each oil extraction plant. In addition to processing, seed variety and environmental factors (fertilization and climatic conditions) can also affect SBM intestinal digestibility.

CONCLUSION

The results of the present study can be used to provide additional information for formulating the protein fractions in rations for ruminants. These results also show that SBM was more resistant to ruminal degradation compared with SFM, subsequently more protein escaped the rumen as RUP in SBM. The results suggest that there are opportunities to improvement the protein digestibility of SFM, which could be achieved by changing heat treatment parameters and decrease ruminal degradability.

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