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The effect of chemical processing of soybean meal on *in vitro* ruminant intestinal available protein

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Abstract

The aim of this study was to investigate the effects of chemical processing of soybean meal on the amount of available crude protein at the duodenum (uCP) using a new modified gas technique. Evaluated samples were two samples of unprocessed soybean meal and one sample of processed soybean meal (heat processing with xylose) containing 495, 480 and 446 g/kg crude protein (CP), respectively. To perform the gas test, rumen fluid was collected before the morning feeding from two rumen fistulated Holstein dairy cows (640 ± 38 kg, body weight). Feed samples (200 mg) and blanks (only 30 ml of buffered rumen fluid) incubated simultaneously in three repeats and three runs for 8 and 24 hours. At the end of the each incubation time, the uCP was calculated as non-ammonia N which was calculated by subtracting the amount of ammonia N released in the incubation medium of the total incubated N (sum of N content of feed sample and ammonia N in blanks). Effective uCP (EuCP) was calculated via an exponential equation using the estimated uCPs at 8 and 24 h post incubation. Ratio of effective uCP (at the passage rate of 0.1/h) to the CP content of feed samples for processed soybean meal and two untreated soybean meals were 0.873, 0.747 and 0.785 (SEM=0.0355), respectively. These results showed that the processing had no significant effect on the amount of EuCP of soybean meal (P> 0.05). However, the amount of relative EuCP for processed soybean meal was 10-15 percent greater than untreated soybean meals.

Keywords: soybean meal, protein
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Introduction

Nitrogen efficiency in ruminants, especially in high yielding dairy cows is low (Calsamiglia et al., 2010) and environmental nitrogen pollution through dairy farms is becoming a serious challenge. There are several methods to estimate ruminal protein degradability; between these methods, recently Edmunds et al. (2012) described a new gas production technique that facilitates to estimate sum of microbial protein and ruminally undegraded protein (that is approximately equivalent with the utilizable crude protein at the duodenum (uCP)) in the incubation medium. This technique eliminate the confounding effects of various parameters such as *de novo* synthesis of microbial protein, microbial markers, loss of the feed particles through bag pores, soluble proteins and endogenous nitrogen on evaluation of feed proteins. Moreover, this new gas test estimated the nutritional value of feed proteins using CP content of feed sample and concentration of ammonia nitrogen in the incubation medium which are easily measurable. Therefore, the aim of this study

was to evaluate the effects of chemical processing of soybean meal on the amount of uCP using this new modified gas technique.

Materials and Methods

Commercial untreated soybean meals (soybean meal 1 and 2) and a processed soybean meal (heat processing with xylose) were used as feed samples. These three feed samples were incubated simultaneously in complete randomized design. The experiment was conducted with six replicates in three runs. In each run, a blank (buffered rumen fluid without feed sample) was incubated in eight replicates. A new gas production technique according to Edmunds et al. (2012) was followed. The rumen fluid was collected manually before the morning feeding from two rumen fistulated lactating Holstein dairy cows (640 ± 38 kg, body weight) that were fed a diet that containing corn silage (250 g/kg DM), alfalfa hay (250g/kg DM), and concentrate (500g/kg DM consisted of 30% barley grain, 20% corn grain, 10% cotton seed meal, 10% soybean meal, 10% sugar beet pulp, 18% wheat bran and 2% vitamin and mineral supplement). Rumen fluid was filtered through four layers of cheesecloth and then was mixed with buffered mineral solution (Menke and Steingass, 1988) in ratio of 1:2.Incubations were performed in 125 ml serum bottles. 200mg of each feed sample was weighed (0.001g Precision Balance) into incubation bottles then; 30 ml of buffered rumen fluid (BRF) was added to each bottle and oxygen was removed from the bottles headspace using CO2 stream. Then, each bottle was sealed with rubber stopper and aluminum cap. The incubations were performed in a water bath at 39 °C for 8 and 24 h. Blanks were contained only BRF and treated in the same manner as the samples. Half of the replicates of each treatment (and blanks) at 8 h and remained bottles at 24 h post incubation were transferred to a water-ice mixture to stop fermentation. Afterwards, the bottles were opened and 5 ml of liquid phase was pipetted into 50 ml serum bottles that were contained 5 ml of 0.2 N HCl. Then, these bottles were sealed with rubber stopper and aluminum cap and stored in refrigerator at 4 °C for measurement of ammonia concentration. For each of feed samples the uCP value was calculated according to the equations proposed by Edmunds et al. (2012).Data were analyzed using GLM procedure of SAS (9.2, 2002).

Results and discussion

The amounts of CP and uCP values of the feed samples are shown in the Table 1. The two untreated soybean meals had higher CP content than processed soybean meal. The uCP value of processed soybean meal was greater than untreated soybean meals at 8 h post incubation but, differences were not significant ($P > 0.05$). Also, the differences between uCP values of feed samples at 24 h post incubation were not significant ($P > 0.05$). The processed soybean meal had higher EuCP value compared with two untreated soybean meals however; differences were not significant ($P > 0.05$). Ratio of EuCP to the CP content of feed sample for processed soybean meal was greater than untreated soybean meals but, differences were not significant ($P > 0.05$). The amounts of uCP decreased as the incubation time increased. This study was conducted to evaluate the effects of heat processing with xylose on the amount of utilizable crude protein applied by soybean meal at the duodenum using a new gas production technique. Our results indicated that heat processing with xylose cannot significantly affect uCP and EuCP values of soybean meal. However, uCP and EuCP value of processed soybean meal were numerically greater than untreated soybean meals. It has been demonstrated that an increase in rumen undegradable protein at the expense of ruminally

degradable protein can reduced production of microbial protein. But, the used technique in our experiment and the nature of feed samples provided the conditions that N was not limiting factor for producing microbial protein (Edmunds et al., 2012). In the current study, higher CP content of untreated soybean meals may affect the uCP estimates. It has been concluded that at the case of replacing soybean meal with high RUP sources, the lack of an overall increase in non-ammonia nitrogen flow to the small intestine despite increasing in non-ammonia non-microbial nitrogen flow was a result of decreasing in microbial nitrogen flow. Heat processing temperature and xylose supplementation level are other factors that affecting ruminal protein degradability. In the current study, the heat processing temperature might be not enough to affect soybean meal protein degradability. Vaga et al. (2014) examined the effects of various heat processing methods on uCP values of field beans and lupines using gas production technique. They used processed protein feeds in a total mixed ratio and shown that heat processing of field beans and lupines effectively increased uCP values. Therefore, measuring the uCP values of processed soybean meals when those are used in a total mixed ratio may be useful for determining possible changes occurred with more accuracy.

Table1. Effects of heat processing with xylose on CP, uCP and EuCP (g/kg DM) values of soybean meal and its relative amounts of EuCP.

Feed sample	CP	uCP, 8 h	uCP, 24 h	EuCP	EuCP/CP
processed soybean meal	446.281[c]	429.273	230.064	388.841	0.873
untreated soybean meal 1	480.780 [b]	417.084	233.562	379.911	0.785
untreated soybean meal 2	495.723 [a]	404.699	232.446	369.808	0.747
SEM	1.578	9.329	19.236	16.912	0.035

SEM: standard error of the mean. Within columns, means followed by different letters are significantly different by Tukey's test at 5% probability.

Conclusion

This study indicated that the heat processing with xylose had no significant effect on uCP and EuCP values of soybean meal but, the amounts of them were increased numerically. The used new gas production technique provided good, fast and inexpensive estimates of uCP value of soybean meal products and shown that it can be used to evaluate protein feeds without interference effects caused by endogenous nitrogen and de novo synthesis of microbial protein. Nevertheless, more researches are needed to identify factors that affecting the accuracy of the technique.

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