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PAPER

The effects of supplemental protease enzymes on production variables in lactating Holstein cows

Ekin Sucu,[1,2] Amir Nayeri,[1] Maria V. Sanz-Fernandez,[1] Nathan C. Upah,[1] Lance H. Baumgard[1]

[1]Department of Animal Science, Iowa State University, Ames, IA, USA
[2]Zootekni Bölümü, Uludag Üniversitesi, Bursa, Turkey

Abstract

A study was conducted to examine the effects of supplemental dietary protease enzymes on production variables in dairy cattle. Ninety-six multiparous lactating Holstein cows (624±62 kg body weight and 154±104 days in milk) were blocked according to parity, days in milk, and previous milk production and randomly assigned to a control total mix ration (TMR) or a TMR containing a blend of supplemental protease enzymes (PE; 4 g/cow/d) in a crossover design with two 21-day experimental periods. Daily pen milk yield and dry matter intake (DMI) were recorded and milk composition from all cows was determined on d 15, 17, 19 and 21 of each period. There was no treatment effect on milk yield (37.6 kg/d), but supplemental PE-fed cows consumed less DMI ($P<0.05$) compared to controls and therefore tended to have improved feed efficiency ($P=0.06$). Feeding supplemental PE decreased blood urea nitrogen ($P<0.05$) compared to the control cows. However, feeding PE had no effect on milk fat and protein content but tended ($P=0.08$) to increase milk lactose concentration and tended ($P=0.10$) to decrease milk urea nitrogen levels and somatic cell score. Results indicate that supplemental PE may enhance production efficiency and improve parameters of nitrogen status.

Introduction

Optimising the balance between cost and nutritive value of feed is critically important to the sustainability of agriculture. Inclusion of dietary enzymes has the potential to decrease input costs and increase feed conversion while simultaneously decreasing waste product output. Protein is often the most expensive feed component and protein quality and digestibility can vary with different thermal processing techniques. In addition, inefficient nitrogen utilisation in animal agriculture is becoming a major environmental concern and livestock manure is thought to account for about 50% of total atmospheric ammonia (VandeHaar and St-Pierre, 2006). Theoretically, proteases can be added to a diet with the purpose of increasing dietary protein hydrolysis, thus facilitating improved nitrogen utilization. When animals utilise nitrogen more efficiently it may be possible to decrease the dietary protein content, lower feed costs and reduce the environmental nitrogen load.

Most commercial enzyme products contain more than one active enzyme and designed to target fibre digestion. These products are blends with varying concentrations of xylanases, -glucanases, and cellulases, as well as amylases, proteases, and lipases. In recent years, several studies published on enzyme blends have demonstrated positive effects on production (Yang *et al*., 1999, 2000; Kung *et al*., 2000) and environment (Yang *et al*., 1999; Knowlton *et al*., 2002).

Fewer studies have been conducted to specifically evaluate proteases and protein digestion. In broiler chickens, adding supplemental proteases improved true nitrogen digestibility (Ghazi *et al*., 2003). Studies have also reported enhanced protein digestibility and feed efficiency (O'Doherty and Forde, 1999) in pigs fed supplemental protease enzymes. *In vitro* and *in vivo* ruminant trials suggest that adding proteases can enhance fibre degradation by attacking some of the cell wall nitrogen-containing components that are physical barriers to rumen degradation (McAllister *et al*., 1993; Colombatto *et al*., 2003a). This has been confirmed by others who observed that proteases (without any measureable cellulase or xylanase activity) could enhance digestibility of dry matter and neutral detergent fibre (NDF) of alfalfa hay (Colombatto *et al*., 2003b; Eun and Beauchemin, 2007; Colombatto and Beauchemin, 2009), rice straw (Eun *et al.,* 2006) and maize silage (Eun and Beauchemin, 2007). Similarly, Eun and Beauchemin (2005) also indicated that adding proteases to a low-forage diet increased the total tract NDF digestibility in dairy cows.

It is presently unclear how proteases will affect dairy production efficiency. Study objectives were to examine the effects of supplementing proteases on production parameters in Holstein cows.

Corresponding author: Dr. Ekin Sucu, Zootekni Bölümü, Uludag Üniversitesi, 16059 Bursa, Turkey.
Tel. +90.224.2941559 - Fax: +90.224.4428152.
E-mail: ekins@uludag.edu.tr

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Materials and methods

Animals and diets

Ninety-six lactating Holstein cows (2.7±1.6 parity, 153.8±103.7 dry matter intake, 40.3±5.9 kg milk/d, 624±62 kg body weight) were assigned to one of two treatments at the Iowa State University Dairy Farm (Ames, IA, USA). The ingredients and chemical composition of the experimental diet are listed in Table 1. Diets were isonitrogenous, isoenergentic, and balanced to meet or exceed predicted requirements (National Research Council, 2001) of energy, protein, minerals, and vitamins for current stage of production and primarily consisted of corn silage (39%) and concentrate (48%).

All procedures were reviewed and approved by the Iowa State University Institutional Animal Care and Use Committee.

Enzyme product

A developmental proprietary blend of protease enzymes used in the current study was in granular form and compliant with current specifications for pharmaceuticals in America. Although the enzyme activity of proteases has not been determined in our experiment, the product used is alkaline protease enzymes classified as a serine endopeptidase of the subtilisin family (EC 3.4.21.62) and cysteine protease (EC 3.4.22.2).

Experimental design

Cows were randomly assigned to one of four pens (24/pen) and fed diets with or without supplemental protease enzymes (PE; Rumagentin™, Feed Sources LLC, Alta Loma, CA, USA) during two 21-d periods in a contin-

uous random crossover design. There was a 7-d washout period, between the two experimental periods, in which all cows received the control total mix ration (TMR). All pens were fed the TMR once daily (07.30 h) and orts were recorded one hour prior to feeding. The granular form of enzymes was mixed with a ground-corn grain carrier (Mid-State Milling, State Center, IA, USA). Ground corn was added to TMR (at mixing) at a rate of 0.91 kg/cow/d as-fed and contained either no treatment (plain ground corn) or PE to provide product at 4 g/head/d.

Milk and blood sampling

Milk samples were collected from each cow during the morning milking on day 15, 17, 19 and 21 relative to treatment initiation of each period. The sample was stored at 4°C with a preservative (Bronopol Tablet, DandF Control System, San Ramon, CA, USA) until analysis by Dairy Lab Services (Dubuque, IA, USA) using the Association of Official Analytical Chemists approved infrared analysis equipment and procedures for milk components. Blood samples were obtained via coccygeal venipuncture during both periods on d -1 and 21 relative to treatment initiation using heparinized vaccutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA). Plasma was harvested following centrifugation at 1500 g for 15 min, and subsequently stored at -20°C until analysis. Plasma was analysed for blood urea nitrogen and measured by an enzymatic colorimetric method using a commercial kit (Teco Diagnostics, Anaheim, CA, USA).

Calculations

All milk yield and dry matter intake (DMI) data was condensed to weekly means prior to analysis. Fat corrected milk (FCM) and solids corrected milk (SCM) were calculated as described by the National Research Council (2001) and Tyrrell and Reid (1965) using the following equations:

4.0% FCM=0.4 × milk yield (kg) + 15 [milk fat (kg)/100] × milk yield (kg)

SCM=12.24 × milk fat yield (kg) + 7.10 × milk protein yield (kg) + 6.35 × milk lactose yield (kg) - 0.0345 × milk yield (kg)

Feed efficiency was calculated as milk yield/DMI and SCM/DMI.

Statistical analysis

The effects of treatment on pen DMI, milk yield and feed efficiency were analysed using the PROC MIXED procedure of SAS, with week as a repeated measure (SAS, 2005). Milk components were not analysed as repeated measure. Pen was the experimental unit on all analysed data. All data, except blood urea nitrogen (BUN), were covariately adjusted to respective pre-supplementation milk yield values (d -7 to -1) and BUN was covariately adjusted to pre-supplementation BUN values. Results are reported as least squares means±SEM and in all cases, differences among means were declared as significant at $P<0.05$, whereas trends were discussed at $P<0.10$, unless stated otherwise.

Results and discussion

Supplemental PE-fed cows had a decrease in overall DMI (3.7%, $P<0.01$) compared to control cows (Table 2). There were no differences in overall milk yield (37.7 kg/d), 4.0% FCM (31.1 kg/d), or 4.0% SCM (34.1 kg/d). Treatment had no effect ($P>0.05$) on milk fat content (3.5%), milk protein content (3.3%), or somatic cell count (346, 1000/mL). However, PE-fed cows tended to have increased milk lactose (4.8 *vs* 4.7%, $P=0.08$), decreased somatic cell score (3.7%, $P=0.10$) and milk urea nitrogen (MUN) content (3.3%, $P=0.10$) compared to the controls (Table 2). There was no difference ($P=0.14$) in feed efficiency (1.6) calculated as milk yield/DMI. Conversely, feeding supplemental PE tended ($P=0.06$) to improve overall feed efficiency (5.7%) when calculated as SCM/DMI (Table 2). Cows fed PE had decreased BUN (10.2%, $P<0.05$) compared to control cows.

The precise mode of action of PE in ruminant diets had not yet been clearly delineated. However, evidence suggests improved nutrient digestibility when a variety of feeds were treated with proteolytic enzymes (Colombatto *et al*.,

Table 1. Ingredient formulation and chemical composition of the diet.

Ingredient, % of DM	
Alfalfa hay	8.5
Corn silage	39.4
Corn grain, ground	3.4
Whole cottonseed	8.9
Soybean meal, 48%	11.2
Lactation premix	
Soybean hulls	8.8
Corn grain, ground	8.5
Corn distillers, w/solubles	7.1
Limestone	1.1
Animal fat	0.64
Magnesium sulfate	0.58
Urea	0.19
Sodium bicarbonate	0.52
Salt	0.43
Dicalcium phosphate	0.19
Vitamin/mineral pack	0.33
Chemical composition, %	
Crude protein	17.0
Undegradable protein	33.2
Degradable protein	66.8
ADF	22.9
NDF	34.2
NE_L, Mcal/kg	1.67

DM, dry matter; ADF, acid detergent fibre; NDF, neutral detergent fibre; NEL, net energy for lactation.

Table 2. Effects of supplementing protease enzymes on production parameters in lactating Holstein cows.

Parameter	Control	PE	SEM	P
DMI, kg/d	24.3*	23.4*	0.1	<0.01
Milk yield, kg/d	37.6	37.8	0.6	0.82
4.0% FCM, kg/d	30.9	31.2	0.9	0.81
4.0% SCM, kg/d	33.9	34.2	0.5	0.67
Milk composition				
Fat, %	3.55	3.52	0.06	0.80
Protein, %	3.27	3.23	0.04	0.47
Lactose, %	4.73	4.76	0.01	0.08
SCC, 1000/mL	389.00	303.00	46.00	0.28
SCS	2.16	2.08	0.02	0.10
MUN, mg/dL	15.10	14.60	0.20	0.10
Feed efficiency				
MY/DMI	1.54	1.62	0.03	0.14
SCM/DMI	1.41	1.49	0.02	0.06
BUN, (mg/dL)	12.80*	11.50*	0.40	<0.01

PE, protease enzymes; DMI, dry matter intake; FCM, fat corrected milk; SCM, solids corrected milk; SCC, somatic cell count; SCS, somatic cell score; MUN, milk urea nitrogen; MY, milk yield; BUN, blood urea nitrogen. *P<0.05.

2003b; Eun and Beauchemin, 2007; Colombatto and Beauchemin, 2009). According to the manufacturer, the specific blend of PE used in the current study was produced by strains of *Bacillus subtilis* and *B. licheniformis*, with extracts of *carica papaya* and *ananas comosus*. *B. subtilis* had broad specificity and hydrolysed peptide amides (Aehle, 2004). In a previous *in vitro* study (Eun *et al*., 2007), a protease product (*B. subtilis*) increased gas production by 6-8% with alfalfa hay, and NDF degradability increased by 11%.

In this study, milk yield was not affected by PE, but this was expected as increasing diet nutrient extraction would not be expected to increase milk synthesis in cows that are already in positive energy and nutrient balance (Baumgard *et al*., 2006). However, because of the slight PE-induced decrease in DMI, PE-fed cows tented to have increased feed efficiency and we assume this response is likely attributed to improved nutrient digestibility. Our improved feed efficiency response agrees with Eun and Beauchemin (2005) who demonstrated that supplemental protease fed dairy cows had decrease feed intake. However, in contrast to our results, milk yield also decreased in the aforementioned study (Eun and Beauchemin, 2005). Reasons for the inconsistencies between experiments are not clear but one possibility may be basal dietary differences, regardless reasons for differences are of obvious practical interest.

No treatment differences were observed in milk fat or protein content. However, numerically higher milk lactose content tended to be slightly increased in PE-fed cows and this is presumably the result of increased organic matter digestion. Eun and Beauchemin (2005) demonstrated that even though the enzyme product used in their study contained no measurable fibrolytic activity there were increase in acid and neutral detergent fibre, and hemicellulose digestibility. A possibility is that PE helped remove structural cell wall proteins and allowed for more extensive microbial access to degradable fibre (Nsereko *et al.,* 2000; Colombatto *et al*., 2003a). Although typically associated with acetate and butyrate production, increased ruminally digestible fibre may have also increased delivery of glucogenic precursors to the liver and eventually improved carbohydrate status. This result confirms previous observations by Eun and Beauchemin (2005), who reported increased lactose content of milk from cows fed supplemental proteases. The lack of an effect of treatment on milk fat and protein contents agrees with Kung *et al*. (2000) who reported no effect of enzyme treatment on milk fat or solids non-fat percentages.

Both BUN and MUN can be used as a proxy for rumen ammonia levels (Broderick and Clayton, 1997). High MUN or BUN levels can suggest excess ruminal ammonia and inefficient nitrogen utilization. In this study, the tendency for lower MUN and significantly less BUN is likely attributed to the improvements in microbial protein synthesis in response to PE supplementation. In addition, proteases may increase the release of ammonia-N from dietary proteins and the release of usable energy by increased fiber digestion resulting in improved nitrogen utilization. Yang *et al.* (1999) reported that enzymes enhanced microbial protein synthesis and protein degradability.

Animal agriculture can contribute to nitrogen pollution via urea excretion due to the over feeding of protein and subsequently decrease efficiency of nitrogen utilization (Jonker *et al*., 1998). Urea excreted in the urine is directly proportional to both the amount of BUN and that of MUN. Therefore, urinary ammonia can be predicted either from MUN or BUN (Baker *et al*., 1995; Jonker *et al*., 1998; Kohn *et al*., 2002). The MUN and BUN levels in the present study indicated that dietary protein was not fed in excess and adding dietary PE likely decreased environmental nitrogen excretion. In contrast, Eun and Beauchemin (2005) observed that efficiency of nitrogen use tended to decrease (P=0.11) and concentration of ammonia in the rumen tended to increase both for low- and high-forage diets supplemented with proteases. The same authors also reported that proteases had no effect on urinary nitrogen excretion. Reasons for the inconsistency in nitrogen variables between trials are not clear but a better understanding of this variation is needed by the industry.

Conclusions

Results from this trial indicate that supplementing a blend of PE at a rate of 4 g/h/d during established lactation decreased dry matter intake and tended to improve feed efficiency. The decrease in BUN seen in the treatment group coincides with the tendency for a decrease in MUN in the same group. This may suggest some interaction with nitrogen utilization but more research is warranted to investigate potential effects.

References

Aehle, W., 2004. Enzymes in industry: production and applications. Wiley-VCH, Weinheim, Germany.

Baker, L.D., Ferguson, J.D., Chalupa, W., 1995. Responses in urea true protein of milk to different protein feeding schemes for dairy cows. J. Dairy Sci. 78:2424-2434.

Baumgard, L.H., Odens, L.J., Kay, J.K., Rhoads, R.P., Van Baale, M.J., Collier, R.J., 2006. Does negative energy balance (NEBAL) limit milk synthesis in early lactation? pp. 181-187 in Proc. 21st Southwest Conf. Nutr. Manag., Tempe, AZ, USA.

Broderick, G.A., Clayton, M.K., 1997. A statistical evaluation or animal nutritional factors influencing concentrations or milk urea nitrogen. J. Dairy Sci. 80:2964-2971.

Colombatto, D., Beauchemin, K.A., 2009. A protease additive increases fermentation of alfalfa diets by mixed ruminal microorganisms in vitro. J. Anim. Sci. 87:1097-1105.

Colombatto, D., Hervas, G., Yang, W.Z., Beauchemin, K.A., 2003a. Effects of enzyme supplementation of a total mixed ration on microbial fermentation in continuous culture, maintained at high low pH. J. Anim. Sci. 81:2617-2627.

Colombatto, D., Morgavi, D.P., Furtado, A.F., Beauchemin, K.A., 2003b. Screening of exogenous enzymes for ruminant diets: Relationship between biochemical characteristics in vitro ruminal degradation. J. Anim. Sci. 81:2628-2638.

Eun, J.S., Beauchemin, K.A., 2005. Effects of a proteolytic feed enzyme on intake, digestion, ruminal fermentation, milk production. J. Dairy Sci. 88:2140-2153.

Eun, J.S., Beauchemin, K.A., 2007. Assessment of the efficacy of varying experimental exogenous fibrolytic enzymes using in vitro fermentation characteristics. Anim. Feed Sci. Tech. 132:298-315.

Eun, J.S., Beauchemin, K.A., Hong, S.H., Bauer, M.W., 2006. Exogenous enzymes added to untreated or ammoniated rice straw: effects on in vitro fermentation characteristics degradability. Anim. Feed Sci. Tech. 131:86-101.

Eun, J.S., Beauchemin, K.A., Schulze, H., 2007. Use of an in vitro fermentation bioassay to evaluate improvements in degradation of alfalfa hay due to exogenous feed enzymes. Anim. Feed Sci. Tech. 135:315-328.

Ghazi, S., Rooke, J.A., Galbraith, H., 2003. Improvement of the nutritive value of soy-

bean meal by protease α-galactosidase treatment in broiler cockerels broiler chicks. Brit. Poultry Sci. 44:410-418.

Jonker, J.S., Kohn, R.A., Erdman, R.A., 1998. Using milk urea nitrogen to predict nitrogen excretion utilization efficiency in lactating dairy cattle. J. Dairy Sci. 81:2681-2692.

Knowlton, K.F., McKinney, J.M., Cobb, C., 2002. Effect of a direct-fed fibrolytic enzyme formulation on nutrient intake, partitioning, excretion in early late lactation Holstein cows. J. Dairy Sci. 85:3328-3335.

Kohn, R.A., Kalscheur, K.E., Russek-Cohen, E., 2002. A comparison of analytical methods the influence of milk components on milk urea nitrogen recovery. J. Dairy Sci. 85:227-234.

Kung, L. Jr., Treacher, R.J., Nauman, G.A., Smagala, A.M., Endres, K.M., Cohen, M.A., 2000. The effect of treating forages with fibrolytic enzymes on its nutritive value lactation performance of dairy cows. J. Dairy Sci. 83:115-122.

McAllister, T.A., Phillippe, R.C., Rode, L.M., Cheng, K.J., 1993. Effect of the protein matrix on the digestion of cereal grains by ruminal microorganisms. J. Anim. Sci. 71:205-212.

National Research Council, 2001. Nutrient requirements of dairy cattle. 7th rev. ed. National Academy Press, Washington, DC, USA.

Nsereko, V.L., Morgavi, D.P., Beauchemin, K.A., Rode, L.M., 2000. Inhibition of ruminant feed enzyme polysaccharidase activities by extracts from silages. Can. J. Anim. Sci. 80:523-526.

O'Doherty, J.V., Forde, S., 1999. The effect of protease alpha-galactosidase supplementation on the nutritive value of peas for growing finishing swine. Irish J. Agr. Food Res. 38:217-226.

SAS, 2005. SAS/STAT users guide, ver. 9.3. SAS Inst. Inc., Cary, NC, USA.

Tyrrell, H.F., Reid, J.T., 1965. Prediction of the energy value of cow's milk. J. Dairy Sci. 48:1215-1223.

VandeHaar, M.J., St-Pierre, N., 2006. Major advances in nutrition: relevance to the sustainability of the dairy industry. J. Dairy Sci. 89:1280-1291.

Yang, W.Z., Beauchemin, K.A., Rode, L.M., 1999. Effects of an enzyme feed additive on extent of digestion milk production of lactating dairy cows. J. Dairy Sci. 82:391-403.

Yang, W.Z., Beauchemin, K.A., Rode, L.M., 2000. A comparison of methods of adding fibrolytic enzymes to lactating cow diets. J. Dairy Sci. 83:2512-2520.