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# Determining the Effectiveness of Microbial Protease on Production Variables in Lactating Holstein Cows

## A.S. Leaflet R2706

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### Summary and Implications

Lactating Holstein cows were assigned to a control diet or a diet containing a blend of supplemental protease enzymes (Rumagentin™, Feed Sources LLC, Alta Loma CA) at the Iowa State University Dairy farm. Study objectives were to examine the effects of proteases on milk yield, milk composition, feed intake and feed efficiency in lactating Holstein cows. Our results indicate that protease enzymes enhanced feed conversion efficiency and nitrogen utilization of dairy cows.

### Introduction

Commercial enzymes are a tool to enhance the efficiency of livestock production. The inclusion of enzymes for ruminant diets has primarily centered on fibrolytic enzymes. However, protein is typically the most expensive component in dairy rations. In addition, the plant protein is sometimes less degradable and can vary based on plant type, maturity and protein matrix. Recently, a number of *in vitro* studies have demonstrated that proteases increased dry matter and NDF digestibility of alfalfa hay and rice straw. On the other hand, little work has evaluated the impact of protease enzymes in ruminants, and specifically dairy production variables.

### Materials and Methods

Ninety-six lactating Holstein cows ( $2.7 \pm 1.6$  parity,  $153.8 \pm 103.7$  days in milk,  $40.3 \pm 5.9$  kg milk/d,  $624 \pm 62$  kg BW) were housed in a free-stall barn (ISU Dairy) and assigned to one of two treatments. Cows were milked twice daily (0800, 2000 h) and milk yields were recorded at each milking. All cows were fed a total mixed ration (TMR) once daily (0730 h) and orts/weigh-backs were recorded prior to morning feeding (0630 h). The diet primarily consisted of corn silage, alfalfa hay and concentrate. The TMR did not contain supplemental by-pass protein (e.g. Soyplus, Soybest, blood meal) and was formulated by Dairy Health Services (Sanborn, IA) to meet or exceed the predicted requirements (NRC, 2001) for energy, protein, minerals and vitamins. All procedures were reviewed and approved by the Iowa State University Institutional Animal Care and Use Committee.

Cows were split into four pens (24/pen) and fed diets with or without supplemental protease enzymes (Rumagentin™, Feed Sources LLC, Alta Loma CA) during two 21 d periods in a continuous random cross over design. There was a 7 d adaptation period to the control diet without bypass protein. During the first period (P1), half the cows (2 pens) received the treatment diet (control plus Rumagentin™) while the other half (2 pens) received the control diet. After P1, there was a 7 d washout period in which all cows (4 pens) received the control diet. During the second period (P2), treatment assignment switched: cows that received the treatment diet in P1 received the control diet and vice versa. The granular form of enzymes was mixed with a ground-corn carrier (Mid-State Milling State, Center, IA). Ground corn was added to the TMR (at mixing) at a rate of 0.91 kg/cow/d and contained either the enzyme or the control treatment (plain ground corn) to provide product at a level of 4 g/head/d. Feed efficiency was calculated using solids corrected milk yield/dry matter intake.

Milk samples from each cow were collected during the a.m. milking on d 15, 17, 19 and 21 relative to treatment initiation during both periods. Samples from each collection were stored at 4°C in vials containing a preservative (bronopol tablet; D&F Control System, San Ramon, CA) until analysis. Samples were analyzed by Dairy Lab Services (Dubuque, IA) using AOAC 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 vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Plasma was harvested following centrifugation at 2100 x g for 15 min, and subsequently stored at -20°C until analysis. Plasma was analyzed for blood urea nitrogen by an enzymatic colorimetric method using a commercial kit (Teco Diagnostics, Anaheim, CA).

The effects of treatment on pen feed intake, feed efficiency, milk yield, and milk components were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc. Cary NC), with week as a repeated measure and pen (treatment) as the random statement. Pen was the experimental unit on all analyzed data. All data were covariately adjusted using their respective pre-supplementation values (d -7 to -1). Results are reported as least squares means 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 protease-fed cows had reduced dry matter intake (0.93 kg/d;  $P < 0.05$ ) compared to cows fed control diets (Table 1). There was a tendency ( $P = 0.06$ ) for an increased (5.37%) feed efficiency (SCM/dry matter intake (DMI)) in the protease-fed cows compared to control cows. However, gross feed efficiency (milk yield/DMI) did not differ between treatments ( $P = 0.14$ ). The decreased feed intake and thus increased feed efficiency could be due to increased fiber digestion in the rumen. Protease enzymes may decrease cell wall proteins, enabling faster access to fiber by the rumen microorganisms.

Lactation performance, milk composition and blood parameters are presented in Table 2. There were no differences in production parameters between treatments ( $P$

$> 0.05$ ). However, protease-fed cows had lower concentration of blood urea nitrogen (BUN; 10.02%;  $P < 0.05$ ) and tended to have higher milk lactose (0.06%;  $P = 0.08$ ), lower milk urea nitrogen levels (MUN; 3.4%;  $P = 0.10$ ), and lower somatic cell score (SCS; 3.7%,  $P = 0.10$ ) in comparison to the control fed cows. The improved BUN and MUN status may have been due to increased nitrogen utilization in response to protease supplementation.

**Acknowledgments**

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**Table 1. Effects of Rumagentin™ on feed intake and feed efficiency of Holstein cows.**

Variable	Control	Rumagentin™	SEM	<i>P</i>
DMI, kg/d <sup>1</sup>	24.32	23.39	0.10	<0.01
Feed efficiency <sup>2</sup>	1.41	1.49	0.02	0.06
Gross feed efficiency <sup>3</sup>	1.54	1.62	0.03	0.14

<sup>1</sup>Dry matter intake

<sup>2</sup>Solids corrected milk (SCM) / DMI

<sup>3</sup>Milk yield / DMI

**Table 2. Effects of Rumagentin™ on lactational performance, milk and blood composition of Holstein cows.**

Variable	Control	Rumagentin™	SEM	<i>P</i>
Lactation performance				
Milk yield, kg/d	37.55	37.75	0.57	0.82
4.0% Fat-corrected milk, kg/d	30.88	31.19	0.85	0.81
4.0% SCM <sup>1</sup> , kg/d	33.89	34.20	0.47	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
Total solids, %	12.03	11.96	0.12	0.98
SCC <sup>2</sup>	389	303	45	0.28
SCS <sup>3</sup>	2.16	2.08	0.02	0.10
MUN <sup>4</sup> , mg/dL	15.11	14.60	0.15	0.10
Blood composition				
BUN <sup>5</sup> , mg/dL	12.77	11.49	0.40	<0.01

<sup>1</sup>Solids corrected milk

<sup>2</sup>Milk somatic cell count

<sup>3</sup>Milk somatic cell score = log of SCC

<sup>4</sup>Milk urea nitrogen

<sup>5</sup>Blood urea nitrogen