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# EFFECT OF FEEDING SUPPLEMENTAL EXOGENOUS AMYLASE ON THE PERFORMANCE OF HIGH YIELDING DAIRY COWS

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**ABSTRACT**

The objective of this study was to determine the effect of an exogenous rumen-resistant amylase preparation on live weight, milk production and milk composition in Holstein Friesian dairy cows (n = 70) in a dairy farm experiment. According to the Hungarian feeding practice corn silage-alfalfa haylage-dried corn meal based diet was used in the diets. Milk production was recorded every day. Chemical analyses were made from the morning milked samples once a week. There was a 3-week long preliminary and a 12-week long experimental period in the trial. Cows used in the experiment were in the 71st (control) and 72nd (experimental) day of lactation. All the animals were weighed at the beginning (control: 651 kg/cow; experimental: 657 kg/cow) and at the end of the trial (control: 685 kg/cow; experimental: 697 kg/cow). The exogenous rumen-resistant α-amylase used in this trial significantly ($p < 0.05$) improved milk production (control: 37.7 ± 6.96 kg *vs*. experimental: 38.7 ± 6.97 kg) and significantly ($p < 0.001$) decreased lactose content of milk (4.60 % *vs*. 4.55 %). The exogenous rumen-resistant α-amylase had no influence on the fatty acid composition of milk fat.

**Key words:** cattle, dairy cows, animal nutrition, exogenous α-amylase, milk production, milk composition

## 1 INTRODUCTION

Dairy cows producing 30 to 50 kg milk per day require approximately 2.5 to 4.0 kg glucose daily, but only a small amount (0.5 to 1.0 kg/day) of glucose is absorbed in the small intestine (Flachowsky and Lebzien, 1997). Blood plasma and liver glucose pool in the cow limited to 520 to 550 g, thus 1.0 to 3.0 kg glucose has to be synthesized through the gluconeogenesis pathway for the milk production mentioned. Besides improving gluconeogenesis, increasing the grain content of the diet is often used in practice. However, rapid digestion of excessive amounts of starch in the rumen can result in ruminal acidosis and reduce dry-matter intake and production (Owens *et al.*, 1998). Thus, while it may be desirable to achieve high levels of starch digestion in the rumen, avoiding ruminal fermentation conditions that lead to acidosis is also important. For these reasons the pressure is growing to reduce starch in dairy cow rations, making optimization of starch digestibility an important area of research (Nozière *et al.*, 2014). Feed enzymes are a radical innovation in dairy cow nutrition. Several studies have demonstrated that exogenous α-amylase preparations resistant to ruminal degradation are able to improve OM digestibility (Hristov *et al.*, 2008; Gencoglu *et al.*, 2010) and providing better milk efficiency by optimizing starch utilization in the rumen of dairy cows. Amylase can help to hydrolyse slowly fermentable corn starch shifting the digestion more towards the rumen. This provides more energy for microbial growth of cellulose degrading bacteria and thus increases fibre digestibility in the rumen. This characteristic especially alleviates the energy gap in the first 150 days of lactation (Weiss *et al.*, 2011). Therefore the objective of the present work was to evaluate the effect of an exogenous α-amylase preparation on live weight, milk production and milk composition in dairy

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cows. Increased dietary starch concentration decrease the apparent transfer of dietary polyunsaturated fatty acids to milk, suggesting an increased channeling of fatty acids to adipose tissue (Cabrita *et al.*, 2007). Additionally, dietary effects on milk fatty acid profiles were also investigated.

## 2 MATERIAL AND METHODS

### 2.1 COWS, FEEDS AND MANAGEMENT

Trials were established at the commercial dairy farm of Solum Co. in Komárom (Hungary). In a randomized complete block design multiparous Holstein Friesian dairy cows were used either in the control (n = 35) and experimental (n = 35) groups which were in the second and third lactation (DIM: 71 days control and 72 days experimental). Average daily milk yield 3 weeks prior to experimental period was 42.9 ± 7.0 kg/day for the control; 42.8 ± 6.8 kg/day for the experimental group. Cows were given a corn silage-alfalfa haylage-dried corn meal based diet (Table 1 and Table 2). An exogenous α-amylase preparation (Ronozyme® Rumistar, DSM) was given to the cows in the experimental group daily. Supplement was added to concentrate (12 g/cow/day). Diets were offered *ad libitum* as total mixed diets twice daily at 11.00 and 17.00 h. There was a three-week preliminary feeding period prior to milk production experiment which was lasted 12 weeks. Cows were accustomed to the feeding of enzyme preparation during preliminary feeding period. Cows were milked twice daily and individual milk yields were recorded at each milking. Milk composition was determined on consecutive morning and evening samples collected once weekly. Cows were weighed at the beginning and at the end of the experiment after the morning milking.

### 2.2 CHEMICAL ANALYSIS

The composition of milk was analyzed by the Hungarian Dairy Research Institute (Mosonmagyaróvár, Hungary), where the fat, protein, lactose and dry matter contents of the milk were measured. Milkoscan FT 120 (Foss Electric) equipment was used for the analysis. The chemical content of the feeds were analyzed according to the Hungarian Feed Codex (2004). Starch content of feed was measured with a polarimeter (Carl Zeiss, Jena, Germany) as described in the Hungarian Feed Codex (2004). Fatty acid profile of the feed and milk samples were determined using Agilent Technologies 6890N (Agilent Technologies, Foster City, CA, USA) gas chromatography according to Hungarian Standards (MSZ ISO 5508:1992). The α-amylase in the assay solution was quantified by using an α-amylase standard curve and the activity was expressed as kilo novo units (KNU) per kilogram.

***Table 1:*** *Ingredients and chemical composition of the diets*

| Item | Control | Amylase |
|---|---|---|
| Ingredient composition, % of DM | | |
| Corn silage | 31.8 | 31.8 |
| Alfalfa haylage | 13.3 | 13.3 |
| Grass hay | 8.2 | 8.2 |
| Dry corn meal | 19.6 | 19.6 |
| Sunflower meal | 7.6 | 7.6 |
| Soybean meal | 4.5 | 4.5 |
| Rapeseed meal | 5.2 | 5.2 |
| Molasses | 3.6 | 3.6 |
| Concentrate * | 2.5 | 2.5 |
| Amylase (g/cow/d) ** | 0.0 | 12.0 |
| Chemical composition, g/kg of DM | | |
| CP | 173 | 175 |
| NDF | 342 | 335 |
| ADF | 197 | 199 |
| ADL | 43 | 45 |
| EE | 42 | 40 |
| NFC | 372 | 379 |
| Starch | 260 | 260 |
| Sugar | 95 | 100 |

* produced by Vitafort Co. (Dabas, Hungary)
** distributed by DSM Hungary Ltd. (Újhartyán, Hungary)

### 2.3 CALCULATIONS

Fat-corrected milk was calculated as FCM (kg/d) = 0.4 × milk, kg/d + 15 × fat, kg/d). Energy-corrected milk was calculated as ECM (kg/d) = milk production kg × (383 × fat% + 242 × protein% + 165 × lactose% + 20.7)/3.140. (1 litre (L) of milk = 1.033 kg of milk).

### 2.4 STATISTICAL ANALYSIS

Evaluation of data was performed by one-factor variant analysis (Kolmogorov-Smirnov test, Levene's test, t-test) with SPSS 19.0 Windows Program (SPSS Inc., Chicago, USA).

## 3 RESULTS AND DISCUSSION

### 3.1 FEED

Composition, analysed nutrition content and fatty acid profile of control and experimental diets (TMR) are summarised in Table 1 and Table 2. Starch content of the diet was in correspondence with Hungarian farm practice (26 % of starch in DM). The assayed α-amylase activity for the control concentrate without enzyme was below limit of detection, and that for the experimental concentrate with enzyme was 561 (472 and 650) KNU/kg of DM. That equates to activity of 323 (271 and 374) KNU/kg of TMR DM for the experimental diet and no enzyme activity for the control diet.

***Table 2:*** *Fatty acid profile of the diets (g/100 g fatty acid)*

| Item | Control | Amylase |
|---|---|---|
| Caprylic acid $C_{8:0}$ | 0.02 | 0.02 |
| Capric acid $C_{10:0}$ | 0.02 | 0.02 |
| Lauric acid $C_{12:0}$ | 0.23 | 0.23 |
| Tridecanoic acid $C_{13:0}$ | 0.24 | 0.23 |
| Myristic acid $C_{14:0}$ | 0.63 | 0.79 |
| Palmitic acid $C_{16:0}$ | 26.23 | 27.53 |
| Heptadecanoic acid $C_{17:0}$ | 0.13 | 0.14 |
| Stearic acid $C_{18:0}$ | 7.61 | 8.34 |
| Arachidic acid $C_{20:0}$ | 0.47 | 0.45 |
| Heneicosanic acid $C_{21:0}$ | 0.02 | 0.02 |
| Behenic acid $C_{22:0}$ | 0.32 | 0.30 |
| **Saturated fatty acids** | **35.88** | **38.07** |
| Myristoleic acid $C_{14:1}$ | 0.07 | 0.08 |
| Palmitoleic acid $C_{16:1}$ | 0.19 | 0.35 |
| Oleic acid $C_{18:1}$ | 23.70 | 23.12 |
| Elaidic acid 9t-$C_{18:1}$ | 0.02 | 0.02 |
| Vaccenic acid c-$C_{18:1}$ | 0.58 | 0.50 |
| Eicosenoic acid $C_{20:1}$ | 0.19 | 0.18 |
| **Monounsaturated fatty acids** | **24.75** | **24.25** |
| Linoleic acid $C_{18:2}$ | 33.02 | 31.35 |
| Linolenic acid $C_{18:3}$ | 4.87 | 4.46 |
| Eicosadienoic acid $C_{20:2}$ | 0.03 | 0.03 |
| Eicosapentaenoic acid $C_{20:5}$ | 0.03 | 0.03 |
| Docosapentaenoic acid $C_{22:5}$ | 0.04 | 0.04 |
| **Polyunsaturated fatty acids** | **37.99** | **35.91** |
| Other fatty acids | *1.38* | *1.77* |

### 3.2 BODY WEIGHT

The BW of animals were increased during the trial (Table 3). Mean values of daily live weight changes were improved in the supplemented group which could probably be explained with the more favourable feed conversion.

### 3.3 MILK YIELD AND MILK COMPOSITION

Milk yield was significantly ($p < 0.05$) increased by 1.0 kg/d per cow when the diet was supplemented with enzyme preparation (Table 3). This result is consistent with Harrison and Tricario (2007) and Klingerman *et al.* (2009). Changes in ruminal fermentation and plasma metabolites suggesting that improved nutrient metabolism may be the cause for increased milk production in α-amylase supplemented cows. No effect of treatment ($p > 0.05$) was observed on the milk DM, milk fat and milk protein while a significant decrease ($p < 0.001$) was measured in lactose content of milk when cows were supplemented with the enzyme preparation (Table 3). This result is differred from Nozière *et al.* (2014) who found increased lactose content attributable to the α-amylase supplement. No effect of treatment ($p > 0.05$) was observed on the fatty acid content of the milk (Table 4).

## 4 CONCLUSIONS

α-Amylase supplementation has the potential to improve milk yield without a reduction in milk fat or milk protein yield. Further research on high-producing cows

***Table 3:*** *Effect of amylase addition on BW, milk yield and composition*

| Item | Control | Amylase |
|---|---|---|
| BW, kg | | |
| At the beginning of trial | 651 | 657 |
| At the end of trial | 685 | 697 |
| Milk yield, kg/d | 37.7 ± **6.96**$^{b}$ | 38.7 ± **6.97**$^{a}$ |
| 4% FCM yield, kg/d | 30.9 | 31.6 |
| ECM, kg/d | 31.3 | 31.9 |
| Milk fat, % | 2.80 ± **0.74** | 2.78 ± **0.79** |
| Milk protein, % | 3.12 ± **0.25** | 3.11 ± **0.25** |
| Milk lactose, % | 4.60 ± **0.16**$^{A}$ | 4.55 ± **0.22**$^{B}$ |
| DM, % | 11.34 ± **0.82** | 11.32 ± **0.94** |

$^{a, b}$ $p < 0.05$; $^{A, B}$ $p < 0.001$

***Table 4:*** *Fatty acid profile of milk produced in the morning (g/100 g fatty acid; n = 12)*

| Item | Control | Amylase |
|---|---|---|
| Caprylic acid $C_{8:0}$ | 1.03 ± 0.05 | 1.02 ± 0.04 |
| Capric acid $C_{10:0}$ | 2.70 ± 0.20 | 2.61 ± 0.21 |
| Undecylic acid C11:0 | 0.34 ± 0.02 | 0.34 ± 0.02 |
| Lauric acid $C_{12:0}$ | 3.62 ± 0.30 | 3.30 ± 0.13 |
| Tridecanoic acid $C_{13:0}$ | 0.21 ± 0.02 | 0.20 ± 0.01 |
| Myristic acid $C_{14:0}$ | 11.91 ± 0.47 | 11.69 ± 0.11 |
| Pentadecylic acid C15:0 | 1.21 ± 0.71 | 1.22 ± 0.02 |
| Palmitic acid $C_{16:0}$ | 32.23 ± 1.26 | 32.72 ± 1.11 |
| Heptadecanoic acid $C_{17:0}$ | 0.76 ± 0.06 | 0.74 ± 0.02 |
| Stearic acid $C_{18:0}$ | 9.63 ± 0.32 | 9.56 ± 0.21 |
| Arachidic acid $C_{20:0}$ | 0.15 ± 0.01 | 0.17 ± 0.01 |
| Heneicosanic acid $C_{21:0}$ | 0.03 ± 0.00 | 0.04 ± 0.01 |
| **Saturated fatty acids** | **63.83 ± 2.46** | **63.61 ± 2.11** |
| Myristoleic acid $C_{14:1}$ | 1.16 ± 0.07 | 1.16 ± 0.04 |
| Palmitoleic acid $C_{16:1}$ | 2.47 ± 0.17 | 2.43 ± 0.05 |
| Heptadecenoic acid C17:1 | 0.25 ± 0.03 | 0.26 ± 0.01 |
| Oleic acid $C_{18:1}$ | 22.95 ± 1.71 | 22.79 ± 0.60 |
| Elaidic acid 9t-$C_{18:1}$ | 1.14 ± 0.25 | 1.25 ± 0.20 |
| Vaccenic acid c-$C_{18:1}$ | 0.55 ± 0.11 | 0.60 ± 0.02 |
| Eicosenoic acid $C_{20:1}$ | 0.12 ± 0.01 | 0.18 ± 0.02 |
| **Monounsaturated fatty acids** | **28.64 ± 1.92** | **28.67 ± 1.31** |
| Linoleic acid $C_{18:2}$ (n-6) | 2.18 ± 0.15 | 2.17 ± 0.10 |
| CLA (c9, t11) | 0.43 ± 0.05 | 0.44 ± 0.02 |
| Alpha-linolenic acid $C_{18:3}$ (n-3) | 0.39 ± 0.05 | 0.40 ± 0.02 |
| Gamma-linolenic acid C18:3 (n-6) | 0.02 ± 0.00 | 0.02 ± 0.00 |
| Eicosadienoic acid $C_{20:2}$ (n-6) | 0.04 ± 0.00 | 0.04 ± 0.00 |
| Dihomo-gamma-linolenic acid C20:3 (n-6) | 0.12 ± 0.01 | 0.12 ± 0.01 |
| Arachidonic acid C20:4 (n-6) | 0.21 ± 0.01 | 0.19 ± 0.01 |
| Eicosapentaenoic acid $C_{20:5}$ (n-3) | 0.03 ± 0.00 | 0.03 ± 0.00 |
| Docosadienoic acid C22:2 (n-6) | 0.02 ± 0.01 | 0.02 ± 0.00 |
| Docosatetraenoic acid $C_{22:4}$ (n-6) | 0.04 ± 0.00 | 0.04 ± 0.00 |
| Docosapentaenoic acid $C_{22:5}$ (n-3) | 0.05 ± 0.01 | 0.06 ± 0.00 |
| **Polyunsaturated fatty acids** | **3.53 ± 0.25** | **3.53 ± 0.11** |
| Other fatty acids | 4.00 | 4.19 |

is necessary to assess the usefulness of this exogenous α-amylase on milk production.

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