INFLUENCE OF EXOGENOUS AMYLASE ON MILK PRODUCTION AND COMPOSITION IN DAIRY COWS

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SUMMARY

The effect of an exogenous rumen-resistant amylase preparation on live weight, milk production and milk composition in Holstein Friesian dairy cows (n=70) was examined in a Hungarian commercial 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. The average days in milk (DIM) at the start of the experiment were 71 days (control) and 72 days (experimental), respectively. 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 α-amylase supplementation significantly ($p<0.05$) improved milk production (control: 37.7±6.96 kg *vs*. experimental: 38.7±6.97 kg), No treatment effect ($p>0.05$) was observed on milk dry matter (DM), milk fat and milk protein but significant decrease ($p<0.001$) was measured in lactose content of milk when cows were supplemented with the enzyme preparation. Exogenous α-amylase supplementation slightly improved ($p>0.05$) live weight changes of the cows during the experiment (control: 0.40 kg/day vs. experimental: 0.48 kg/day).

ÖSSZEFOGLALÁS

Tóthi, R. – Tóth, T.: AMILÁZ ENZIMKIEGÉSZÍTÉS ETETÉSÉNEK HATÁSA A TEJELŐ TEHENEK TERMELÉSÉRE ÉS A TEJ ÖSSZETÉTELÉRE

A szerzők Holstein-fríz tehenekkel (n=70) elvégzett magyarországi üzemi kísérletben vizsgálták egy α-amiláz készítmény etetésének hatását a tehenek élősúly változására, tejtermelésére és a tej fontosabb táplálóanyag-tartalmára (szárazanyag-, fehérje-, zsír- és tejcukor tartalom) vonatkozóan. Az etetett kontroll és kísérleti takarmányadag a magyarországi gyakorlatnak megfelelően kukoricaszilázs, lucernaszenázs és szárított kukoricadara alapú volt. A tejtermelés mérése naponta, a reggeli fejésből származó tejminták kémiai vizsgálata pedig hetente egy alkalommal történt. A kísérletet 3 hét előetetési, majd ezt követően 12 hét vizsgálati szakaszból állt. A kísérletbe vont állatok a laktáció 71. (kontroll), illetve 72. (kísérleti) napján voltak. Valamennyi állat súlya a kísérlet elején (kontroll: 651 kg/tehén; kísérleti: 657 kg/tehén), illetve végén (kontroll: 685 kg/tehén; kísérleti: 697 kg/tehén) megállapításra került. Az α-amiláz enzim etetése szignifikánsan ($p<0,05$) javította a tehenek tejtermelését (kontroll: 37,7±6,96 kg *vs*. kísérleti: 38,7±6,97 kg), de nem volt szignifikáns hatással ($p>0,05$) a tej szárazanyag-, zsír- és fehérje-tartalmára. Ugyanakkor a tejcukor tartalom szignifikáns ($p<0,001$) mértékben csökkent (4,60% *vs*. 4,55%). Az α-amiláz enzimkiegészítés kismértékben ($p>0,05$) javította a tehenek élősúly változását a kísérleti ideje alatt (kontroll: 0,40 kg/nap vs. kísérleti: 0,48 kg/nap).

INTRODUCTION

High producing dairy cows require an energy-dense diet to fulfill their production potential. Therefore starchy cereals are so prevalent in the lactating dairy cow rations. Starch provides approximately 50% of the energy found in corn silage and 75% of the energy in corn grain; therefore the major source of dietary starch for lactating cows is corn (*Allen,* 2012). Corn, oats, barley, and wheat were fed to lactating cows in 94, 18, 14, and 7% of herds, respectively. The first (and the main) site of starch digestion is in the rumen where the starch is fermented by the rumen microbes (*Kotarski et al.,* 1992). The process of starch digestion in the rumen involves α-amylase and isoamylase that are produced by rumen bacteria (*Ruminobacter amylophilus* and *Streptococcus bovis*, followed by *Prevotella ruminicola* and some *Butyrivibrio fibrisolvens* strains). While protozoa and fungi are known to contribute to ruminal starch digestion, their roles are still not clearly defined (*Tricarico et al., 2008*). In order to hydrolyse starch, bacteria must either actively secrete amylase or produce surface associated amylases to hydrolyse starch for transport into the bacterial cell (*Kotarski et al.,* 1992). Starch digestion is usually not considered to be limiting in the rumen and ruminal digested starch is a major source of energy for both the ruminal microbes and the host animal. *In vivo* rumen degradable starch ranges from 60 % for maize and sorghum, to 85 % for oats and up to 90 % for rye, wheat and barley (*Nocek and Tamminga,* 1991). Some researchers have suggested that starch digested post-ruminally may be used more efficiently by the animal than starch digested in the rumen (*Owens et al.,* 1986; *Nocek and Tamminga,* 1991; *Harmon and McLeod,* 2001). Some literature data suggest that there may be limitations in starch digestion, potentially because of inadequate pancreatic amylase secretion (*Nocek and Tamminga,* 1991). However, no animal performance studies have clearly demonstrated that this is the case. The rate and extent of starch digestion in the rumen appear to have an impact on total tract starch digestibility and animal performance (*Lykos et al.,* 1997).

The use of exogenous enzyme supplements for dairy cattle has received much attention during the last several years. Recent studies evaluating the effects of exogenous enzyme supplements for ruminants have mainly focused on fibrolytic activities and rarely on starch digesting activities (*Burroughs et al.,* 1960; *Perry et al.,* 1966). Thus, the lack of information on the effects of amylase-based supplements for ruminants is apparent in the literature. The addition of exogenous amylase to the ration is one method of enhancing ruminal digestibility of both starch and non-starch carbohydrates. Amylase hydrolyses starch in the rumen into mixed oligosaccharides, which are normally fermented to volatile fatty acids by the rumen microflora. The effect of α-amylase on rumen fermentation is believed to be caused by these hydrolysis products providing substrates to non-amylolytic organisms, thereby modifying bacterial populations and volatile fatty acids (VFAs) production (*Tricarico et al.,* 2008). In a study by *Klingerman et al.* (2009), α-amylase enzyme formulations had a relatively stable α-amylase activity in a 24-h *in vitro* ruminal fermentation, which suggested that the enzymes were not subject to extensive degradation by rumen microbes. Several studies have demonstrated that exogenous α-amylase preparations are able to improve organic matter (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). Although the exact mechanism is not known, the addition of amylase to lactating rations has shown an increase in NDF digestibility in the trials of *Gencoglu et al*. (2010) and *Weiss et al.* (2011). Until now *in vivo* experiments with α-amylase have resulted in variable responses in dairy cow performance. *DeFrain et al.* (2005) found that exogenous α-amylase improved energy balance in transition cows but did not affect rumen fermentation. However, *Hristov et al.* (2008) found no benefit in microbial protein synthesis or nutrient digestion when α-amylase was included in diets containing alfalfa hay or silage as the primary forage. In Hungary the main grain source in the diet of lactating dairy cows is ground corn. But the digestibility of starch provided by ground corn is often low, which reduces the digestible energy concentration of the diet. It was assumed that adding exogenous amylase to diets based on corn silage-alfalfa haylage-dried corn based diet would increase milk production and affect milk composition.

MATERIAL AND METHODS

Animals 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. The average DIM at the start of the experiment was 71 days. 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. The diet consisted of a total mixed ration (TMR) based on corn silage, alfalfa haylage and dried corn (*Table 1.*). An exogenous α-amylase preparation (Ronozyme® Rumistar) produced by a genetically modified strain of *Bacillus licheniformis* (DSM 21564) was given to the cows in the experimental group (RSE treatment) daily.

The RSE treatment was designed to provide 561 Kilo Novo Units (KNU) amylase activity per kg grain mix DM and 323 KNU amylase activity per kg of TMR DM. One KNU is the amount of enzyme that releases in a 2-step α-amylase/α-glucosidase reaction, 6 μmol of p-nitrophenol per minute from 1.86 mM ethylidene-G7-p-nitrophenyl-maltoheptaoside at pH 7.0 and 37°C (*Jung* and *Vogel*, 2008). The amylase for the ration (12 g/cow/day) was provided in a dry form (Ronozyme RumiStar, DSM Inc., Basel, Switzerland) and blended into the concentrate grain mix during formulation at the feed mill. During this trial all cows were fed *ad libitum* twice daily at 11.00 and 17.00 h. Water and mineral salt were freely available. Refusals from the previous day were measured and removed prior to feeding. Cows were milked twice daily at approximately 05.00 and 16.30 h and milk production was recorded automatically via computer. Milk samples were taken weekly throughout the trial at consecutive afternoon and morning milkings. Cows were weighed at the beginning

Table 1.

Ration composition and analysed ration nutrient composition

Item (1)	Control (2)	Experimental (3)
Ingredient composition, % of DM (4)		
Corn silage (5)	31.8	31.8
Alfalfa haylage (6)	13.3	13.3
Grass hay (7)	8.2	8.2
Dry corn meal (8)	19.6	19.6
Sunflower meal (9)	7.6	7.6
Soybean meal (10)	4.5	4.5
Rapeseed meal (11)	5.2	5.2
Molasses (12)	3.6	3.6
Glycerine (13)	2.5	2.5
Concentrate* (14)	3.7	3.7
Amylase (g/cow/d)** (15)	0.0	12.0
Chemical composition, g/kg of DM (16)		
CP (17)	173	175
NDF (18)	342	335
ADF (19)	197	199
ADL (20)	43	45
EE (21)	42	40
NFC (22)	372	379
Starch (23)	260	260
Sugar (24)	95	100

* produced by Vitafort Co. (Dabas, Hungary) (24) **distributed by DSM Hungary Ltd. (*Újhartyán*, Hungary) (25)

1. táblázat A kísérlet során etetett takarmányadag komponensei és kémiai összetétele
tétel (1); kontroll (2); kísérleti (3); a takarmányadag komponensei a szárazanyag %-ában (4) kukoricaszilázs (5); lucernaszenázs (6); réti széna (7); kukoricadara (8); napraforgódara (9); szójadara (10); repcedara (11); melasz (12); glicerin (13); koncentrátum (14); amiláz (15); kémiai összetétel; g/kg szárazanyag (16); nyersfehérje (17); NDF (18); ADF (19); ADL (20); nyerszsír (21); nem rostszerű szénhidrátok, NFC (22); keményítő (23); cukor (24); gyártó Vitafort Vitafort Zrt. Első Takarmánygyártó és Forgalmazó Részvénytársaság, Dabas, Magyarország (24); forgalmazta DSM Nutritional Products Hungary, Gyártó és Kereskedelmi Korlátolt Felelősségű Társaság, Újhartyán, Magyarország (25)

and at the end of the experiment after the morning milking. Experimental periods were 12 weeks. Milk production and milk composition were evaluated after a 3 weeks adaptation period to the experimental diets.

Chemical analysis and calculations

The composition of milk was analysed 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. Silage and TMR samples were collected 3 times a week and stored at -20°C. At the end of each week, frozen samples were thawed and composited. Samples of each concentrate mix and hay were collected once a week. Dry matter of all weekly samples was determined following drying for 48 h in a forced-air oven at 60°C, and used for weekly DM adjustments of TMR mixing. The chemical content of the feeds were analysed 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). Amylase activity was measured by DSM Nutritional Products Analytical Services Center (Basel, Switzerland) as described by *Jung and Vogel (2008)*.

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).

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).

RESULTS AND DISCUSSION

Composition and analysed nutrition content of control and experimental diets (TMR) are summarised in *Table 1*. According to Hungarian feeding practice corn silage-alfalfa haylage-dried corn based diet was fed in the experiment. Starch content of the diet was in correspondence with Hungarian farm practice (26% of starch in DM).

Effect of amylase addition on milk production and composition are summarised in *Table 2*. Based on the results milk yield was significantly ($p<0.05$) increased by 1.0 kg/d per cow when the diet was supplemented with enzyme preparation (*Table 2.*). Previous studies have evaluated incorporation of amylase into normal starch rations. The addition of amylase to a 26% starch TMR increased milk production and DMI in the trial of *Klingerman et al.* (2009). Other trials have shown an increase (*Tricarico et al.,* 2005) and a tendency for an increase (*Harrison and Tricarico,* 2007) in milk yield. In the digestibility experiment of *Tricarico et al*. (2006) the amylase enzyme supplement did not alter the rate or extent of starch digestion of the corn silage, but increased the extent of starch digestion of the dry ground corn. In the experiment of *Nozière at al*. (2014) ruminal digestibility of starch increased from 75% in control to 81% with amylase supplementation. *Tricarico et al*. (2006) suggested also that the magnitude of the increase in starch digestibility

of the corn portion of the diet is by itself too small to produce the large increase in milk production. The majority of the increase in milk production can probably be attributed to the effects of the enzyme supplement on proportions of ruminal VFAs. These observations suggest that the mechanism by which supplemental amylase enhances butyrate production in the rumen is probably by modifying the ruminal microbial population. The hypothesis of *Tricarico et al*. (2006) is that addition of supplemental amylase will continually provide soluble sugars that will give a competitive advantage to butyrate producing organisms over lactic acid producing starch digesters. Approximately 74 to 90% of the butyrate produced in the rumen is metabolized by the rumen epithelial tissue (*Remond et al*., 1995) with a large portion converted to ketone bodies, mainly β-hydroxybutyric acid. The increased concentration of β-hydroxybutyric acid in blood represents an additional source of energy for milk synthesis in enzyme-supplemented animals. Both *Tricarico et al.* (2005) and *DeFarin et al.* (2005) noted changes in ruminal fermentation suggesting that improved nutrient metabolism may be the cause for increased milk production in amylase supplemented cows.

No effect of treatment ($p>0.05$) was observed on the milk DM, milk fat and milk protein (Table 2). When exogenous amylase was included in a normal starch ration, *Tricarico et al.* (2005) also reported no difference in milk protein, while *Klingerman*

Table 2.

Effect of amylase addition on body weight (BW), milk production and composition

Item (1)	Control (2)	Experimental (3)
BW, kg (4)		
At the beginning of trial (5)	651±59.4	657±77.9
At the end of trial (6)	685±59.5	697±83.1
Milk yield, kg/d (7)	37.7 ± 6.96^{b}	38.7 ± 6.97^{a}
4% FCM yield, kg/d (8)	30.9	31.6
ECM, kg/d (9)	31.3	31.9
Milk fat, % (10)	2.80±0.74	2.78±0.79
Milk protein, % (11)	3.12±0.25	3.11±0.25
Milk lactose, % (12)	4.60 ± 0.16^{A}	4.55 ± 0.22^{B}
Urea, mg/100 g (13)	18.80±2.71	19.03±2.75
DM, % (14)	11.34±0.82	11.32±0.94

^{a,b} figures with different superscript in the same row differ significantly ($p<0.05$) (15)
^{A,B} figures with different superscript in the same row differ significantly ($p<0.001$) (16)

2. táblázat Az amiláz kiegészítés hatása a tehenek élősúlyára, tejtermelésére és a termelt tej összetételére

tétel (1); kontroll (2); kísérleti (3); testsúly, kg (4); a kísérlet kezdetén (5); a kísérlet végén (6); tejtermelés, kg/nap (7); 4 % zsírtartalommal átszámított tejmennyiség, kg/nap (8); energia tartalomra korrigált tej, kg/nap (9); tejzsír, % (10); tejfehérje, % (11); tejcukor, % (12); karbamid, mg/100 g (13); szárazanyag, % (14); az azonos sorban különböző betűvel jelölt értékek között szignifikáns különbség van ($p<0,05$) (15); az azonos sorban különböző betűvel jelölt értékek között szignifikáns különbség van ($p<0,001$) (16)

et al. (2009) reported higher protein yield in amylase supplemented cows. *Tricarico* (2007, unpublished data) dosed 0, 12, 24, or 36 g/day of an amylase preparation to 24 Holstein cows fed a corn silage-ground corn diet. The addition of 12 g/day of amylase increased ground corn digestibility after 24 hrs, increased milk fat yield, and increased milk protein yield.

In our treatment significant decrease ($p<0.001$) was measured in lactose content of milk when cows were supplemented with the enzyme preparation (*Table 2.*). This result is differed from *Nozière at al.* (2014) who found increased lactose content attributable to the α-amylase supplement. *Weiss et al.* (2011) reported that cows fed a diet with reduced starch and amylase treatment had lower lactose percentages compared to cows fed the high starch ration. They also found an effect of starch on lactose yield with cows fed the higher starch ration yielding greater lactose than those fed the reduced starch rations. While not statistically significant, cows on the amylase treatment gained 6 kg more body weight (BW) than control cows (*Table 2.*) we suspect that this increase in weight change may have been due to amylase treatment.

CONCLUSIONS

Based on the results of this study the addition of supplemental amylase to a diet containing corn silage and ground corn has the potential to improve milk yield without a reduction in milk fat or milk protein yield. Further research is required to determine the potential effects of amylase on ruminal fermentation.

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