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The Use of Exogenous Enzymes in Dairy Cattle on Milk Production and their Chemical Composition: A Meta-Analysis[#]

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ABSTRACT

Ortiz-Rodea, A., Noriega-Carrillo, A., Salem, A.Z.M., Castelan Ortega O. and González-Ronquillo, M. 2013. The use of exogenous enzymes in dairy cattle on milk production and their chemical composition: a meta-analysis. Animal Nutrition and Feed Technology, 13: 399-409.

We performed a meta-analysis to evaluate the effect of the addition of exogenous enzymes in ruminant feeding on milk production and chemical composition. We analysed the observations of 29 experiments, which included 52 treatments, 9 enzymes, and 1187 animals; with this information, we arranged a comprehensive database. The dose and study were used as experimental approaches. We observed that the addition of enzyme has no effect on the increment in milk yield production (P=0.16), fat content (P=0.88), lactose (P=0.39) or protein (P=0.95). The study showed that the variable milk yield is not a good parameter for determining with respect to the administration of exogenous enzymes (R^2=0.001). As a conclusion, it is necessary to reconsider the use of exogenous enzymes in domestic ruminants when the focus is to improve milk production and their chemical composition.

Key words: Enzymes, Meta-analysis, Milk yield, Ruminants

INTRODUCTION

Animal feeding is considered the major source of economic expenditures when referring to the production of milk and dairy products because they require high external inputs that allow us to keep elevated and constant production levels. Thus, milk production is not limited to dairy cattle only; also participating are domestic species such as sheep, goats, and in some regions such as Southeast Asia and Europe, native species such as the buffalo. Therefore the amount of feed required to maintain these productive farms, increases constantly and the agricultural surface area in the best of the cases is only maintained or it is decreasing. This is where the problem arises to maintain production and quality of milk yield and milk products with the least amount

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of feed inputs. That is why it is necessary to make the nutrition of the animal more efficient, maximizing rumen activity and seeking to achieve sustainable production units. Thus ruminants exhibit endogenous enzymatic digestion, which allows them to obtain nutrients from food with complex structure (Pariza and Cook, 2010). Because of the benefits observed with these enzymes, several studies have tried to replicate this natural action mechanism by the addition of exogenous enzymes. The aim of this study was to conduct a meta-analysis to evaluate the effect of the addition of exogenous enzymes in feed for dairy cattle and its effects on the milk yield production and chemical composition

MATERIALS AND METHODS

Database development

The information search was focused on studies of exogenous enzymes supplementation in dairy cattle, and their effects on milk yield production and chemical composition to approach the number of studies recommended for this type of methodology (St Pierre, 2001). A database was conducted from experiments where both enzyme and dairy cattle, were specified from research published in scientific journals (Sauvant *et al.*, 2008). This included publications which were obtained from the ISI Web of Science database, Scopus, Redalyc, Routledge-Taylor and Francys Group, Science Direct and SpringerLink using the following keywords: exogenous enzymes, ruminants, milk yield, "enzymes and exogenous and ruminants," "enzymes and milk production," "enzymes and ruminants or dairy cattle." Additionally in the database, the following variables were recorded: number of animals in the study, basal diet, the enzyme used and its source, trade name of the enzyme, route of administration, dosage of enzyme (g/kg $LW^{0.75}$), milk yield production (kg/kg $LW^{0.75}$), and their chemical composition: protein, fat content, lactose (g/100g), and treatment duration (days).

We obtained a total of 29 studies, which included 52 experimental doses (Table 1) that provided the data for developing the basis of analysis. Sources of enzymes used in the studies were cellulase, xylanase, endoxylanase, amylase, protease, hemicellulase, exoglucanase, endoglucanase and glucanase. A total of 1187 animals were used for the studies analysed.

Statistical analysis

Analysis of the database was performed using a statistical approach meta-analysis (St-Pierre, 2001; Sauvant *et al.*, 2008). Using the MIXED procedure of SAS (version 9.2, SAS Institute Inc., 2008), the mixed model analysis used was $Y_{ij}=B_0 + B_1X_{ij} + s_i + b_iX_{ij} + e_{ij}$, where Y_{ij}=dependent variable, B_0=general intercept of all experiments (milk yield, fat, lactose and protein content), B_1=coefficient of linear regression coefficient of Y on X (exogenous enzyme), X_{ij}=value of the continuous predictor variable (exogenous enzyme dosage), s_i=random effect of study i, b_i=random effect of study i on X in study i, and e_{ij}=residual error not explained.

Table 1. Studies included in the meta-analysis of the effect of the addition of exogenous enzymes on milk yield production and chemical composition of dairy cattle.

Study	Reference	No. of animals	Basal feed	Enzyme	Enzyme source	Commercial enzyme product	Administration way	Enzyme dose (g/kg LW$^{0.75}$)	Trial duration (days)	kg LW $^{0.75}$
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
1	Arriola *et al.*, 2011	60	Forage, concentrate	Cellulase, xylanase and esterase	*Trichoderma longibrachiatum*	-	Sprayed on to TMR	0 and 3.4	63	118.19-120.32
2	Beauchemin *et al.*, 1999	4	Forage, concentrate	Cellulase, pectinase, xylanase	-	*Pro-Mote*®	Sprayed on to the TMR	0 and 2.50	23	122.14
3	Beauchemin *et al.*, 2000	6	Concentrate	Cellulase, endocellulase, endoglucanase, exoglucanase, xylanase		Natugrain® 33-L	Added to the concentrates	0, 1.22 and 3.67	21	128.88-129.33
4	Bernard et al, 2010	44	Corn silage and alfalfa or T85 haylage	Celllulase	-	*Promote ®N.E.T.*	Applied to TMR	0 and 0.006	56	127.78-137.20
5	Bowman *et al.*, 2002	8	Forage, concentrate mixture	Cellulase, xylanase	-	*Promote ®N.E.T.*	Added to concentrate and premix, pelleted	0 and 0.0015	28	128.58
6	DeFrain *et al.*, 2005	24	Hay, haylage, concentrate	Endoglucanase, xylanase	*Saccharomyces cerevisiae* and *Aspergillus oryzae*	-	Added to TMR	0 and 0.017	21	114.04-115.27
7	Dhiman *et al.*, 2002	50	Hay, silage, concentrate	Cellulase, xylanase	-	*Bovizyme*®	Applied to the forage	0, 0.002 and 1.3	270	128.91
8	Elwakeel *et al.*, 2007	24	Silage, hay, concentrate	Fibrolytic	-	-	Mixed in ration	0 and 5-15	56	122.59-124.55

Table 1. Contd. .....

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
9	Eun *et al.*, 2005	8	Forage, concentrate	Protease	*Bacillus licheniformis*	*Protex®6L*	Sprayed on to the pelleted supplement	0 and 1.25	21	134.63
10	Ferraretto *et al.*, 2011	45	Forage	Amylase	-	*Ronozyme RumiStar®*	Added to concentrate	0 and 3.8	70	139.72-140.87
11	Gado *et al.*, 2009	20	Corn silage, concentrate	Amylase, cellulase, protease, xylanase	Anaerobic ruminal bacteria	*ZADO®*	Added to TMR	0 and 40	84	105.74
12	Gencoglu *et al.*, 2010	36	Corn silage, alfalfa silage, concentrate	Amylase	-	*Ronozyme RumiSta®r*	Sprinkled on to concentrate	0 and 1250	84	134.92-135.94
13	Holtshausen *et al.*, 2011	60	Silage, hay, concentrate	Endoglucanase, xylanase	-	*Econase® RDE*	Added to TMR	0 and 0.5-1	70	114.04-115.27
14	Klingerman *et al.*, 2009	28	Concentrate, silage, hay, haylage	Amylase	*Saccharomyces cerevisiae, Aspergillus oryzae, A. oryzae*	*7B enzyme formulation*	Sprayed on to concentrate	0, 0.88 and 4.40	21	143.03-144.18
15	Knowlton *et al.*, 2002	34	Forage, concentrate	Celllulase	Commercial preparation from fungal extracts	-	Added to TMR	0 and 0.204	28	117.73-126.35
16	Knowlton *et al.*, 2007	24	Corn silage, alfalfa silage	Cellulase, phytase	Fungal extracts	-	Mixed with a corn grain carrier	0 and 0.297	31	120.59-121.43
17	Kung *et al.*, 2000	60	Corn silage, alfalfa, pelleted concentrate	Cellulase, hemicellulase, xylanase	*Trichoderma longibrachiatum*	-	Sprayed on to forages	0, 2 and 10	84	119.10-129.77
18	Kung *et al.*, 2002	30	Corn silage, alfalfa, pelleted concentrate	Cellulase, xylanase	-	-	Sprayed on to forages	0 and 10	84	129.03-129.62

Table 1. Contd. …..

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
19	Lewis *et al.*, 1999	70	Alfalfa silage, hay, concentrate	Cellulase, xylanase	-	*Cornzyme®*	Applied to the forage	0, 1.25 and 5.0	21 and 112	121.23-131.54
20	Miller *et al.*, 2008	72	Concentrate	-	*Trichoderma longibrachiatum*	*Roxazyme® G2 Liquid*	Applied to barley or sorghum	0, 2.15 and 4.3	75	111.86-112.80
21	Pinos-Rodríguez *et al.*, 2005	40	Forage, concentrate	Xylanase	*A. niger y T. viridae*	*Fibrozyme®*	Added to concentrate mixture	0 and 1.3	120	121.23
22	Reddish *et al.*, 2007	24	Silage, hay, concentrate	Cellulase, xylanase	-	-	Mixed by hand into the TMR	0 and 0.016	21	119.86
23	Rode *et al.*, 1999	20	Silage, hay, concentrate	Cellulase, xylanase	-	*Pro-Mote®*	Diluted and added to concentrate	0 and 1.3	84	126.65-131.40
24	Sutton *et al.*, 2003	4	Silage, concentrate	Endoglucanase, xylanase	*Trichoderma longibrachiatum*	-	Sprayed on to the TMR	0 and 2	35	124.7
25	Vicini *et al.*, 2003	233	Silage, hay, concentrate	Fibrolytic	*Trichoderma longibrachiatum*	-	Sprayed on forage and mixed ration	0, 0.00038 and 2	84	121.23
26	Weiss *et al.*, 2011	28	Silage, concentrate	Amylase	-	*Ronozyme RumiStar ®*	Added to concentrate mix	0 and 0.125	98	123.35-127.54
27	Yang *et al.*, 1999	4	Concentrate, silage, hay	Cellulase, xylanase	-	*Pro-Mote®*	Sprayed on to hay	0, 1 and 20.8	21	124.10-125.15
28	Yang *et al.*, 2000	43	Concentrate, hay, silage	Cellulase, xylanase	*Trichoderma longibrachiatum*	-	Sprayed on to TMR or applied to concentrate	0 and 0.5	105	136.09
29	Zheng *et al.*, 2000	48	Forage, concentrate	Xylanase	-	*Bovizyme®*	Sprinkled on to the forage	0 and 1.25	126	122.74-130.21

TMR: Total Mixed Ration.

The variable in the study was determined in the level CLASS. This presented no quantitative data, and we determined the structure for unstructured matrix of the covariance (TYPE=UN), and this was specified in the random model to avoid positive correlation between intercepts and slopes. In addition, we calculated the standard deviation, the P value, the standard error mean (SEM) and the coefficient of determination. In reference to the graphic representation of the results of the meta-analysis, an adjustment of the response variables was made, taking into account the random effect of the study. Similarly, variables were standardized in relation to the metabolic live weight ($LW^{0.75}$) to avoid variation between studies.

RESULTS

The mixed analysis showed 67 enzyme dosages with a range of 0.0002 to 3.48 g/kg $LW^{0.75}$. However, for the variable response milk yield production, there were no differences ($P>0.16$) between doses. Also, the coefficient of determination was lower, between the enzyme doses and the milk yield production response as shown in Fig. 1.

The chemical composition of milk showed no significant differences ($P=0.88$) for fat component (Fig. 2), presenting a low coefficient of determination, which indicates the poor relationship between the administration of the enzyme and milk fat composition, with a negative effect. Similar results were obtained when the component lactose was determined (Fig. 3), ($P=0.39$). Also there were no differences ($P=0.95$) in the crude protein content in milk (Fig. 4) due to the addition of enzymes to the feed.

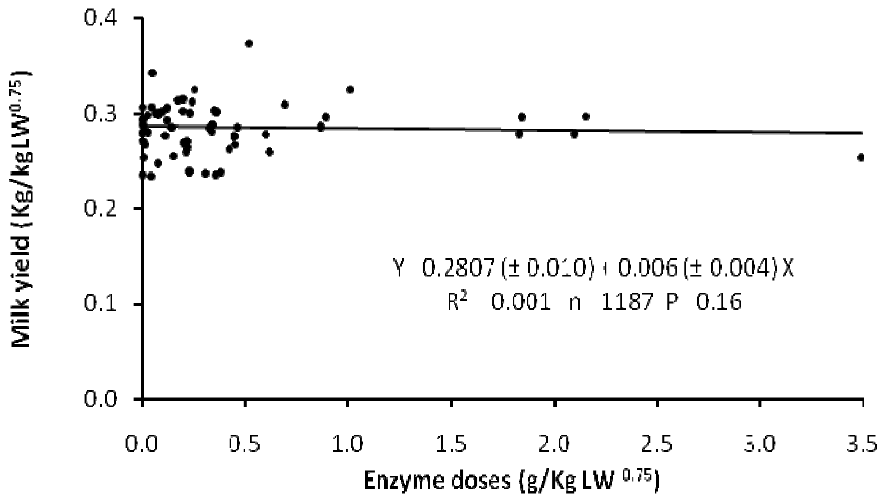

Fig. 1. Effect of the addition of exogenous enzymes (g/kg $LW^{0.75}$) on milk yield production (kg/kg $LW^{0.75}$) in dairy cattle.

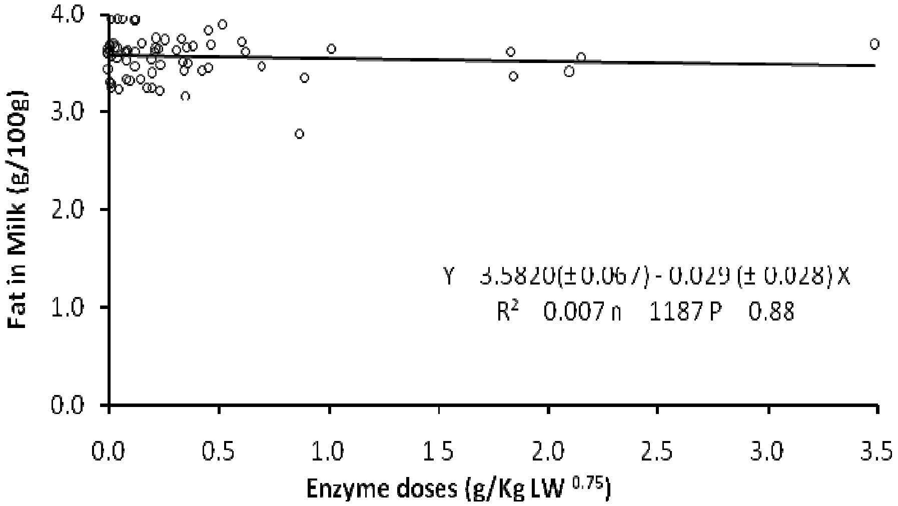

Fig. 2. Effect of exogenous enzymes intake (g/kg $LW^{0.75}$) on the composition of milk fat (g/100g) in dairy cattle.

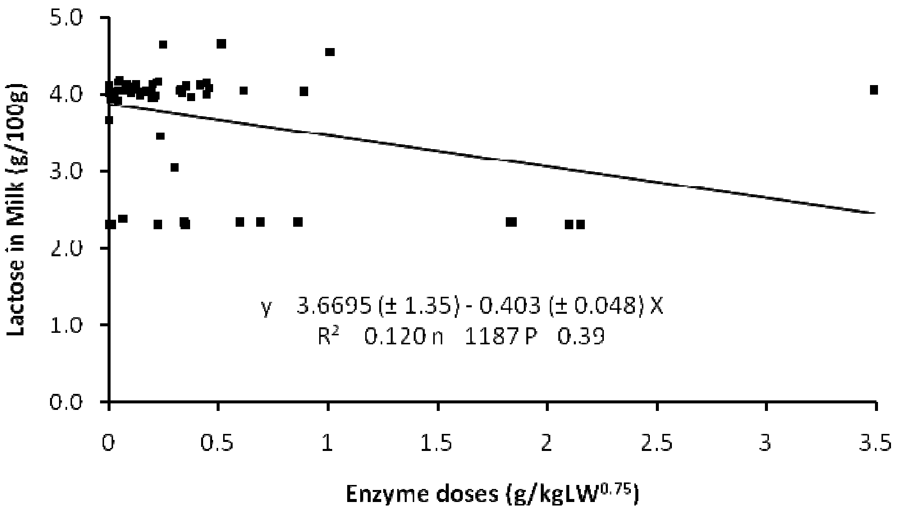

Fig. 3. Effect of exogenous enzymes intake (g/kg $LW^{0.75}$) on the content of lactose in dairy cattle milk (g/100g).

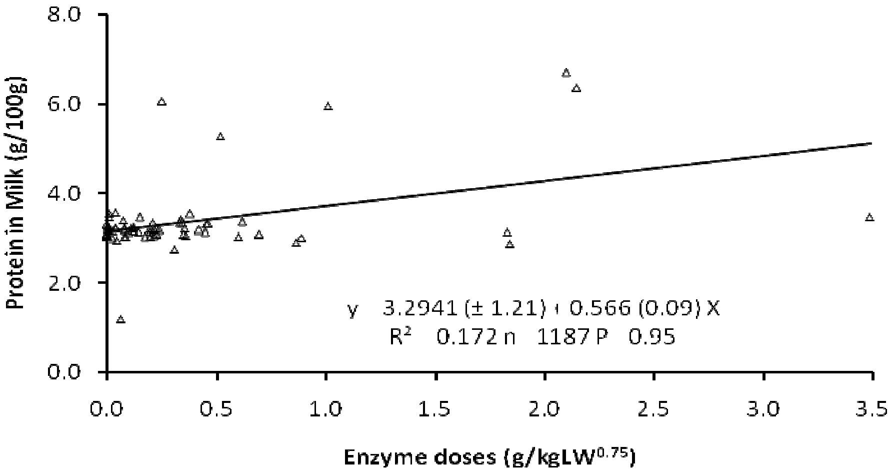

Fig. 4. Effect of exogenous enzymes intake (g/kg $LW^{0.75}$) on milk protein content (g/100g) in dairy cattle.

DISCUSSION

The analysis indicates there was no effect of the addition of exogenous enzymes in the animal feed offered to the variables on milk yield production; these results are consistent with Flores *et al.* (2008), Bowman *et al.* (2002), Beauchemin *et al.* (1999) and Rode *et al.* (1999). In contrast, studies by Titi and Stella found an effect when utilizing enzymes, with an increase in the amount of milk yield produced by goats fed with supplementation of yeast culture. Similar results are shown by Kung *et al.* (2000), Lewis *et al.* (1999), Zheng *et al.* (2000) and Yang *et al.* (1999; 2000) in dairy cattle. The results that show no effect when supplemented by enzymes can be influenced by the dose and type of enzyme. Kung *et al.* (2000) suggests that overdose of enzymes causes decreased chewing due to an increase in the digestibility of the feed; this, in turn, decreases the production of saliva, ruminal pH and thus generates less fiber digestion, resulting in less amount of milk yield produced; meanwhile Treacher *et al.* (1996) suggests that excessive doses of enzymes affect the ruminal micro-organisms adhering to the substrate and also promote the release of anti-nutritional factors as secondary compounds, thereby reducing the microbial digestion. Moreover the combination of exogenous enzymes (Morgavi *et al.*, 2001) is capable of withstanding the ruminal and intestinal proteolysis, such is the case of compounds derived from *Trichoderma longibrachiatum* fungus.

The analysis showed no effect in the milk fat content by the addition of enzyme, however, the slope was negative, indicating that a higher enzyme doses diminish the milk fat content; this effect coincides with Kung *et al.* (2000), Rode *et al.* (1999), and Stella *et al.* (2007) who found a decrease in the milk fat content of animals that were fed various doses of enzyme. Meanwhile Beauchemin *et al.* (1999), Flores *et al.* (2008), and Titi *et al.* (2004) indicate no effect in the milk fat content by the addition of enzyme. In contrast Bowman *et al.* (2002) found an increased milk fat component when supplemented by enzyme in the food of dairy cattle. The lactose content was not affected by the addition of exogenous enzymes; these results are consistent with Beauchemin *et al.* (1999) and Rode *et al.* (1999), but differ from Bowman *et al.* (2002) who found an increased lactose content. The milk protein content coincides with Flores *et al.* (2008), Titi *et al.* (2004), and Rode *et al.* (1999) who indicate no effect on the amount of enzyme protein in milk. The absence of increased protein content in milk can be caused by changes in protein metabolism in the rumen; studies by Yang *et al.* (1999) mention that the fibrolytic enzymes increase the degradation of dietary protein in the rumen, which in turn increases the synthesis of microbial crude protein. Meanwhile Rode *et al.* (1999) found that the increase in the endogenous protein is due to the catalytic effect of enzymes on the exogenous protein, causing insufficient protein levels on step. This greater amount of imbalance and microbial protein of lower protein content of the input step has an effect on amino acids in milk, which according with Chalupa *et al.* (2000) is 50 to 55% of amino acids originating from microbial protein and from 45 to 50% amino acids provided by the rumen undegradable protein. On the other hand Kung *et al.* (2000) found a negative effect on the protein with the inclusion of enzymes; on the contrary Bowman *et al.* (2002), showed an increase in this parameter.

CONCLUSION

The parameter milk yield production and their components of fat, lactose and protein have no effect to the administration of exogenous enzymes. It is necessary to reconsider its use in ruminants when the aim is to increase milk yield production and their chemical composition.

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