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Effects of Live Yeast and *Aspergillus niger* Meal Extracted Supplementation on Milk Yield, Feed Efficiency and Nutrients Digestibility in Holstein Lactating Cows

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Abstract: Twenty four Holstein cows in early lactation (days in milk: 24±4) were allocated equally to one of four treatments. Treatments were as follows: control basal diet without supplement; LY basal diet supplemented with 10 g of live yeast; ANX basal diet supplemented with 30 g of *Aspergillus niger* extracted meal and LY+ANX basal diet supplemented with 10 g LY and 30 g ANX. Daily individual milk production and feed intake recorded and weekly milk samples were taken for milk composition analyse. Fecal grab samples were collected during 3 consecutive days at the end of experiment period from each cow. The experimental period lasted 75 days. Milk production was improved by 6.8% for group fed the LY compared control diet ($p<0.05$). Fat-corrected milk, feed efficiency, body weight and body condition score changes and DM intake were similar between groups. Greater protein yield and percentage were observed in the LY and LY+ANX groups compared to the control group ($p<0.05$). No differences were observed in other milk composition between groups. The apparent digestibility of DM and OM were higher ($p<0.05$) in the LY+ANX supplemented diet compared to the control. Similar to DM and organic matter digestibility, greater NDF apparent digestibility was observed in the LY+ANX supplemented diet versus the control that might be due to synergistic effects of feeding LY and ANX together. We concluded that LY might have potential as an effective direct-fed microbial to increase milk production in early lactating cows.

Key words: Live yeast, *Aspergillus niger* meal extracted, milk yield, feed efficiency, digestibility, lactating cows

INTRODUCTION

Interest in the use of safe feed additives as rumen manipulators to increase animal productivity has become widespread over the last 15 years. Yeast cultures and live yeast such as *Saccharomyces cerevisiae* are widely used in diets of lactating dairy cows. Results of numerous studies with yeast supplementation to diets of lactating cows are variable and inconsistent (Moallem *et al*., 2009; Dann *et al*., 2000; Arambel and Kent, 1990).

In some studies, yeast culture increased DMI (Moallem *et al*., 2009; Nocek and Kautz, 2006; Dann *et al*., 2000) but others reported no response in DMI with yeast culture (Bruno *et al*., 2009; Di Francia *et al*., 2008; Kung *et al*., 1997; Swartz *et al*., 1994).

Similarly, the effects of yeast on milk production have been variable. In some studies, greater milk production was reported (Bruno *et al*., 2009; Moallem *et al*., 2009; Arambel and Wiedme, 1986) whereas in other investigations no effect of yeast on production was observed (Dann *et al*., 2000; Wang *et al*., 2001). Other benefits of yeast product supplementation in ruminant nutrition have been as increase in milk fat percentage (Moallem *et al*., 2009) and improvement of nutrient digestibility (Jouany *et al*., 1998; Wohlt *et al*., 1991). In addition to yeast culture, fungal fermentation extract (*Aspergillus oryzae*) are widely used to improve the production of dairy cows.

Huber *et al*. (1994) reported that a culture of *Aspergillus oryzae* improved milk yields of lactating cows. However, some researchers have not been shown this benefit (Higginbotham *et al*., 1994). Most of studies on fungi additives have been performed with *Saccharomyces cerevisiae* and *Aspergillus oryzae* whereas there is no data available on the effects of including an *Aspergillus niger* extract in the diet of dairy cows.

The addition of a mixture of Live Yeast (LY) and ANX might have a synergistic effect over the use of either LY or ANX alone. Therefore the objective of the current study was to determine the effects of LY and ANX on milk yield, intake and feed efficiency in early lactating cows.

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MATERIALS AND METHODS

About 24 (Holstein cows (8 primiparous and 16 multiparous) in early lactation (DIM: 24±4 days) were allocated equally (2 primiparous and 4 multiparous in each treatment group) to one of four treatments on the basis of DIM, the average milk production during pre-treatment period, parity and body weight. The treatments were as follows: control: cows were fed a basal diet (Table 1) supplemented with 100 g of wheat bran per day per cow; LY: cows were fed the basal diet supplemented with 10 g of LY (Biosaf, Lesaffre Feed Additives, Lille, France), premixed with 90 g of wheat bran; ANX: cows were fed the basal diet supplemented with 30 g of *Aspergillus niger* exteracted meal (Bospro, PetAg Inc., Hampshire, USA), premixed with 70 g of wheat bran and LY+ANX: cows were fed the basal diet supplemented with 10 g LY and 30 g ANX, premixed with 60 g of wheat bran. According to the manufacture's information, LY contained *Saccharomyces cerevisiae* SC47 strain which the quantity of yeast given by 1 g of product was 10^{10} cfu. Bospro is made of fermentation soluble meal extracted from *Aspergillus niger* which used in this study (Table 2).

Cows housed in the individual tie stalls and were fed the total mixed ration (basal diet) twice daily at 7 and 17 h for *ad libitum* intake with free access to water. The supplementations were top-dressed on the total mixed ration at morning meal. The experimental period lasted 75 days (15 days adaptation), started in early-December and continued until at the end of February 2008. Daily individual feed intake recorded. Feed efficiency was determined by calculating the kilograms of FCM (3.5%) yield per kilogram of DMI for each animal in each treatment group. Cows were weighed at the start and the end of the experiment. Body condition score (1-5 scale; Edmonson *et al*., 1989) was determined every 15 days.

Feed and orts samples were collected weekly and composited monthly. The samples were grounded through a 1 mm screen (Philadelphia, PA) and were analyzed for DM, CP (AOAC, 1990) and NDF (Van Soest *et al*., 1991). Cows were milked 3 times daily and milk production was recorded. Milk composition was analyzed weekly for milk protein, fat, lactose, solid not fat and urea by Milk-O-Scan (Foss Electric, Denmark). Individual feed and fecal grab samples were collected prior to the feeding for a 3 days at the end of the experiment and composited to measure total tract apparent nutrient digestibility. Fecal samples were dried in a 60°C forced air oven for 72 h and then at 100°C for 24 h. Samples were then grounded through a 2 mm screen (Philadelphia, PA) and analyzed for DM, CP, NDF and ash content. Acid Insoluble Ash (AIA) was used as an internal marker to determine apparent total tract digestibility (Van Keulen and Young, 1977). Blood samples were collected from each cow at the end of experiment after the morning meal by puncture of the median coccygeal vein into evacuated tubes containing heparin (Becton Dickinson, Franklin Lakes, NJ). Samples were immediately placed on ice and tubes were centrifuged at 3000×g for 15 min at for plasma separation. Plasma was frozen at -20°C and later analyzed for glucose, Nonesterified Fatty Acids (NEFA), triglyceride, urea N, phosphorous, magnesium and potassium concentrations. These parameters were analyzed using appropriate kits and an ABX Mira Auto analyzer (ABX Mira, cedex 4, France).

Data such as milk yield, milk composition and DMI were analyzed as repeated measurements using Proc Mixed (SAS Institute, 2000) with the following model:

$$Y = \mu + T_i + P_j + C(T \times P)_{ijk} + time_l + DIM_{ijklmn} + pmilk_{ijklmn} + E_{ijklmn}$$

Table 1: Ingredient and nutrient composition of diet

Ingredients	Amount (DM%)
Corn silage	19.51
Alfalfa hay (chopped)	19.51
Corn grain (ground)	9.76
Beet pulp	7.10
Barely grain (ground)	14.19
Soybean meal	12.42
Canola meal	9.76
Wheat bran	2.18
Fat powder^{-1}	1.90
Sodium bicarbonate	0.92
Calcium carbonate	0.93
Dicalcium-phosphate	0.45
White salt	0.45
Mineral and vitamin premix[2]	0.92
Chemical composition	
NEl (Mcal kg^{-1})	1.65
CP	17.78
RDP (CP%)	67.80
NDF	32.20
ADF	26.00
Ca	0.93
P	0.50

[1]Rumen protected palm fat, Energizer RP100, [2].Mineral and vitamin premix contained: 195 g kg^{-1} of Ca, 80 g kg^{-1} of P, 21 g kg^{-1} of Mg, 2.2 g kg^{-1} of Mn, 3 g kg^{-1} of Fe, 300 mg kg^{-1} of Cu, 100 mg kg^{-1} of Co, 120 mg kg^{-1} of I, 11 mg kg^{-1} Se, 60,0000 IU of vitamin A kg^{-1}, 200,000 IU of vitamin D3 kg^{-1}, 2000 IU of vitamin E kg^{-1}, 400 mg of antioxidant kg^{-1}. [3]Calculated using NRC recommendations

Table 2: Nutrient composition of Bosporo

Chemical composition[1]	DM basis
CP	17.5
Fat	1
Fiber	38
Ash	9
Moisture	9
Carbohydrate	25.5
Cobalt	0.0004
Iodine	0.01
Total	100

[1]Catalogue

Where:

μ = Overall mean
T_i = Treatment effect
I = 1-4
$C (T \times P)_{ijk}$ = Cow_k nested in treatment and cow nested in $parity_j$
$time_l$ = Date of measurement
DIM_{ijklmn} = Days in milk as a covariate variable
$pmilk_{ijklmn}$ = Average milk production during pre-treatment period as a covariate variable
E_{ijklmn} = Random residual

The interactions such as treatment x parity, treatment x time, treatment x parity, parity x time and treatment x parity x time were tested for each dependent. Not to be significant for each interaction as mentioned above, therefore they were omitted from the model. For nutrient digestibility, BW and BCS changes and blood metabolites no repeated measures were used otherwise, these values were analyzed using the GLM procedure of SAS Institute (2000). The level of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

There were no significant interactions such as parity by treatment, parity by time, treatment by time and treatment by time and parity thus, only least squares means for main effects are shown (Table 3 and 4). Least squares means for milk production, 3.5% FCM, changes in BW and BCS and feed efficiency for the four treatment groups are shown in Table 3. Also because no treatment x parity effect was significant for any independent variable, the results for primiparous and multiparous cows are present together. There were no significant differences observed for daily DMI between cows fed a control diet and those fed supplemented diets. Changes in BW and BCS were unaffected by the experimental diets. The average daily milk production was greater in the LY group than control group ($p<0.01$). Although, the LY supplemented cows had higher milk production ($p<0.01$), no differences in FCM 3.5% occurred between treatments (Table 3). Feed efficiency as was expressed by milk yield of FCM 3.5% from DMI was similar between groups. Milk composition and yield are shown in Table 3. The average fat yield and fat percentage were not different between groups. Greater protein yield and protein percentage were observed in cows fed LY and LY+ANX than those fed control ($p<0.005$). No differences were observed in other milk composition and yield (lactose, SNF, TS and urea) between groups.

The apparent digestibility of DM and OM were higher ($p<0.05$) in the LY+ANX supplemented diet compared to the control. CP digestibility tended to be higher ($p = 0.10$) in cows receiving LY. Similar to DM and OM digestibility, greater NDF apparent digestibility was observed in the LY+ANX supplemented diet versus the control. Concentrations of glucose, Nonesterified Fatty Acids (NEFA), triglyceride, urea N, phosphorous, magnesium and potassium were not influenced ($p>0.05$) by experimental diets (Table 5). The difference in mean daily milk production in LY supplemented group was 6.8% greater compared to the control group. Significant increase in daily milk production of cows supplemented with LY, have been previously reported (Bruno *et al*., 2009; Moallem *et al*., 2009; Nocek and kautz, 2006). However, some researchers reported no response in milk yield (Schingoethe *et al*.,

Table 3: Least squares means of DMI, milk yield and composition, feed efficiency and changes in BW and BCS of cows fed experimental diets[1]

	Treatments				
Parameters	Control	LY	ANX	LY+ANX	SEM[2]
Cows (n)	6.00	6.00	6.00	6.00	
DMI (kg day^{-1})	21.54	21.44	20.41	21.83	1.29
Milk yield (kg day^{-1})	37.16[b]	39.69[a]	37.11[b]	39.60[a]	0.03
FCM 3.5% (kg day^{-1})	38.49	39.22	36.94	37.47	0.59
Milk composition					
Fat (%)	3.76	3.44	3.32	3.19	0.24
Protein (%)	2.76[b]	2.90[a]	2.72[a]	2.88[a]	0.02
Lactose (%)	4.74	4.59	4.51	4.58	0.03
SNF (%)	7.59	8.02	7.80	7.94	0.28
TS (%)	11.35	11.46	11.12	11.13	0.13
Urea (mg dL^{-1})	17.75	17.07	17.5	17.05	0.47
Milk composition yield (kg day^{-1})					
Fat	1.38	1.36	1.23	1.26	0.03
Protein	1.04[b]	1.13[a]	1.00[b]	1.14[a]	0.09
Lactose	1.76	1.82	1.67	1.81	0.03
Feed efficiency[3]	1.78	1.82	1.76	1.71	0.04
BW changes (kg day^{-1})	-0.67	-0.83	-0.43	-0.43	0.10
BCS changes	-0.21	-0.26	-0.19	-0.21	0.10

[1]Treatment groups include; basal diet (control), basal diet supplemented with Live Yeast (LY), *Aspergillus niger* meal extracted (ANX) or a mixture of LY and ANX (LY+ANX); [2]Standard error of means. [3]Kg of FCM 3.5% kg^{-1} of DM, [a, b]Means within a row lacking a common superscript letter differ ($p<0.05$)

Table 4: Least squares means of apparent nutrient digestibility of cows fed experimental diets[1]

Digestibility	Control	LY	ANX	LY + ANX	SEM[2]
DM	65.03[b]	69.07[a]	67.54	69.97	0.51
OM	65.61[c]	71.12[b]	68.17	76.04	0.43
CP	64.02	72.28	65.34	69.46	0.23
NDF	55.76[c]	62.16[b]	58.69	69.73	0.62

[1]Treatment groups include: basal diet (control), basal diet supplemented with live yeast (LY), *Aspergillus niger* meal extracted (ANX) or a mixture of LY and ANX (LY+ANX), [2]Standard error of means, [a,b,c] Means within a row with different superscripts differ ($p<0.05$)

Table 5: Least squares means of blood plasma chemistry of cows fed experimental diets[1]

Items	Control	LY	ANX	LY + ANX	SEM[2]
NEFA (mmol L^{-1})	0.53	0.54	0.53	0.51	0.06
Triglyeeride (mg dL^{-1})	8.53	8.99	6.55	8.71	1.29
Glucose (mg dL^{-1})	48.30	43.37	48.71	45.65	2.66
Urea nitrogen (mg dL^{-1})	16.98	16.21	16.55	16.21	0.77
Phosphorous (mg dL^{-1})	5.61	5.85	5.51	5.71	0.28
Magnesium (mg dL^{-1})	1.73	1.53	1.53	1.62	0.08
Potassium (mg dL^{-1})	4.36	4.25	4.07	4.31	0.08

[1]Treatment groups include: basal diet (control), basal diet supplemented with Live Yeast (LY), *Aspergillus niger* meal extracted (ANX) or a mixture of LY and ANX (LY+ANX), [2]Standard error of means

2004; Dann *et al*., 2000; Arambel and Kent, 1990). Piva *et al.* (1993) suggested that LY appears to be more beneficial in high concentrate diets in early lactation cows. Such a benefit effect can be depicted by the potential of LY to decrease ruminal lactic acid concentrations and to stabilize ruminal pH as well as stimulate cellulolytic bacterias (Chaucheyras-Durand and Fonty, 2002; Stella *et al*., 2007; Nocek and Kautz, 2006). Also variable effects of LY on production of dairy cows were reported under different management and dietary conditions (Piva *et al*., 1993). However in present study FCM 3.5% were similar between supplemental groups.

In the current study, the treated cows produced significantly more milk than controls, changes in BW and BCS as well as DMI were unaffected by treatments. Similar results were reported by some researchers (Bruno *et al*., 2009; Piva *et al*., 1993; Arambel and Kent, 1990) but in other reports (Moallem *et al*., 2009; Wohlt *et al*., 1998), supplemental LY increased DMI.

FCM 3.5% were not affected by treatments which are corresponding with the study of Arambel and Kent (1990). Not to affect the average of milk fat percentage by treated groups is similar to results of Moallem *et al.* (2009); Dann *et al*. (2000); Arambel and Kent (1990), however some researchers reported enhancement of milk fat content by the cow treated with yeast (Wang *et al*., 2001). In contrast to milk fat content, the average protein percentage and yield in response to yeast in the reports were not variable. In a study conducted with early lactation in dairy goats (Stella *et al*., 2007) and in dairy cows (Moallem *et al*., 2009; Dann *et al*., 2000; Arambel and Kent, 1990), no difference was reported for animals fed LY and control diet. The higher protein percentage may be due to the higher CP digestibility in this group of treated cows (receiving LY) compared to the others and the higher protein yield could be also attributable to the higher milk production and higher protein content in this group. Bruno *et al*. (2009) reported similar milk fat percentage but increased production of milk and milk true protein might indicate that changes in rumen fermentation as a result of feeding LY increase the supply of glucogenic and aminogenic and but not lipogenic substrates.

Moallem *et al*. (2009) showed that feeding LY to dairy cow during hot season increased numerically but not significantly the amounts of digested DM (5.4%), OM (5%), protein (2.8%) and NDF (6.9%). Some other researchers also reported no effect of yeast on digestibility (Arambel and Kent, 1990; Wohlt *et al*., 1998). In this study, greater significantly DM, OM and NDF digestibility and numerically CP digestibility in response to LY supplementation compared to control was observed.

It has been suggested that LY has potentially to scavenge oxygen and then create anaerobic condition in rumen, therefore might contribute to change bacteria numbers and affect the rate of digestibility (Moallem *et al*., 2009; Chaucheyras-Durand *et al*., 2008; Mwenya *et al*., 2004).

Providing a source of vitamins and some other growth factors by the live yeast that once lyses occur in the rumen and needed for bacterial growth may attribute to greater digestibility (Arambel and Kent, 1990). Grater DM, OM and NDF digestibility in cows fed LY+ANX compared to LY supplementation may be due to a synergistic effect over the use of LY and ANX together. Concentrations of glucose, Nonesterified Fatty Acids (NEFA), triglyceride, urea N, phosphorous, magnesium and potassium were similar between treatments.

Not to affecting BCS and DMI by the treatment might be interpreted with lack of changes in energy metabolites of plasma (Bruno *et al*., 2009). They reported similar results when cows were fed *Saccharomyces cerevisae* during early lactation suggesting that feeding LY did not improve energy status of these animals in which DMI remained the same. Banadaky *et al*. (2003) reported that LY decreased plasma concentrations of magnesium and potassium in lactating cows but in present study neither LY nor ANX could affect concentration of these minerals.

CONCLUSION

Results from present study show that feeding LY supplementation to Holstein cows increased milk yield. Feed intake, feed efficiency and FCM 3.5% were not affected by the supplements. Greater percentage of milk protein and protein yield were observed in the LY group. The apparent DM, OM and NDF digestibility of cows fed a mixture of LY and ASN were higher compared to those fed LY which may be due to a synergistic effect over the use of LY and ASN together. Additional studies are warranted to determine the effect of feeding LY, ANX or their combination on ruminal metabolites in cattle of different physiological statuses.

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