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Effect of hydrolyzed and live yeast supplementation during transition period on colostrum and milk composition and blood biochemical parameters in dairy cows

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Abstract The main purpose of this study was to assess live yeast and hydrolyzed yeast effects on blood biochemical parameters, colostrum quality and performance of dairy cows during transition period. Fifteen pregnant Holstein dairy cows were randomly divided into three groups during three weeks pre- and post-parturition. Cows were assigned to treatments as: 1) control group fed basal diet, 2) cows fed basal diet plus 6.0 g/d/head live yeast and 3) cows fed basal diet plus 20 g/d/head hydrolyzed yeast as on top. Live yeast supplementation resulted in higher dry matter intake and milk production compared with hydrolyzed yeast and the control group. Cows received live yeast had the highest milk fat percentage and those in the control group had the lowest lactate dehydrogenase and alkaline phosphatase activities and those received hydrolyzed yeast had the highest IgG and lactoferrin in the colostrum. Cows received live yeast had the highest serum albumin and those in the control group had the lowest serum albumin. Cows received hydrolyzed yeast had the highest globulin among treatments. There was no difference among treatments for blood triglyceride, cholesterol, non-esterified fatty acids and beta-hydroxy butyric acid. Cows in the control group had the highest aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities in the serum. Live yeast and hydrolyzed yeast supplementation reduced aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in the serum. Cows receive hydrolyzed yeast had higher IgG level in the serum. Dietary supplementation of live yeast could improve feed intake and milk yield post parturition, but hydrolyzed yeast enhance immunity components of colostrum. Pre-parturition, supplementation of 20 g/d/head hydrolyzed yeast in the diet of dairy cows recommended for enhancing immunity status of cow and her newborn and post-parturition, supplementation of 6.0 g/d/head live yeast recommended for better production performance.

Keywords: hepatic enzyme, IgG, immunity, lactoferrin, milk yield

Introduction

Numerous metabolic and endocrine changes occur in the body of cow during the late dry period, which result in parturition and lactation (Wankhade et al., 2017). The period of three weeks before and after parturition is referred to as the transition period, in which the body of high producing dairy cow encounters with an intense stressful condition. During this period, dry matter intake is limited and nutrients demand on a daily basis increase and farmers could not meet the nutrient requirements. As a result, the body of dairy cow experiences a negative energy balance which subsequently endangers the animal health, production and reproduction during early lactation (Grummer, 1995; Wankhade et al., 2017). During the hot season, these conditions become -

more complex (Burdick et al., 2011). Many farmers feed their cows a diet containing more concentrate and less roughage during the transition period. A low fiber and high starch ration leads to ruminal subclinical acidosis, which finally results in the loss of appetite and lower feed intake and milk production, lower fertility rate and higher production of lipopolysaccharide (Wankhade et al., 2017). Lipopolysaccharides as a constituent of the cell wall structure in gram-negative bacteria, cause inflammation in various tissues, especially the liver and mammary glands (Opal et al., 1999), which has a significant negative effect on the health and performance of dairy cows.

There are some options to overcome this problem, including feeding of probiotics such as live yeast (Perdomo et al., 2020; Sallam et al., 2020) or prebiotics such as hydrolyzed yeast (Adili et al., 2020). It has been claimed that yeast culture and its products could enhance the nutrient digestibility, production, and immune response in dairy cows (Nocek et al., 2011; Adili et al., 2020; Perdomo et al., 2020; Sallam et al., 2020), sheep (Hassan et al., 2020), horse (Jacobs et al., 2017) and in cell cultures (Wojcik, 2014). Hydrolyzed yeast is used as an appetite enhancer and immune modulator in non-ruminant animals (Silva et al., 2009; Yalçin et al., 2010). Two important components in hydrolyzed yeast could impact on the immune system responses, i.e., mananooligosaccharides and β -glucans (Kröger et al. 2017), which could also reduce inflammation.

Live yeast and hydrolyzed yeast have been fed to dairy cows exposed to heat stress, and are reported to alleviate the signs of heat stress by decreasing the rectal temperature and respiration rate, or increasing the performance by improvement in diet digestion (Dias et al., 2018) or changes in feeding behavior (Bach et al., 2007). In the literature, there is limited information concerning the effects of dietary supplementation of live yeast (*Saccharomyces cerevisiae*) and hydrolyzed yeast on blood biochemical parameters and performance of dairy cows in the hot climate or season, especially during the transition period. Therefore, the purposes of this study was to determine the effects of live and hydrolyzed yeast on metabolic profile, colostrum quality and performance of dairy cows under heat stress during the transition period.

Materials and methods

Location and ethical statement

The experiment was conducted at a farm located in Buinzahra region (Qazvin province, Iran) from late July to early September. All experimental procedures were ethically approved by the Department of Animal Science (Tehran, Iran). Live yeast, *Saccharomyces cerevisiae* strain SC20-CNCM I-1077 (10×10^9 CFU/g) was obtained from Levucell Co. (Paris, France) and autolyzed yeast of *Saccharomyces cerevisiae* from Behan Kimia Enzyme Co. (Tehran, Iran).

Animals and treatments

Fifteen pregnant Holstein dairy cows (4 years old, 2 parities, body weight 670 ± 25 kg) were randomly divided into three groups three weeks pre- and post-parturition and received basal diet with or without live yeast or hydrolyzed yeast as on top. Treatments were; Group 1: as control received basal diet without yeast supplementation; Group 2: received 6.0 g/d/head live yeast; Group 3: received 20 g/d/head hydrolyzed yeast. Levels of live yeast and hydrolyzed yeast were chosen based on the companies' recommendations. After parturition, the cows were offered their daily diets which consisted of 43% alfalfa and corn silage and 57% concentrate mixture. Daily dry matter intake and milk production in the third week of lactation were recorded. The diet was formulated according to CNCPS (Van Amburgh et al., 2010) recommendations for nutritional needs of lactating cows. The diet was offered three times at 6:00 AM, 2:00 PM and 10:00 PM as a total mixed ration.

Blood sampling procedure

Blood samples were taken from the tail vein on day 21 of lactation and transferred to sterile glass tube for serum separation. After clotting, the samples were centrifuged at 1500 × *g* for 15 min and the serum samples were stored at -70 °C until analysis (Aung et al., 2019).

Measurements of colostrum and milk components

Cows were milked three times at 5:00 AM, 1:00 PM and 9:00 PM daily by machine milking system. Colostral sample, collected after cow was fully milked out within 4 h of calving, was mixed thoroughly. Milk samples were collected at each milking during three consecutive days in the third week of the experiment. Colostrum and milk samples (100 mL) were refrigerated, and four 25-mL aliquots of each sample were taken and stored at -20°C for fat, protein, lactose, milk urea nitrogen, and solids non- fat. Fat measurement was based on Gerber method (Kleyn et al., 2001). Protein and lactose contents were analyzed according to Kjeldahl (AOAC, 2005) and Bertrand, respectively. Five mL of the milk samples were treated with 5 mL of 25% (wt/vol) trichloroacetic acid for the determination of milk urea nitrogen (Nocek et al., 2011).

Biochemical measurements

Serum biochemical metabolites included glucose, triglyceride, albumin, total protein, globulin (calculated by total protein – albumin), hepatic enzymes included aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) an-

d also milk urea nitrogen and lactate dehydrogenase in colostrum were analyzed using Pars Azmoon kits (Parsazmoon Co., Tehran, Iran) and spectrophotometric device model (Jasco V-570, Japan) according to the instructions of the kit manufacturer. Beta-hydroxy-butyrate acid (BHBA) and non-esterified fatty acids were determined by The Randox Ranbut Colorimetric Assay Kits (London, UK). Measurement of the serum IgG concentration was done using ELISA kits (Thermo Life Sciences, Basingstoke, UK).

Immunoglobulin G (IgG) in colostrum was determined with Single Radial Immunodiffusion method (Mancini et al., 1965). Colostrum dilutions were made with saline by 10-fold as colostrum is viscous. Lactoperoxidase and lactoferrin were determined in colostrum using Abbexa ELISA kits (Abbexa Co., London, UK).

Statistical analysis

Statistical analyses were done using Proc GLM of SAS 9.1 software (SAS, 2005) based on completely randomized design. The test of Kolmogorov-Smirnov was applied to evaluate the data normality before analysis of variance was performed. The Duncan's multiple range test was used to compare the means. Statistical differences were declared at P≤0.05.

Results

Dry matter intake, colostrum composition, milk production and composition are presented in Table 1. There were differences among treatments for dry matter intake and milk yield (P<0.05), with no effect of treatments on milk efficiency. Live yeast supplementation resulted in higher dry matter intake and milk production compared with hydrolyzed yeast and the control group.

Crude protein percentage and lactoperoxidase content in colostrum was not affected by treatments, but other components were significantly affected. Cows receiving live yeast had the highest fat percentage and those in the control group had the lowest fat percentage, lactate dehydrogenase and alkaline phosphatase activities in colostrum. Cows receiving hydrolyzed yeast had the highest colostral IgG and lactoferrin concentrations.

Table 1. Effects of live yeast and hydrolyzed yeast supplementation on the dry matter intake (DMI), milk yield and colostrom and milk composition in dairy cows

Parameters	Treatments			SEM	*P*-value
	Control	Live yeast	Hydrolyzed yeast		
Dry matter intake (kg/d)	23.5[b]	24.6[a]	24.3[ab]	0.13	0.035
Milk Yield (kg/d)	37.0[b]	38.9[a]	37.8[b]	1.08	0.045
Milk efficiency (kg milk/kg DMI)	1.57	1.57	1.55	0.051	0.322
Colostrum composition					
Crude protein (%)	13.91	13.69	13.75	0.325	0.368
Fat (%)	5.89[b]	6.38[a]	5.93[b]	0.051	0.041
Lactate dehydrogenase (U/L)	1540[a]	1369[b]	1387[b]	15. 5	0.033
Alkaline phosphatase (U/L)	1461[a]	1302[b]	1289[b]	14.3	0.045
Immunoglobulin (g/L)	84[b]	87[b]	96[a]	2.39	0.015
Lactoferrin (g/L)	3.28[b]	3.39[b]	4.56[a]	0.029	0.018
Lactoperoxidase (mg/L)	38.4	39.3	38.9	1.12	0.321
Milk composition (%)					
Protein	2.98	2.93	2.95	0.051	0.765
Fat	2.86	2.78	2.81	0.086	0.402
Lactose	4.71	4.64	4.69	0.031	0.178
Solids not-fat	8.51	8.46	8.47	0.067	0.834
Fat: protein	0.96	0.94	0.95	0.032	0.354
Milk urea nitrogen (mg/dL)	15.02	13.96	14.24	0.311	0.202

a,b: Within rows, mean values with common letter(s) are not different (P>0.05).

The effects of experimental diets on blood biochemical parameters are shown in Table 2. Live yeast and hydrolyzed yeast had no effect on serum total protein, but albumin and globulin levels were affected by treatments. Cows receiving live yeast had the highest serum albumin level and those in the control group had the lowest level (P<0.05). Cows receiving hydrolyzed yeast had the highest globulin concentration among treatments. There was no difference among treatments for serum triglyceride, cholesterol, non-esterified fatty acids and beta-hydroxy butyric acid. Cows in the control group had the highest AST, ALT and ALP activities in the serum.

As presented in Figure 1, there was a significant difference (P<0.05) in serum IgG concentration between the cows receiving hydrolyzed yeast and the control group. Cows receiving hydrolyzed yeast had the highest IgG level. There was no difference in IgG concentration between cows receiving live yeast and the control group (P>0.05).

Table 2. Effects of live yeast and hydrolyzed yeast supplementation on blood biochemical parameters in dairy cows

Blood parameters	Treatments			SEM	*P*-value
	Control	Live yeast	Hydrolyzed yeast		
Total protein (g/dL)	9.13	9.31	9.38	0.184	0.154
Albumin (g/dL)	3.49^{b}	3.65^{a}	3.59^{ab}	0.044	0.028
Globulin (g/dL)	5.64^{b}	5.66^{b}	5.79^{a}	0.056	0.043
Triglycerides (mg/dL)	13.18	13.88	14.01	0.605	0.251
Cholesterol (mg/dL)	289.1	274.3	280.8	8.76	0.419
Non-esterified fatty acids (mmol/L)	0.332	0.297	0.311	0.019	0.270
Beta-hydroxy butyric acid (mg/dL)	0.928	0.890	0.907	0.099	0.383
Aspartate aminotransferase (U/L)	75.35^{a}	68.81^{b}	70.42^{b}	1.28	0.018
Alanine aminotransferase (U/L)	37.22^{a}	31.04^{b}	30.54^{b}	1.14	0.019
Alkaline phosphatase (U/L)	108.24^{a}	103.61^{b}	102.48^{b}	1.59	0.011

a,b: Within rows, mean values with common letter(s) are not different (P>0.05).

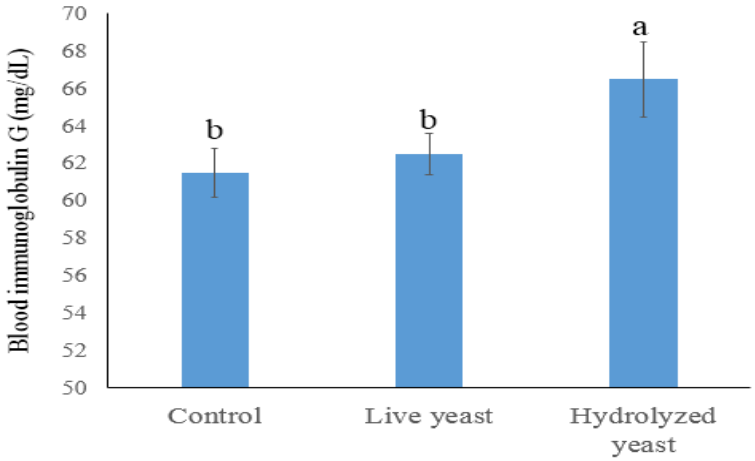

Figure 1. Immunoglobulin G concentration in the serum of cows feeding on the diets supplemented with live or hydrolyzed yeast.

Discussion

Live or hydrolyzed yeast are important additives in dairy cattle feeding, but comparison between these additives and their effects on ruminal fermentation and blood biochemical parameters and performance during the transition period, especially in the hot season is scare. This study was done to compare the effects of these additives in early lactation cows under heat stress condition.

Our finding of a significant effects of live yeast or hydrolyzed yeast supplementation on dry matter intake (Table 1), is inconsistence with several authors (Piva et al., 1993; Wohlt et al., 1998; Soder and Holden, 1999; Schingoethe et al., 2004; Bagheri et al., 2009) who reported no response in dry matter intake by yeast supplementation in dairy animals. Live yeast has been reported to have scavenger activity on oxygen in the rumen (Chaucheyras-Durand et al., 2012), which could reduce the rumen redox and is an important part of its mechanism of action to stimulate the microbial growth and ruminal pH stabilization (Marden et al., 2008). Moreover, live yeast could improve ruminal fiber digestion by increasing the cellulolytic bacteria (Chaucheyras-Durand et al., 2016). Ruminal pH stabilization and high rate of fiber digestion by yeast supplementation leads to higher feed intake and finally higher milk production (de Ondarza et al., 2010).

Live yeast supplementation increased milk production. Significant increases in milk production after live yeast or hydrolyzed yeast supplementation to diet were also reported previousely (Callaway and Martin, 1997; Wohlt et al., 1998; Bruno et al., 2009). Yeast can provide growth factors which stimulate the growth of amylolytic and cellulolytic bacteria with subsequent increase in milk production (Callaway and Martin, 1997). Other researchers have shown benefits of supplementing live yeast (Moallem et al., 2009; Salvati et al., 2015) on lactation performance in dairy cows under heat stress. Based on the report of de Ondarza et al. (2010), the response of milk yield by dairy cows to dietary yeast supplementation is depended on fermentable amount of neutral detergent fiber and fiber passage rate. The results of this study are consistence with other researchers (Moallem et al., 2009; Dias et al.,

2018), who reported higher milk yield in the dairy cows under high ambient temperatures being fed with diets supplemented with live yeast. Our finding is inconsistent with reports of Soder and Holden (1999), Schingoethe et al. (2004), Bagheri et al. (2009) and Sallam et al. (2020), who reported that live yeast or yeast extract had no beneficial effects on milk production.

In agreement with our finding, Bagheri et al. (2009) and Moallem et al. (2009) reported that yeast supplementation had no significant effect on milk composition of dairy cows. They also observed no differences in milk protein percentage and milk protein yield. Conversely, both a decrease in milk fat (Stella et al., 2007) and an increase in milk fat (Giger-Reverdin et al., 1996) were reported in lactating dairy goats fed live yeast. A summary meta-analysis (over 110 papers, Desnoyers et al., 2009) demonstrated that yeast supplementation could increase milk yield without significant effects on milk composition.

Fat percentage in the clostrum was higher in cows receiving live yeast which may be related to fiber digestion and higher acetate production as previously studies reported in lactating cows (Callaway and Martin, 1997; Dias et al., 2018). Lactate dehydrogenase and ALP activity in colostrum was the highest in the control group, which infers an effect of heat stress on higher oxidative stress and somatic cell counts in the colostrum. Live and hydrolyzed yeast supplementation reduced the activities of these enzyme. Concentration of IgG and lactoferrin was the highest in cows receiving hydrolyzed yeast. It may be related to the presence of immunomodulatory compounds such as α-D-glucan and β-D-glucan which are found in autolyzed yeast, and could affect immunoglobulin production (Kogan and Kocher, 2007; Adili et al., 2020). Lactoferrin is antimicrobial peptide and its level in colostrum is important. Lactoperoxidase, also as an antimicrobial agent, could also enhance the health status of the newborn calf; however, in the present study, these were not affected by the treatments.

Serum biochemical components are usually measured to monitor the metabolic disorder or health status of dairy herds (Ametaj et al., 2009). In agreement with our finding, Galip (2006) reported that serum total protein was not influenced in the calves receiving yeast. Similar to our finding, Piva et al. (1993) reported that serum tryglyceride, cholesterol and total protein were not influenced by yeast supplementation. In contrast, Ayad et al. (2013) showed an increase in total serum protein after dietary yeast supplementation in the dairy cows. Ayad et al. (2013) also reported that the addition of live yeast to the diet of dairy cows increased plasmatic total protein and albumin, whereas triglyceride and creatinine concentrations were significantly decreased; there was a slight decrease in cholesterol concentrations. Live yeast supplementation increased serum albumin compared with the control group. The level of albumin in serum is very important, because directly reflects the state of nitrogen in the diet.

Live yeast and hydrolyzed yeast supplementation impacted on hepatic enzyme activity in the serum; this is inconsistent with a previous finding in which yeast supplementation had no significant effect on AST or gamma-glutamyl transpeptidase levels of dairy cows (Aung et al, 2019). In normal circumstances, AST, ALT and ALP are found mostly inside the liver cells, but if the liver is inflamed or injured, liver cells become disrupted and cytoplasmic contents, especially enzymes, are released into the blood stream (Schmidt and Schmidt, 1967; Contreras-Zentella et al., 2015). Hence, the levels of ALT, AST and ALP were considered as useful biomarkers for detection of liver damages. In the control group, it seems that heat stress resulted in oxidative stress and increased the hepatic enzymes in the blood. Serum activity of hepatic enzymes decreased in the cows that received supplementary live yeast and hydrolyzed yeast, indicating the yeast potential to decrease the oxidative stress in the heat stressed cows. In agreement with our finding, Du et al. (2022) reported that yeast supplementation could enhance antioxidant capacity in the serum of cows, which finnaly could reduce the hepatic damage.

Live yeast and hydrolyzed yeast supplementations had no significant effect on serum BHBA. One source of circulating BHBA is butyrate that is produced in the ruminal epithelium (DeFrain et al., 2004). However, little evidence is available from either *in vivo* (Harrison et al., 1988) or *in vitro* (Sullivan and Martin, 1999) studies that yeast products increase butyrate production. Hepatic ketogenesis is a key source of circulating BHBA in early lactation cows. These changes, in addition to significant effects on serum BHBA, indicate an increase in fat mobilization. Also, hepatic ketogenesis has a direct effect on lipid metabolism when consuming yeast product.

As shown in Figure 1, hydrolyzed yeast, but not live yeast, increased the blood IgG level. Immunomodulatory compounds such as α-D-glucan and β-D-glucan in hydrolyzed yeast may affect the immune response and immunoglobulin production (Kogan and Kocher, 2007; Adili et al., 2020). Moreover, α-D-glucan and β-D-glucan bind and prevent colonization of pathogenic bacteria in the digestive tract and could interact with the immune cells directly (Stier et al., 2014). The result of our study is agreement with report of Yalçin et al. (2010) who showed that hydrolyzed yeast supplementation could increase the antibody levels. Oligosaccharides are also present in hydrolyzed yeast and may increas the antibody production. It was assumed that oligosaccharides in hydrolyzed yeast could bind to macrophage reception sites by recognizing specific sugars found in epithelial surface glucoproteins. After recognition, the binding could trigger a cascading reaction that activate macrophages and release cytokines, and finally activate the acquired immune response (Silva et al., 2009). Moreover, Lei et al. (2013) observed that yeast cell wall can bind the compounds such as lipopolysaccharides and prevent its translocatio-

n into the circulation. Lipopolysaccharides could effectively initiate the inflammatory responses, which ultimately reduce the activities of the immune system and immunoglobulin production.

Conclusion

The results of this study showed that live yeast supplementation could improve nutritional quality of colostrum and hydrolyzed yeast supplementation could enhance the immune components in colostrum. Feeding hydrolyzed yeast (20 g/d/head) was recommended for enhancing the immunity status of dairy cows and feeding live yeast (6.0 g/d/head) for better production performance.

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Conflict of interest

There is no conflict of interest.

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