

# Effects of live and hydrolyzed yeast supplementation during transition period on blood IgG content and INF- $\gamma$ gene expression in dairy cows

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## Abstract

This study was carried out as a trial to improve the immune response of dairy cows following the dietary supplementation of live and hydrolyzed yeast during transition period. Cows (n=20) were randomly divided into five groups 3 weeks pre-parturition and received basal diet with or without live yeast or hydrolyzed yeast as on top until 3 week post-parturition as follows: Group 1: as control received basal diet without yeast products; Group 2: received 0.5 g live yeast; Group 3: received 1.0 g live yeast; Group 4: received 10 g hydrolyzed yeast and Group 5: received 20 g hydrolyzed yeast. There were no significant differences ( $P>0.05$ ) among treatments for dry matter intake and milk production. There was a difference ( $P<0.05$ ) for blood IgG level in cows received live yeast at two doses as compared to control group. The highest mean was for cows received live yeast at dose of 2 g. Hydrolyzed yeast had no significant effect on the relative interferon- $\gamma$  (INF- $\gamma$ ) gene expression as compared with the control. It was concluded that live yeast could, but hydrolyzed yeast at the levels applied in this study could not influence on immune response of dairy cows fed supplemented diet during transition period.

**Keywords:** dairy cow; hydrolyzed yeast; live yeast; transition period; interferon- $\gamma$

## Introduction

The period of changing a dairy cow from the non-lactating to lactating state was defined as transition period. The transition period is an interval from 3 weeks prepartum to 3 weeks postpartum (Goff and Horst, 1997). During transition period, the numerous changes in metabolic and endocrine statuses occur in the body of cow that results in parturition and lactogenesis (Grummer, 1995; Drackely, 1999). Dry matter intake is limited during this period and nutritional management usually could not meet body requirements. Occurred physiological changes could increase the requirement and it is not possible to fortify these requirements by diet. Therefore, the transition cow will be at a high risk in health and severely suppressed immune function soon after parturition (Bell, 1995; Grummer, 1995; Goff and Horst, 1997). The proper management during this period could enhance the immune system function and immune responses (Lowry *et al.*, 2005; Chae *et al.*, 2006; Rode, et al., 1999; Gordon, 2004), and could decrease the metabolic and infectious diseases incidences (Axford, 2001; Hsueh *et al.*, 2010). It has been claimed that  $\beta$ -glucan of live and hydrolyzed yeast could enhance the immune response in dairy cows (Magalhaes *et al.*, 2008), pigs (Xiao *et al.*, 2004; Eicher *et al.*, 2006) and cell cultures (Gantner *et al.*, 2003; Majtán *et al.*, 2005; Brown, 2006). Interferon-gamma (IFN- $\gamma$ ) is a cytokine with an important role in induction and modulation of immune responses (Xiao et al., 2004). Immunoglobulin G (IgG), the most abundant type of antibody, is found in all body fluids and protects tissues against infections (Broadway *et al.*, 2015). In the literature there is limited information concerning the effects of dietary supplementation of live and hydrolyzed yeast on immune response of dairy cows in the pre- and post-parturition (Broadway *et al.*, 2015). Therefore, this study was carried out as a trial to improve the immune response of dairy cows following the dietary supplementation of live and hydrolyzed yeast during transition period.

## Material and Methods

### *Animals and treatments*

Dry dairy cows (n=30, 3-8 years old and 1-6 parities) were kept in a private farm in Qazvin province (Qazvin, Iran) during the period from January to December, 2017. Cows were housed in controlled conditions and vaccinated against the epidemic and parasitic diseases common in the region. Live yeast ( $10 \times 10^9$  CFU/g) was prepared from Levucell Co. (Paris, France) and hydrolyzed yeast prepared from Kimia Co. (Tehran, Iran). Cows (n=20) were randomly divided into five groups three weeks before parturition and received basal diet (suitable for close up) with or without live yeast or hydrolyzed yeast as on top as follows: Group 1: as control received basal diet without yeast supplementation; Group 2: received 0.5 g live yeast; Group 3: received 1.0 g live yeast; Group 4: received 10 g hydrolyzed yeast and Group 5: received 20 g hydrolyzed yeast. After parturition, cows of each group received basal diet supplemented or not-supplemented with live yeast and hydrolyzed yeast. After parturition, cows were offered daily ration which mainly consisted of 40% Alfalfa and corn silage and 60% concentrate mixture. Daily dry matter intake and milk production in the third week of lactation were recorded.

### *Blood sampling procedure*

The blood samples were taken from tail vein at day 21 of lactation and divided to two parts. A part was poured in sterile glass tube for IgG measurement and other part was poured in EDTA gel containing tube for INF- $\gamma$  gene expression. The former samples were centrifuged at  $1500 \times g$  for 15 min and the serum samples were stored at  $-70^\circ C$  until measurement of the serum IgG level using ELIZA kits (Thermo Life Sciences, Basingstoke, UK).

### *INF- $\gamma$ gene expression analysis*

Gene expression analysis for INF- $\gamma$  was done according to the method described by Sweeney et al. (1998) in Kharazmi Laboratory (Tehran, Iran). Blood samples for total mRNA extraction were placed in liquid nitrogen and then stored at  $-80^\circ C$ . RNA samples were extracted and cDNA was synthesized according to the method described by BioNeer Company (Seoul, South Korea). INF- $\gamma$  and  $\beta$ -ACTIN sequences were prepared using gene data bases of NCBI (National center for biotechnology information). The  $\beta$ -ACTIN gene was used as an internal control. After preparing the sequences of  $\beta$ -ACTIN and INF- $\gamma$  on NCBI, the gene-specific primers were designed by primer express software and synthesized by BioNeer Company (Seoul, South Korea). Generation analysis and Melting Curve was done using a Real-Time PCR System (Applied Bio systems, Foster City, CA).

### *Statistical Analysis*

Statistical analyses were done using GLM procedure of SAS for Windows version 9.1 (SAS Institute Inc., Cary, NC). The test of Kolmogorov-Smirnov was applied to evaluate the data normality before analysis of variance was performed. Duncan multiple test was used to compare the means. Season was

subjected to the model and its effect was not significant, therefore it was removed from the model. Statistical differences were declared at  $P < 0.05$ .

**Results**

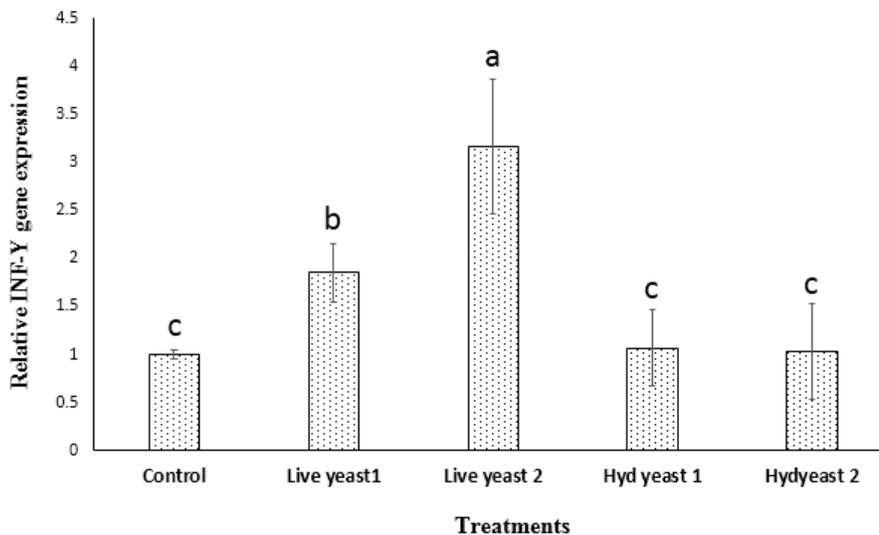
Daily dry matter intake and milk production are reported in Table 1. There was no significant differences ( $P > 0.05$ ) among treatments for dry matter intake and milk production. As presented in Table 1, there was a difference ( $P < 0.05$ ) for blood IgG level in cows received live yeast at two doses compared to control group. There was no difference between control group and those received hydrolyzed yeast ( $P > 0.05$ ).

Relative INF- $\gamma$  gene expression is presented in Figure 1. There were differences among treatments for relative INF- $\gamma$  gene expression. The highest mean was for cows received live yeast at dose of 1.0 g. Hydrolyzed yeast had no significant effect on the relative INF- $\gamma$  gene expression as compared with the control group.

**Table 1.** Daily dry matter intake, milk production and blood IgG levels of cows

Treatment	Daily dry matter intake (kg/d)	Average milk production (kg/day)	Blood IgG (mg/ml)
Control	24.4	37.5	61.4 <sup>b</sup>
Live yeast 1	25.5	38.1	65.2 <sup>a</sup>
Live yeast 2	25.6	38.0	66.0 <sup>a</sup>
Hydrolyzed yeast 1	25.5	37.7	62.1 <sup>b</sup>
Hydrolyzed yeast 2	25.2	37.5	60.8 <sup>b</sup>
SEM	1.64	1.52	1.71

a, b Means within a column with different superscripts are significantly different ( $P < 0.05$ ).  
 Live yeast 1: received 0.5 g live yeast; Live yeast 2: received 1.0 g live yeast; Hydrolyzed yeast 1: received 10 g hydrolyzed yeast and Hydrolyzed yeast 2: received 20 g hydrolyzed yeast.



**Figure 1.** The relative INF- $\gamma$  gene expression

Live yeast 1: received 0.5 g live yeast; Live yeast 2: received 1.0 g live yeast; Hyd yeast 1: received 10 g hydrolyzed yeast and Hyd yeast 2: received 20 g hydrolyzed yeast.

**Discussion**

The transition phase (-3 to 3 weeks postpartum) is characterized by increased nutritional demands and suppressed immune function (Nonnecke et al., 2003). Therefore, feed supplements that can improve nutrient utilization and immune function in the pre-parturition period are needed.

In our study dry matter intake was not affected by yeast supplementation (Table 1). In consistence with that, some authors (Piva et al., 1993; Wohlt et al., 1998; Soder and Holden, 1999; Schingoethe et al., 2004; Bagheri et al., 2009) reported no response in dry matter intake by yeast supplementation in dairy animals. The dietary yeast supplementation may increase the ruminal counts of cellulolytic bacteria, but this

increase may have not influenced the total fiber digestion and finally dry matter intake (Harrison *et al.*, 1988).

As shown in Table 1, there is no difference among treatments for daily milk production. Our finding is consistent with reports of Soder and Holden (1999), Schingoethe *et al.* (2004) and Bagheri *et al.* (2009). They reported that live yeast or yeast extract had no beneficial effects in milk production. In contrast, some researchers (Piva *et al.*, 1993; Callaway and Martin, 1997; Wohlt *et al.*, 1998; Bruno *et al.*, 2009) speculated that live yeast and yeast culture resulted in increases of milk production in dairy cows. Authors (Robinson and Garrett, 1999; Bruno *et al.*, 2009) showed that milk response to yeast supplementation usually ranges between 1 and 2 kg/d.

As seen in Table 1, live yeast increased, but hydrolyzed yeast had no effect on blood IgG level. Kogan and Kocher (2007), also Medzhitov and Janeway (2000) reported that  $\alpha$ -D-glucan and  $\beta$ -D-glucan are the major components of yeast cell wall and act as immunomodulating compounds. The  $\alpha$ -D-glucan and  $\beta$ -D-glucan could bind pathogenic bacteria to prevent colonization in the digestive tract and able to interact with immune cells directly (Ruiz-Herrera, 1992).

Live yeast supplementation increased, but hydrolyzed yeast had no effect on INF- $\gamma$  gene expression. Our finding is consistent with observations of (Majtán *et al.*, 2005; Brown, 2006; Gantner *et al.*, 2003) who reported that yeast components could promote cytokines release in macrophages such as IL-1, IL-2, and IL-6, and could modulate the immune cells (Medzhitov and Janeway, 2000). Increased in the production of INF- $\gamma$  in T lymphocyte has been reported in swine fed diet containing yeast products (Xiao *et al.*, 2004). Also, Eicher *et al.* (2006) reported an increase in the concentration of TNF- $\alpha$  in some tissues of pigs fed diet containing yeast products during a challenge of lipopolysaccharide. Thus, live yeast and yeast-based products may alter the production of cytokines and activate the immune system because of the effects of  $\beta$ -glucans on immune cells.

An important stressful event in the life of a cow is during the final period and immediately after parturition. During stress, the activations of the hypothalamic-pituitary-adrenal (HPA) axis and immune system occur and resulted in a loss in cow performance (Burdick *et al.*, 2011). Recently, it was shown that yeast supplementation could mitigate some of the negative effects of stress (Magalhaes *et al.* 2008). Magalhaes *et al.* (2008) reported that dietary yeast supplementation in dairy cattle enhanced performance and minimized the morbidity and mortality rate.

The hydrolyzed yeast used in this study had no significant effect on dry matter intake, daily milk production, blood IgG level and INF- $\gamma$  gene expression. The amount of hydrolyzed yeast was 5 and 10 g and these levels cannot influenced on the ruminal fermentation and immune response. It was concluded that live yeast could, but hydrolyzed yeast at the levels applied in this study could not influence on immune response of dairy cows fed supplemented diet during transition period.

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