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Effects of Yeast Culture (*Saccharomyces cerevisiae*) on Humoral and Cellular Immunity of Jersey Cows in Early Lactation

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Abstract: The aim of the present study is to investigate the effects *Saccharomyces cerevisiae* (Yea-Sacc 1026/Alltech) yeast culture on humoral and cellular immunity of Jersey cows in early lactation. Twenty Jersey cows in the second lactation period were equally divided to control and study groups. Starting from the 45th day of the lactation period, 10 g day^{-1} of *S. cerevisiae* (Yea-Sacc/All-Tech Co.) were administrated to cows for 21 days. Blood were collected via vena jugularis on the 45, 52, 59, 66, 73th days postpartum. From the smears obtained, T and B lymphocyte numbers were recorded and in blood sea Bovine IgG, Bovine IgG_1, Bovine IgG_2, Bovine IgM and Bovine IgA levels were deteected. In conclusion, the results of this study indicated an immunstimulant tendency of *Saccharomyces cerevisia* at 10 g daily intake for dairy cows in early lactation.

Key words: *Saccharomyces cerevisiae*, lactation, humoral immunity, cellular immunity, Jersey cow, Turkey

INTRODUCTION

Saccharomyces cerevisiae have been used as an alternative to antimicrobial feed additives for over 15 years. Supplementing diets with *S. cerevisiae* was shown to increase total Volatile Fatty Acids (VFA) and propionic acid production, besides higher propionate concentration and decreased acetate to propionate ratio (Dawson, 1990; Nisbet and Martin, 1991; Piva *et al*., 1993; Sullivan and Martin, 1999). Higher VFA, especially propionic acid are important in terms of enhanced lactose production, milk volume and overall energy balance (Miller-Webster *et al*., 2002).

Fatty acids have various effects on immune and inflammatory responses, acting as intracellular and intercellular mediators and even the least sophisticated fatty acids such as the volatile fatty acids or the long-chain saturated fatty acids have important roles in cell metabolism, structure and regulation with considerable implications in the immune function when the cells in question are leukocytes (Pompeia *et al*., 2000).

Effects of short chain fatty acids on immune system varies, may be inhibitory or stimulatory (Harris and Webb,1990; Soder and Holden, 1999; Dann *et al*., 2000).

Especially, multiple unsaturated fatty acids and volatile fatty acids generally inhibits lymphocyte proliferation (Soder and Holden, 1999) and it is suggested that this effect of proprionate is related with lipid synthesis inhibition from pyruvate and acetate (Dawson, 1990). It is known that lipid synthesis has a key role in the synthesis of plasma membrane components such as phospholipids and cholesterol (Dawson, 1990; Erasmus *et al*., 1992).

The aim of the present study is to investigate the effects *Saccharomyces cerevisiae* (Yea-Sacc 1026/ Alltech) yeast culture on humoral and cellular immunity of Jersey cows in early lactation.

MATERIALS AND METHODS

This study was carried out in the Karaköy Farm of General Directorate of Agricultural Enterprises (TIGEM), Samsun, Turkey.

Twenty Jersey cows in the second lactation period were used. The cows were fed with a ration consisting of 10 kg of concentrate mixture, 2 kg of triticale hay and 15 kg of maize silage per day/cow. All the cows were at the second lactation term with the intention of preventing the differences which may arise from age and birth number. They were grouped equally forming a study group (Group 1) and a control group (Group 2).

Starting from the 45th day of the lactation period, 10 g day^{-1} of *S. cerevisiae* (Yea-Sacc/All-Tech Co.) were administrated to cows for 21 days in 200 mL of water. *S. cerevisiae* was a live culture consisting 1×10^8 cfu g^{-1} (EU Ref. No. CBS 493.94). Blood were

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collected via vena jugularis on the 45, 52, 59, 66 and 73th days postpartum. Blood smears were prepared from each specimen immediately and serum was seperated immediately and the specimen were stored.

Then, smears were prepared using a commercial streptavidin-biotin peroxidase system (DAKO). The smears were fixed in absolute ethanol and kept at -20°C. Smears were than preincubated in a blocking solution containing 10% goat non-immune serum (DAKO, Carpinteria, CA). Next step was the reaction with primary antibodies (Mouse anti CD3 and CD79) at 37°C for 3 h and rinsing with Phosphate Buffered Saline, pH 7.4 (PBS) at room temperature.

Next, the sections were reacted with biotin-conjugated 2nd step antibody (DAKO, Carpinteria, CA) for 10 min at room temperature and then rinsed in PBS also in room temperature. For inactivation of endogenous peroxidase, sections were incubated in 0.3% H_2O_2/ methanol for 60 min. Following another rinsing stage with PBS at room temperature, reaction with Streptavidin-Biotin-peroxidase Complex (SABC) for 10 min at room temperature was performed (DACO). After sections were incubated DAB (DAKO) for another washing with PBS for 15 min and then counterstained with Harris' hematoxylin. After the preparates were ready, T and B lymphocyte numbers were counted under microscope using immersion oil.

T and B lymphocyte numbers in the experiment and control groups were compared seperately for the periods. Besides, each group were compared within itself for the changes in T and B lymphocyte numbers according to the periods.

Likelihood Ratio Chi-Square Score test was used for all comparisions. Bovine IgG, Bovine IgG_1, Bovine IgG_2, Bovine IgM and Bovine IgA ELISA Quantitation Kit (Bethyl Laboratories Inc. US) were used in the determination of serum antibody levels. Bovine IgG, IgG_1, IgG_2, IgM and IgA, each were suspended with covering solution to the level of 10 microgram mL^{-1}. Prepared microplates were covered with 100 μL of the obtained solution for the antibodies mentioned.

Microplates were left at 37°C for 2 h, washed and dried for three times, 250 μL milk diluent was added and saturation process was achieved by leaving at 37°C for 2 h. Following this period, microplates were washed and dried three times, then 100 μL of 1/100 suspansed serum in addition to 1x milk diluent were added. These were left at 37°C for an hour, washed and dried and 100 μL Anti-bovine Ig-alkalene phosphatase conjugate was added. As previously mentioned, 1 h incubation washing and drying processses were repeated. Afterwards, 100 μL pNPP substrate (1 mg mL^{-1}) was added to microplates, incubated for 30 min and reaction was stopped with the stoper solution (1N H_2SO_4). Antibody levels were read by the ELISA reader at 405 nm. Regression parameters were performed with Ordinary Least Squares for serum immunoglobuline G, immunoglobuline G_1, immunoglobuline G_2, immunoglobuline A and immunoglobuline M levels and obtained data were evaluated and compared with t-test.

RESULTS AND DISCUSSION

T and B lymphocyte values of groups are shown in Table 1 and 2, respectively. Difference between the groups for all the weeks were determined significant for the comparision of T lymphocyte values of the experimental and control groups ($p<0.01$). Same situation was valid in the first and second weeks for the comparisions of the experimental and control groups of B lymphocytes ($p<0.05$) but significance control of the B lymphocyte values of the experimental and control groups in the 3rd and 4th weeks were not statistically significant ($p>0.05$).

Important differences for all weeks were determined in the comparisions of T lymphocyte values of the experimental and control groups within each group ($p<0.01$). Same situation is also valid for the B lymphocyte values within group comparisions ($p<0.01$).

Time dependent variations of the study and control groups for IgG_1, IgG_2, IgG, IgM and IgA levels are presented at Fig. 1-5, respectively.

Variation observed as a decrease at the period starting from the beginning to the first week for the control group was recorded as an increase for the study group ($p<0.05$).

Time dependent changes of both of the groups were observed insignificant from the 2nd week.

Variation in 0-2 time interval for the control group was determined as a decrease, while the contemporary variation for the study group was recorded as an elevation and a significant difference was reported

Table 1: T lymphocyte values

	Tm1		Tm2		Tm3		Tm4		
Groups	n	$\bar{X}$	n	$\bar{X}$	n	$\bar{X}$	n	$\bar{X}$	χ^2
1 (Study)	10.00	16	10.00	11	10.00	17	10.00	16	59.11**
2 (Control)	10.00	11	10.00	3	10.00	5	10.00	6	52.22**
χ^2	14.54**		12.58**		15.36**		16.26**		

Table 2: B lymphocyte values

	Bl1		Bl2		Bl3		Bl4		
Groups	n	$\bar{X}$	n	$\bar{X}$	n	$\bar{X}$	n	$\bar{X}$	χ^2
1 (Study)	10.00	0.10	10.00	0.50	10.00	0.50	10.00	0.10	11.17**
2 (Control)	10.00	0.60	10.00	0.10	10.00	0.00	10.00	0.00	8.62**
χ^2	4.21*		4.22*		1.44		1.43		

*; p<0.05; **;p<0.01

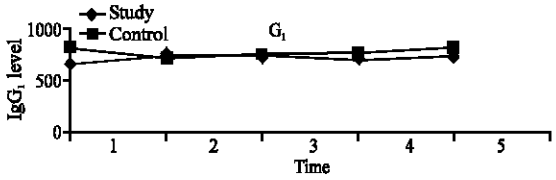

Fig. 1: Time dependent variations at IgG_1 levels of the groups

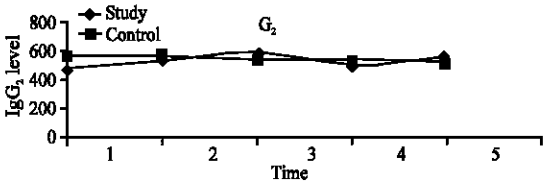

Fig. 2: Time dependent variations at IgG_2 levels of the groups

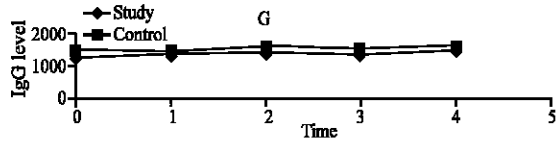

Fig. 3: Time dependent variations at IgG levels of the groups

between the groups from this aspect ($p<0.05$). Similar variations were observed at 2-4 time interval. Time dependent variation in both groups and comparision of this variation between groups for the G parameter were insignificant, however in 0-1 time interval, a statistically insignificant variation as an increase in the study group and as a decrease in the control group were observed.

A time dependent alteration was not recorded in M parameter for the control group. Although, a similar situation was valid for the study group, a statistically insignificant linear elevation was observed at 1-2 time interval.

An insignificant linear elevation and a significant decrease in the quadratic direction in 0-2 time interval for A parameter for the control group were observed. Comparision of the variations between the two groups were determined insignificant, except the variation at 0-1 time interval.

Several studies demonstrated that *S. cerevisiae* feed additives increased the production of acetate, propionate and total VFA in dairy cows (Nisbet and Martin, 1991; Piva *et al*., 1993; Miller-Webster *et al*., 2002). Propionate is used by the cow to produce glucose and can be in short supply during transition periods and lactation. Since glucose is needed to produce energy, less fat might be mobilized and this condition might be advantageous in

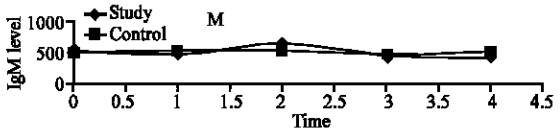

Fig. 4: Time dependent variations at IgM levels of the groups

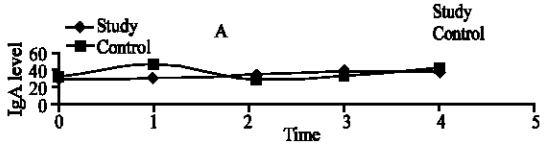

Fig. 5: Time dependent variations at IgA levels of the groups

preventing postpartum metabolic diseases, especially ketosis. According to some researchers, elevation of volatile fatty acids has negative effects on the immune system (Dawson, 1990; Iwanska *et al*., 1999; Dann *et al*., 2000).

The inhibitory effects of acetoacetic acid and betahydroxybutyric acid on bovine bone marrow cells were demonstrated and consequently it was considered that high concentrations of acetoacetic acid and betahydroxybutyric acid may alter the number of leucocytes at circulation, hence may augment the already increased tendency of dairy cattle for environmental mastitis (Iwanska *et al*., 1999).

Many researchers indicated the stimulatory effect of volatile fatty acids on leukocyte function as follows; the effect of proprionate on lymphocyte proliferation was investigated using human blood and rat mesenteric lymph nodules and it was determined that proprionate at concentrations between 2-5 mmol L^{-1}, results with a significant lymphocyte proliferation inhibition (Dawson, 1990). Furthermore, it was reported that short chain fatty acids prevents *Staphylococcus aureus* phagocytosis and extermination by human phagocytes (Williams *et al*., 1991; Sullivan and Martin, 1999).

Butyrate was demonstrated to reduce macrophage ability to stimulate lymphocytes (Bohmig *et al*., 1997; Perez *et al*., 1998). Lymphocytes treated with propionate have impaired lipid synthesis and diminished proliferation and macrophages treated with this fatty acid show changes in metabolism (Curi *et al*., 1993; Schoonjans *et al*., 1995).

Volatile fatty acids, particularly butyrate has been shown to suppress T-cell proliferation to immobilized anti-CD3 monoclonal antibody (Pompeia *et al*., 2000). Butyrate-induced cytotoxicity mainly occurs by induction of apoptosis, an effect probably associated with histone

acetylation (Dangond and Gullans, 1998). The results are not concordant with the above suggestions. The data reveals a stimulation in T lymphocyte activation with *Saccharomyces cerevisiae* supplemention supporting the results of Smirnov *et al.* (1993) and Melentkova *et al.* (1993) whom reported the stimulatory effects of Saccharomyces strains on immune system.

In addition, Koh *et al.* (2002) observed an obvious stimulatory effect of *Saccharomyces cerevisiae* on the immune system of rats. Similarly, Ochigava *et al.* (2006) used *Saccharomyces cerevisia* var.Vini strain in rats suffering from central nervous system symptoms because of *Staphylococcus aureus* and indicated a significant immune stimulation. The difference of the effects of *Saccharomyces cerevisia* may be attributed to the concentrantions of volatile fatty acids they create, because the majority of the studies indicating the inhibitory effects regards high concentrations of these acids which we may not have achieved with 10 g of *Saccharomyces cerevisia* per animal per day.

We have not yet encountered any studies about the effects of *Saccharomyces cerevisia* on the immunoglobuline levels of dairy cows. Data obtained from the present study revealed a short term increase for the IgG, IgG_1 and IgG_2 levels of dairy cows in early lactation. Only a short term decrease was detected for IgA level. Main source of IgA is gastrointestinal system mucosa and IgA is responsible for the local immune defence of the organism at mucus covered outer surfaces.

This temporary decrease in the IgA level do not promise a potential danger for the organism due to the variation staying within the reference limits. On the other hand, Qamar *et al.* (2001) indicated that Saccharomyces boulardii-another saccharomyces strain-stimulated intestinal immunoglobulin A immun reaction against Clostridium difficile toxin in rats. The difference may be attributed to the microorganism presence, the strain difference or the difference of the animal species. But the results are concordant with the researchers, stating that the mentioned yeast stimulates the immune system (Harris and Webb, 1990; Soder and Holden, 1999).

CONCLUSION

In this study, the results of this study indicates an immunstimulant tendency of *Saccharomyces cerevisia* at 10 g daily intake for dairy cows in early lactation.

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