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Effect of Yeast Culture on Serum Lipid and Meat Lipid Values of Rabbits

Nurten Galip and Nilay Seyidoolu
Department of Physiology, Faculty of Veterinary Medicine,
University of Uludag, Uludag, Turkey

Abstract: All over the world, LDL cholesterol and heart diseases are increasing day by day. Natural dietary supplements can assist in lowering cholesterol and protect against heart diseases. Therefore, the aim of this study was conducted to evaluate the effect of *Saccharomyces cerevisiae* live yeast culture supplement on serum lipid, meat lipid, haematological indices and serum clinical enzyme activities of rabbits. Twenty seven, 6-7 weeks age old New Zealand white rabbits were studied in 3 groups, of which each supplemented with 0, 2 and 4 g kg^{-1} yeast. Feed and water were offered *ad libitum* to the rabbits throughout the trial. Blood samples were obtained by ear venipuncture on the 85th day. Serum total lipid and serum LDL decreased ($p<0.05$) by YS. Saccharomyces cerevisiae at a level of 2-4 g kg^{-1} significantly reduced LDL cholesterol by 25-48% and total lipid by 5-17%, respectively. Also, the meat lipid value decreased slightly by 4-6% after yeast consumption 2-4 g kg^{-1}, respectively. Researchers suggest that dietary YS may reduce serum LDL and lipid value. Thus, yeast culture may have positive effect of reducing the coronary artery disease depend on cholesterol. *Saccharomyces cerevisiae* can become viable alternatives to cholesterol drugs.

Key words: Rabbits, yeast culture, serum lipid, meat lipid, cholesterol

INTRODUCTION

For many years to achieve efficiency in animals, studies carried out on feed additives. Antibiotics, hormones and hormone-like substances have adversely affect on the animals food and create health problems for humans. Because of this, studies have started on the new natural resources. This is the most well known natural sources is *Saccharomyces cerevisiae* live yeast culture (SC) that microbial probiotic feed additive. The feasibility and benefit of adding dietary yeast have been observed in studies on rams (Galip, 2006a-c; Paryad and Rashidi, 2009), broiler chickens (Ashayerizadeh *et al*., 2011; Paryad and Mahmoudi, 2008) and rabbits (Onifade *et al*., 1999; Kimse *et al*., 2008; Shrivastava and Jha, 2010). Paryad and Mahmoudi (2008) reported higher population of leucocytes andlymphocytes with lower H/L ratio by addition of yeast. And they suggested that the yeast may stimulate immune system of chicks body. Onifade *et al*. (1999) and Onifade (1997) reported a positive correlation between dietary levels of SC with the hematological indices like haematocrit, erythrocytes and haemoglobin in rabbit and broiler chickens. They purposed that these correlations may be an additional mechanism of growth promotion by supplemental yeast. However, Shareef and Al-Dabbagh (2009) found that blood erythrocyte, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin and mean haemoglobin concentration did not influenced but the haemoglobin parameter was increased significantly by 2% yeast group. In the same research, no change was found in leucocytes and in the percentages of heterophils, lymphocytes, monocytes, eosinophils and basophiles by yeast. Also, Gheisari and Kholeghipour (2006) found that the use of live yeast had no significant effects on hematological indices but haemoglobin. Nevertheless, Saied reported that the hematological indices, heterophills, basophiles, eosinophills, basophiles and hetrophills to lymphocytes ratio were not affected by yeast culture.

According to The World Health Organization, the 20% of strokes and 50% of heart attacks are caused by high bad cholesterol (WHO, 2002; Mendis *et al*., 2005; Roth *et al*., 2011). It has been estimated that some patients who take cholesterol-lowering prescription drugs and follow a low-fat or low-cholesterol diet, do not achieve adequate reductions in their cholesterol levels. In many patients this is due to the presence of elevated triglyceride levels, so physicians must add a second or third drug to lower triglyceride as well (Heber, 1998). Every day, cardiologists are discovering new treatments for patients with severe heart disease (Parvez *et al*., 2006; Lichtenstein *et al*., 2006). Currently, scientists debate the benefits of cholesterol drugs for humans. The growing mistrust of the general public on the pharmaceutical

Corresponding Author: Nurten Galip, Department of Physiology, Faculty of Veterinary Medicine, University of Uludag, 16059, Campus, Bursa, Turkey

industry contributes to the decision of patients not to take lipid lowering drugs. As a result, patients seek alternative drugs or opt to rely on natural therapy in order to control their cholesterol (Lin, 2010; Ong and Cheah, 2008; Mehta, 2005; Theuwissen and Mensink, 2008; Katan *et al*., 2003; US FDA, 2007; FDA, 2010).

Saccharomyces cerevisiae has been shown to survive living in the gastrointestinal tract while eliminating the potentially pathogenic bacteria residing. Since, yeast does not colonize the gastrointestinal tract permanently, it is used as a probiotic (Bekatorou *et al*., 2006). It has been postulated that probiotics binds to bile acids in the intestinal lumen which results in a reduced bile acid pool back to the liver. This binding action stimulates the production of more bile acids derived from cholesterol that is either made endogenously or captured from the circulation. This action may reduce serum cholesterol level (El-Arab *et al*., 2009). There are many conflicting studies on the effect ofsaccharomyces cerevisiae on serum lipid profile in animals. Although, some of studies showed cholesterol reduction (Paryad and Mahmoudi, 2008; Shrivastava and Jha, 2010; Saied *et al*., 2011; Kannan *et al*., 2005), the others demonstrated no benefits (Ozsoy and Yalcin, 2011; Yalcin *et al*., 2008; Hassanein and Soliman, 2010; Yildiz *et al*., 2011).

Obviously, more research is needed to confirm the cholesterol-lowering effects of *Saccharomyces cerevisiae* and its beneficial effects on serum LDL cholesterol and total lipid concentrations. Therefore, the objective of this study demonstrating the potential role of probiotics on serum cholesterol and lipid value, meat lipid value, haemotological indices and serum clinical enzyme activities in rabbits fed diets containing different levels of yeast.

MATERIALS AND METHODS

Animals, groups and feeding: About Twenty seven male New Zealand white rabbits, aged 6-7 weeks with a mean body weight of 500 g were randomly allocated on weight basis to three groups. The rabbits were housed individually in metal cages and provided with separate facilities for feeding and watering. Feed and water were offered *ad libitum* to the rabbits throughout the 85 days trial.

Yea Sacc^{1026} (YS, *Saccharomyces cerevisiae* live yeast culture Altech, Nicholasville: 5×10^{8} CFU g^{-1}) were supplemented with 0.0, 2.0 and 4.0 g kg^{-1} of the basaldiet. Basal diet (pelleted) was formulated to contain 2600 kcal ME kg^{-1} metabolizable energy, 16% crude protein and was designed to meet maintenance requirements according to the National Research Council (NRC, 1977). Chemical composition and ingredients of the diet are provided in Table 1 and 2. Chemical analyses of diets were carried out according to AOAC (1990).

Table 1: Chemical composition of basal diet (DM %)

Chemical composition	Diet
Dry matter	88.86
Crude fiber (%*)	11.70
Crude protein (%*)	15.00
Ether extracts (%*)	4.00
Ash	7.80
ADF	12.20
NDF	24.04
ADL	2.85

*Based on % dry matter

Table 2: Ratio of feed ingredients (%)

Ingredients	Usage rate (%)
Barley	25.00
Corn14	13.60
Rice bran	10.00
Corn bran	12.12
Alfalfa meal	30.04
Soybean meal 46	6.40
Marble dust	1.24
Dcp 18	0.25
Salt	0.90
Methionin	0.10
Organic mineral	0.05
Anticoccidial	0.03
Vitaminpremix*	0.25
Antioccidial	0.03
Total	100.00

*Premix: Vit. A 4,800,000 IU, Vit. D 800,000 IU, Vit. E 14,000 mg, Biotin 18 mg, CH-CL 50,000 mg, Folic acid 400 mg, Niacin 8,000 mg, Pant.Acid 4,000 mg, Riboflavin 2,800 mg, Thiamin 1,200 mg, Pyridoxine 2,000 mg, Vit. K 1,600 mg, Zinc 24,000 mg, Iron 2,000 mg, Iodine 400 mg, Manganese 32,000 mg, Selenium 60 mg, Copper 24,000 mg

The experimental protocols were approved by the Animal Care and Use Committee of Uludag University and are in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals.

Measurements: Blood samples were collected by ear venipuncture on the 85th day from overnight-fasted rabbits. Haematological samples were collected in EDTA treated tubes while samples for biochemical parameters were collected without anticoagulant. Serum concentrations of total lipid, cholesterol, LDL-C, HDL-C, Calcium and activites of Aspartate aminotransferaz (AST), Alkaline Phosphatase (ALP) and Alanin Aminotransferaz (ALP) were determined by Clima MC15 auto analyzer (RAL, Barcelona, Spain). Numbers of erythrocyte and haematocrit values were estimated according to the methods reported by Jain (1986). Also, MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Haemoglobin) and MCHC (Mean Corpuscular Haemoglobin Concentration) were mathematically calculated according to Jain (1986). The levels of haemoglobin were measured spectrophotometrically by the Cyanmethemoglobin Method of Cannon (1958).

At the end of the study, 6 randomly selected rabbits from each group were weighed and slaughtered. Rabbits were dissected according to the protocol described by the World Rabbit Science Association (Blasco and Ouhayoun, 1993) and 90 g of meat were extracted for meat analysis. Measurement of lipid in meat was performed according to the method of Soxhlet Extraction (AOAC, 2000) at the Bursa Central Research Institute for Food and Feed Control.

Statistical analysis: Statistical analyses were performed with SPSS (13.0). Data were tested for normality distribution and variance homogeneity assumptions. All the values were grouped and the means and standard deviations were calculated. One-way ANOVA was applied to the all parameters to examine the difference between groups. Differences were considered significant at $p<0.05$. If the difference between groups was provided to be significant ($p<0.05$), differences evaluated group by Tukey's test (Dowdy and Wearden, 1981). On the other hand, in non-homogenous groups, differences between means were analysed by Kruskal Wallis and following Mann Whitney U-test between groups one by one (Dawson and Trapp, 2001).

RESULTS AND DISCUSSION

Biochemical indices of the three treatment groups (0, 2 and 4 g kg^{-1} yeast) are shown in Table 3. Serum total lipid and LDL cholesterol values were significantly lower in rabbits fed 4 g yeast compared to the other groups. ($p<0.05$). Also, the serum cholesterol value was tended to be lower in rabbits fed 2 g yeast. There was no significant change in the haemotological parameteres as shown in Table 4.

Saccharomyces cerevisiae at a level of 2-4 g kg^{-1} significantly reduced LDL cholesterol by 25-48% and total lipid by 5-17%, respectively ($p<0.05$). Although, statistically not significant, the serum cholesterol value was tended to be lower in rabbits fed 2 g kg^{-1} yeast ($p>0.05$). Also, data published by Shrivastava and Jha (2010), Kannan *et al.* (2005) and Onifade *et al.* (1999) who stated that there was a decrease in plasma cholesterol by dietary yeast. A number of probiotics are known to metabolize bile salts, bile acids and prevent reabsorption and recirculation of bile acids into blood. In this way, probiotics could contribute to the regulation of serum cholesterol concentrations by deconjunction of bile acids. Use of probiotics lead to increased excretion of deconjugated bile acids. Cholesterol is precursor of bile acid hence more molecules are spent for recovery of bile acids (De Smet *et al.*, 1994). As a result of increased synthesis of this acid, it seems that the level of serum cholesterol gets reduced. Also, Klaver and van der Meer (1993) suggested that coprecipitation with bile acids might be of importance for decreasing of serum cholesterol concentrations. Data on meat lipid is summarized in Table 3. The amounts of meat lipid was tended tobe lower in rabbits fed 4 g yeast than in the other groups. The meat lipid value decreased slightly by 4-6% after yeast consumption 2-4 g kg^{-1}, respectively. Researchers could locate any literature concerning the effect of yeast on meat lipid.

Serum glucose value slightly higher in rabbits fed 4 g kg yeast ($p>0.05$, Table 3). Also, Shrivastava and Jha (2010) documented that significant increase in serum glucose value in rabbits fed probiotic as a result of the increased fermentative action of microflora present in probiotic.

Table 3: Biochemical indices and rabbit meat analysis of rabbits fed basal or yeast supplemented diets (mean±standard deviation, n = 27; n =18 for meat lipid analysis)

	Yeast (g kg^{-1})		
Parameters	0 (Diet I)	2 (Diet 2)	4 (Diet 3)
Biochemical indices			
Total lipid (mg dL^{-1})	486.11±13.20^a	462.29±7.580^a	404.78±13.99^b
LDL (mg dL^{-1})	22.15±9.660^a	16.65± 2.56^b	11.54± 6.28^b
Kolesterol (mg dL^{-1})	82.33±16.05	77.43±6.070	80.89±13.13
HDL (mg dL^{-1})	45.00±4.840	42.67±4.120	51.11±3.780
LDL/HDL	0.39±0.220	0.40±0.060	0.29±0.330
HDL/Kolesterol	0.63±0.710	0.55±0.020	0.81±0.150
Glucose (mg dL^{-1})	66.55±7.160	72.86±4.300	74.11±6.910
Calcium (mg dL^{-1})	9.28±0.400	8.72±0.220	9.24±0.330
AST (U L^{-1})	36.22±7.170	35.43±1.770	27.89±4.450
ALT (U L^{-1})	26.89±3.120	23.00±1.760	23.22±2.880
ALP (U L^{-1})	293.11±12.28	286.14±16.52	291.33±11.42
Ca/Creatinin	11.04±1.290	9.43±0.230	10.20±0.500
Rabbit meat analysis			
Meat lipid (%)	8.78 ± 0.84	8.39 ± 0.73	8.29 ± 0.23

ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase. ALP: Alkaline Phosphatase; Different superscripts (a, b) show differences ($p<0.05$) between groups

Table 4: Blood composition in rabbits fed basal diet or yeast supplemented diets (mean±standard deviation, n = 27)

	Yeast (g kg^{-1})		
Haemotological indices	0 (Diet I)	2 (Diet 2)	4 (Diet 3)
Haemoglobin (g dL^{-1})	43.33±1.37	40.33±1.00	41.22±1.29
Haematocrit (%)	11.40±0.17	11.11±0.25	10.71±0.15
Erythrocytes (10^6 uL^{-1})	6.43±0.22	5.7±0.29	5.93±0.22
Leucocytes(10^3 uL^{-1})	4.58±0.37	5.21±0.37	5.36±0.35
MCV (FL)	67.78±2.46	71.66±3.89	70.26±3.27
MCH (pg)	17.81±0.70	19.73±1.05	18.29±0.46
MCHC (%)	26.32±0.75	27.63±0.73	26.36±0.99
Neutrophils (10^3 mL^{-1} of blood)	234±26	229±15	230±18
Lymphocytes (10^3 mL^{-1} of blood)	671±30	659±12	681±20
Monocytes (10^3 mL^{-1} of blood)	31±4	27±4	22±4
Eosinophils (10^3 mL^{-1} of blood)	22±3	22±3	17±2
Basophiles (10^3 mL^{-1} of blood)	41±12	63±8	50±7

MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration

Average values AST, ALT and ALP in serum of rabbits at 18th week of age raised on different level of yeast are shown in Table 3. The AST, ALT and ALP in serum of the rabbits did not differ significantly among the groups. These enzymes are located intracellularly in the body including liver, heart, kidney, etc. Their level in blood is increased when there is a membrane damage in these cells due to degenerative changes. Hence, normal level of these enzymes in blood in probiotic treated rabbits suggests that it has no adverse effect on the cells vital organs. These results are agreement with Onbasilar and Yalcin (2008), Shrivastava and Jha (2010), Saied *et al*. (2011), Ibrahim *et al*. (2010), Ozsoy and Yalcin (2011) and Yalcin *et al*. (2008). In the other study, Onifade *et al*. (1999) observed that AST, ALT and ALP enzymes were significantly lower in rabbits fed yeast than in the group fed unsupplemented diet. And they suggested that a low background rate of these enzymes released into the serum from the liver was a normal functioning of the hepatic tissues.

No significant differences among the treatments were observed in serum calcium and serum calcium/creatinine ratio in rabbits. The present data obtained suggest that yeast addition does not have any positive or negative effect on the mineral metabolism. Also data confirmed by some researchers (Galip, 2006a; Shrivastava and Jha, 2010; Shareef and Al-Dabbagh, 2009). However, Onifade *et al*. (1999) reported that serum calcium and serum calcium/creatinine ratio in rabbits decreased significantly by Yea Sacc^{1026}. According to the same researchers enhanced bone mineralization due to yeast addition was suggested because a high calcium/creatinine ratio could be an indicator of osteoporosis or bone resorption. The data obtained suggest that yeast addition may have a positive effect on the mineral metabolism depending on ration.

There were no differences occurred in haematological variables by added dietary yeast (Table 4). Similar results in haematological variables in broiler fed YS have been reported earlier (Shareef and Al-Dabbagh, 2009; Gheisari and Kholeghipour, 2006; Saied *et al*., 2011). However, decrease in haematocrit value ($p<0.01$) in dairy cows as a result of increased protein requirement because of increased yields of milk by dietary YS has been documented by Wohlt *et al*. (1998). Furthermore, Onifade *et al*. (1999) observed that haematocrit, haemoglobin, MCV and MCH increased significantly ($p<0.05$) by *S. cerevisiae* in rabbits and they suggested that YS enhanced haematopoiesis.

CONCLUSION

In this study, serum total lipid and LDL cholesterol were lowered by yeast. Yeast is a natural product that may be beneficial in treating cholesterol. Thus, there is a decrease likelihood of the health problems by yeast such as heart diseases, vascular diseases, heart attack and stroke depend on cholesterol.

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