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Original article

# Influence of probiotic and yeast culture supplementation on selected biochemical and immunological parameters of growing lambs

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## Abstract

This study was carried out to evaluate the potential effects of 90 days-long dietary supplementation of probiotic and yeast culture on immunity condition of lambs. Fifteen Rahmani growing male lambs (about 5 months old and 23.21±2.75 kg body weight) were randomly allocated to three equal groups consisting of 5 animals each. The animals in the first group, served as a control (group C), were fed a basal diet without any supplementation. The lambs in the second and third group were fed the basal diet supplemented with probiotic (group Y) or yeast culture (group YC), respectively. The probiotic consisted of live yeast (*Saccharomyces cerevisiae*) alone, while the yeast culture was composed of *Saccharomyces cerevisiae* and the media on which it was grown. In group Y and YC, each lamb was supplemented daily with 0.5 g and 7.0 g of live yeast and yeast culture, respectively. Blood samples were collected before feeding the supplements and then every 15 days until the day 90<sup>th</sup>. Total and differential leucocytic counts, total protein, albumin, IgA, IgG and IgM levels were measured in blood. There were insignificant ( $p>0.05$ ) variations in the levels of total and differential leucocytic counts and total protein among the groups throughout the experiment. However, significant differences ( $p<0.05$ ) were found in globulin, IgA, IgG and IgM in both (Y) and (YC) groups, but the effect of yeast culture seems to be better than that of the probiotic. In conclusions, the obtained results indicate that the tested probiotic and yeast culture improve the immunological status of lambs.

**Key words:** lambs, probiotic, yeast culture, *Saccharomyces cerevisiae*, immunoglobulins

## Introduction

The use of probiotics as feed supplements for small ruminants is not a novel concept. For many years, researchers have been interested in manipulating the rumen microbial ecosystem in an attempt to improve health, feed utilization and production efficiency of ruminants (Galip 2006, Seo et al. 2010). Probiotics or direct-fed microbials are described as live microbial feed supplements in a mono or mixed culture of living microorganisms, which beneficially affect the host animal by supporting its microbial balance (Fuller 1989, Abd El-Tawab et al. 2016). They have been used as growth promoters to replace the widely used antibiotics and synthetic chemical feed supplements (Fooks and Gibson 2002). Live yeast including *Saccharomyces cerevisiae* (*S. cerevisiae*) and *Aspergillus oryzae* are very important probiotics in ruminant nutrition (Denev et al. 2007). Over the past few years, an increasing interest in comparison of the effects of *S. cerevisiae* live cell products to *S. cerevisiae* culture products on health parameters in ruminants was observed (Mikulec et al. 2010). A yeast culture (YC) is a yeast-fermented feed additive that consists of both alive and dead yeast cells, the culture media in which the yeast cells grew on and the metabolic bioproducts of the yeast cells during fermentation. Yeast products are included in ruminant feeds for many beneficial effects such as stabilization of the microbial equilibrium of the digestive tract, the immune response stimulation, antimicrobial substances production, competitive exclusion of pathogens, antioxidant and anticarcinogenic effect, alleviation of stress through enhanced immune response and reduction of the occurrence of metabolic diseases, including acidosis (Yoon and Stern 1995, Ma et al. 2010, Tripathi and Karim 2011, Bidarkar et al. 2014, Elghandour et al. 2015, Puniya et al. 2015). Moreover, yeast probiotics have also been found to enhance host immunity through stimulation of immunoglobulins, macrophages, natural killer cells and cytokine production. However, the exact mechanisms by which probiotics exert their beneficial roles have not been fully elucidated (Koop-Hoolihan 2001). Probiotics have been shown to enhance humoral immune responses which, in turn, will increase the animal's ability to resist disease and thereby promote the intestine's immunologic barrier (Kaila et al. 1992, Isolauri et al. 1993). Moreover, they exert their protective effects by multiple immune and non-immune mechanisms by increasing phagocytosis, modifying cytokines production by different cell populations and enhancing immunoglobulin A (IgA) production (Lebeer et al. 2008, Vizoso-Pinto et al. 2009, De LeBlanc et al. 2010). However, the researches about the effects of yeast and yeast cul-

tures on modulation and stimulation of the immune system in lambs are scarce. In vitro trial by Jensen et al. (2008a,b) showed, that a yeast culture product could provide anti-oxidant, anti-inflammatory and immunomodulatory activities. Since then, several experiments have been conducted in lambs (Wójcik 2010, Dabiri et al. 2016), and calves (Magalhães et al. 2008, Harris et al. 2017) to determine the efficacy of the yeast products on the immune response in vivo. It is well known that yeast does not grow in rumen fluid but retains its metabolic activity and viability (Newbold et al. 1996). The importance of viable or metabolically active yeast cells in a YC preparation for optimal stimulatory activities in the rumen has been established by a number of investigators who suggested that metabolic activity and not active reproduction is an integral part of the basic process that leads to a maximal beneficial response to yeast supplementation (Abd El-Tawab et al. 2016).

The aim of the present study was to investigate the potential effects of dietary supplementation of probiotic and yeast culture in growing lambs on selected immunity parameters, including evaluation of: leukogram, serum total protein, albumin (A), globulin (G), A/G ratio and immunoglobulins.

## Materials and Methods

The present experiment was carried out at the Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef province, Egypt. All experimental procedures were approved by the Local Ethics Committee for Animal Experiments.

### Animals and feeding procedures

A total of fifteen Rahmani growing male lambs (about 5 months old and  $23.2 \pm 2.75$  kg body weight) were randomly allocated into three dietary groups, consisting of five animals each. The experiment lasted for three months. The animals from the first group served as a control (group C) and were fed a basal diet without any supplementation. The lambs in the second (group Y) and third (group YC) group were fed the basal diet supplemented with probiotic or yeast culture, respectively. The used probiotic consisted solely of live yeast (Levucell SC 20<sup>®</sup> product, manufactured by LALLEMAND CO., France, each gram provides  $20 \times 10^9$  colony forming unit (CFU)/g of *Saccharomyces cerevisiae*). The yeast culture was composed of *Saccharomyces cerevisiae* and the media, on which it grew, dried in a manner preserving the fermenting activity of the yeast (Diamond V XP<sup>™</sup> dried product, manufactured by Original XP CO., Cedar Rapids, USA, its total

Table 1. Ingredients and chemical composition of the concentrate mixture fed to lambs.

Ingredients	Content (%)
Yellow corn, ground	55.0
Soybean meal	16.3
Wheat bran	26.0
Limestone	2.0
Salt	0.5
Vitamins-minerals premix <sup>1</sup>	0.2
Chemical composition (on DMB)	
ME, Mcal/kg DM	2.90
Crude protein, %	17.3
Crude fiber, %	5.22
NDF, %	20.6
ADF, %	7.18
Calcium, %	0.81
Phosphorus, %	0.60
Sodium, %	0.22

ME: Metabolisable energy. NDF: Neutral detergent fiber. ADF: Acid detergent fiber.

<sup>1</sup>Vitamins-minerals premix contains 20,000,000 IU vitamin A, 200,000 IU vitamin D<sub>3</sub>, 10,000 mg vitamin E, 10,000 mg Fe, 2,500 mg Cu, 20,000 mg Mn, 100 mg Mo, 100 mg Co, 800 mg I, 20,000 mg Zn, and 100 mg Se per kilogram.

microbial activity provides  $50 \times 10^7$  CFU/g). In group 2 (Y) and 3 (YC), each lamb was supplemented daily with 0.5 g of live yeast and 7.0 g of yeast culture till the end of the experiment, respectively. The basal diet consisted of concentrate mixture and wheat straw and was formulated to satisfy the animal's requirements according to NRC for sheep (1985). The ingredients and chemical composition of the concentrate mixture is shown in Table 1. The supplements were mixed with the concentrate mixture. The lambs were fed the calculated amounts of concentrate mixture twice daily in equal portions at 8.00 and 17.00 h, whereas the wheat straw was given *ad libitum*. The amount of concentrate mixture was adjusted biweekly according to the body weight gain. Fresh, clean tap water was available *ad libitum*. Lambs were housed in semi-opened pens.

Before the beginning of the experiment, the lambs were subjected to thorough clinical, hematologic and parasitic examinations. The animals chosen for the study were healthy and free from infectious and parasitic diseases.

### Measurement of immunity parameters

Blood samples were collected from each animal on day 0 (before the administration of supplements), on day 15 from receiving supplementation and then every two weeks until the day 90. Samples were collected from jugular vein into two tubes. The first tube contained EDTA as an anticoagulant and was used for total leucocytic count (WBC) ( $10^3/\mu\text{L}$ ) and differential

leucocytic count estimation. The second one was a clean dry sterile test tube used to obtain clear blood serum, which was carefully separated by blood centrifugation at 3000 rpm for 15 minutes. Then, the serum was immediately stored at  $-20^\circ\text{C}$  until analysis of total protein (TP), albumin, IgA, IgM and IgG levels. Total leukocytes count was estimated by laser and cytometry method using hematology analyzer Advia 2120i Siemens. Differential leukocyte counts were performed by counting 100 cells on blood smears stained with May-Grünwald-Giemsa method and included neutrophils, monocytes, eosinophils and lymphocytes. Concentration of total protein was estimated using biuret reaction and concentration of albumin was estimated with bromocresol green method. The colour intensity of the complex was measured at 540 nm and 630 nm, respectively, by using biochemical analyzer Accent-200 (Cormay).

Blood serum globulin was calculated mathematically by subtracting the total protein values minus albumen values. The A/G ratio was estimated mathematically by dividing the albumins by globulins value. Blood serum IgA, IgG and IgM concentrations were determined by ELISA (enzyme-linked immunosorbent assay) technique according to Rivera et al. (2002).

### Statistical analysis

Data obtained during the experiment were analyzed using SPSS program (SPSS for windows Version 22, SPSS Inc., Chicago, USA). Statistically significant dif-

Table 2. The mean values of total and differential leukocytic counts in probiotic and yeast culture-supplemented lambs (Mean  $\pm$  SD).

Parameter	Group	Days of experiment						
		0	15	30	45	60	75	90
WBCs, $1 \times 10^3/\mu\text{l}$	C	9.81 $\pm$ 1.50	11.90 $\pm$ 1.28	11.16 $\pm$ 1.44	11.38 $\pm$ 1.68	11.65 $\pm$ 0.80	9.70 $\pm$ 1.07	10.80 $\pm$ 0.58
	Y	10.59 $\pm$ 1.71	12.32 $\pm$ 0.14	10.80 $\pm$ 1.95	12.08 $\pm$ 0.72	11.69 $\pm$ 0.98	11.32 $\pm$ 1.55	9.37 $\pm$ 1.79
	YC	9.85 $\pm$ 1.29	12.54 $\pm$ 0.59	12.30 $\pm$ 0.31	11.02 $\pm$ 1.06	9.69 $\pm$ 0.84	9.38 $\pm$ 1.23	9.46 $\pm$ 0.90
Neutrophils, %	C	41.50 $\pm$ 3.51	40.75 $\pm$ 3.86	41.75 $\pm$ 4.79 <sup>a</sup>	41.25 $\pm$ 6.18	41.75 $\pm$ 3.86 <sup>a</sup>	40.50 $\pm$ 2.89	40.50 $\pm$ 5.45
	Y	41.20 $\pm$ 3.35	39.80 $\pm$ 4.15	39.00 $\pm$ 4.85 <sup>b</sup>	38.40 $\pm$ 3.51	38.40 $\pm$ 1.52 <sup>b</sup>	38.20 $\pm$ 2.68	38.00 $\pm$ 2.35
	YC	41.20 $\pm$ 5.36	39.40 $\pm$ 5.18	38.20 $\pm$ 5.07 <sup>b</sup>	39.00 $\pm$ 6.20	38.20 $\pm$ 2.17 <sup>b</sup>	38.20 $\pm$ 5.40	38.40 $\pm$ 3.90
Lymphocytes, %	C	52.25 $\pm$ 3.69	52.50 $\pm$ 3.11	51.00 $\pm$ 2.83 <sup>b</sup>	52.50 $\pm$ 3.11 <sup>b</sup>	53.25 $\pm$ 4.19 <sup>b</sup>	53.75 $\pm$ 3.59 <sup>b</sup>	53.25 $\pm$ 2.22 <sup>b</sup>
	Y	52.00 $\pm$ 3.39 <sup>B</sup>	54.20 $\pm$ 4.97 <sup>B</sup>	55.00 $\pm$ 5.24 <sup>aA</sup>	55.80 $\pm$ 3.77 <sup>aA</sup>	55.80 $\pm$ 2.05 <sup>aA</sup>	55.40 $\pm$ 3.71 <sup>aA</sup>	56.80 $\pm$ 2.59 <sup>aA</sup>
	YC	52.20 $\pm$ 5.63 <sup>B</sup>	54.00 $\pm$ 6.86 <sup>B</sup>	55.60 $\pm$ 3.78 <sup>aA</sup>	55.00 $\pm$ 5.92 <sup>aA</sup>	55.80 $\pm$ 2.17 <sup>aA</sup>	55.60 $\pm$ 5.59 <sup>aA</sup>	55.80 $\pm$ 3.21 <sup>aA</sup>
Monocytes, %	C	4.75 $\pm$ 2.22	4.50 $\pm$ 1.91	4.00 $\pm$ 1.83	4.25 $\pm$ 2.06	4.00 $\pm$ 1.41	4.00 $\pm$ 0.00	4.25 $\pm$ 3.10
	Y	4.80 $\pm$ 3.03	4.00 $\pm$ 1.87	4.00 $\pm$ 1.58	4.80 $\pm$ 1.48	4.20 $\pm$ 0.84	4.80 $\pm$ 0.84	4.00 $\pm$ 0.71
	YC	4.60 $\pm$ 0.89	4.80 $\pm$ 1.64	4.60 $\pm$ 1.95	4.80 $\pm$ 1.92	4.60 $\pm$ 1.52	4.80 $\pm$ 0.84	4.20 $\pm$ 1.10
Eosinophils, %	C	1.50 $\pm$ 0.58	2.25 $\pm$ 2.06	2.25 $\pm$ 0.96	2.00 $\pm$ 1.41	1.00 $\pm$ 0.82	1.75 $\pm$ 2.06	2.00 $\pm$ 1.63
	Y	2.00 $\pm$ 1.22	2.00 $\pm$ 1.00	2.00 $\pm$ 1.00	1.00 $\pm$ 1.58	1.60 $\pm$ 1.14	1.60 $\pm$ 0.55	1.20 $\pm$ 1.10
	YC	2.00 $\pm$ 1.87	1.80 $\pm$ 2.28	1.60 $\pm$ 1.52	1.20 $\pm$ 0.84	1.40 $\pm$ 1.52	1.40 $\pm$ 0.55	1.40 $\pm$ 1.14

<sup>a,b</sup> Means with different superscripts within the same column are significantly different at  $p < 0.05$ .

<sup>A,B</sup> Means with different superscripts within the same row are significantly different at  $p < 0.05$ .

Table 3. The mean values of Total protein, Albumin, Globulin and A/G ratio levels in probiotic and yeast culture-supplemented lambs (Mean  $\pm$  SD).

Parameter	Group	Days of experiment						
		0	15	30	45	60	75	90
Total protein, g/dl	C	6.34 $\pm$ 0.35	6.88 $\pm$ 0.11	7.15 $\pm$ 0.37	6.85 $\pm$ 0.37	6.86 $\pm$ 0.72	6.06 $\pm$ 0.08	6.02 $\pm$ 0.31 <sup>b</sup>
	Y	6.54 $\pm$ 0.19	6.95 $\pm$ 0.32	7.47 $\pm$ 0.53	6.65 $\pm$ 0.38	6.54 $\pm$ 0.43	5.90 $\pm$ 0.29	6.12 $\pm$ 0.26 <sup>b</sup>
	YC	6.22 $\pm$ 0.13	6.56 $\pm$ 0.23	7.32 $\pm$ 0.55	6.69 $\pm$ 0.21	6.00 $\pm$ 0.35	6.18 $\pm$ 0.14	6.74 $\pm$ 0.32 <sup>a</sup>
Albumin, g/dl	C	2.61 $\pm$ 0.17	2.49 $\pm$ 0.16	2.49 $\pm$ 0.30	2.40 $\pm$ 0.25 <sup>b</sup>	2.33 $\pm$ 0.15 <sup>b</sup>	2.24 $\pm$ 0.34 <sup>b</sup>	2.21 $\pm$ 0.19 <sup>b</sup>
	Y	2.60 $\pm$ 0.34	2.60 $\pm$ 0.15	2.73 $\pm$ 0.34	2.87 $\pm$ 0.13 <sup>a</sup>	2.78 $\pm$ 0.14 <sup>a</sup>	2.78 $\pm$ 0.44 <sup>a</sup>	2.75 $\pm$ 0.18 <sup>a</sup>
	YC	2.47 $\pm$ 0.24 <sup>B</sup>	2.56 $\pm$ 0.13 <sup>B</sup>	2.77 $\pm$ 0.56 <sup>B</sup>	2.99 $\pm$ 0.22 <sup>aA</sup>	2.70 $\pm$ 0.15 <sup>aB</sup>	2.75 $\pm$ 0.33 <sup>aB</sup>	2.72 $\pm$ 0.34 <sup>aB</sup>
Globulin, g/dl	C	3.73 $\pm$ 0.20	3.39 $\pm$ 0.22 <sup>b</sup>	3.26 $\pm$ 0.28 <sup>b</sup>	3.64 $\pm$ 0.37 <sup>b</sup>	3.12 $\pm$ 0.83 <sup>b</sup>	3.52 $\pm$ 0.28 <sup>b</sup>	3.31 $\pm$ 0.37 <sup>b</sup>
	Y	3.94 $\pm$ 0.31 <sup>B</sup>	4.35 $\pm$ 0.17 <sup>aA</sup>	4.75 $\pm$ 0.65 <sup>aA</sup>	4.78 $\pm$ 0.43 <sup>aA</sup>	4.16 $\pm$ 0.57 <sup>aA</sup>	4.42 $\pm$ 0.17 <sup>aA</sup>	4.87 $\pm$ 0.40 <sup>aA</sup>
	YC	3.75 $\pm$ 0.35 <sup>B</sup>	4.01 $\pm$ 0.23 <sup>aB</sup>	4.56 $\pm$ 0.31 <sup>aA</sup>	4.70 $\pm$ 0.35 <sup>aA</sup>	4.29 $\pm$ 0.40 <sup>aA</sup>	4.83 $\pm$ 0.31 <sup>aA</sup>	4.31 $\pm$ 0.60 <sup>aA</sup>
A/G ratio	C	0.70 $\pm$ 0.02	0.73 $\pm$ 0.07	0.76 $\pm$ 0.10	0.66 $\pm$ 0.13	0.75 $\pm$ 0.19	0.73 $\pm$ 0.16	0.67 $\pm$ 0.12
	Y	0.67 $\pm$ 0.13	0.60 $\pm$ 0.01	0.59 $\pm$ 0.14	0.60 $\pm$ 0.11	0.57 $\pm$ 0.13	0.81 $\pm$ 0.17	0.56 $\pm$ 0.10
	YC	0.67 $\pm$ 0.12	0.64 $\pm$ 0.06	0.61 $\pm$ 0.15	0.64 $\pm$ 0.14	0.63 $\pm$ 0.12	0.57 $\pm$ 0.14	0.66 $\pm$ 0.16

<sup>a,b</sup> Means with different superscripts within the same column are significantly different at  $p < 0.05$ .

<sup>A,B</sup> Means with different superscripts within the same row are significantly different at  $p < 0.05$ .

ferences between groups and time points were determined by variance analysis (two way- ANOVA test). When the differences were significant, Duncan's multiple range post test was performed. Mean values were considered significantly different at  $p < 0.05$ . Data are expressed as mean values  $\pm$  SD.

## Results

All the animals used in the study did not show any signs of disease or inflammatory condition throughout the experimental period.

The ranges of total leukocytic counts in blood of lambs during experiment (Table 2) were 9.70 $\pm$ 1.07 to 11.90 $\pm$ 1.28 ( $\times 10^3/\mu\text{l}$ ), 9.37 $\pm$ 1.79 to 12.32 $\pm$ 0.14 ( $\times 10^3/\mu\text{l}$ ) and 9.38 $\pm$ 1.23 to 12.54 $\pm$ 0.59 ( $\times 10^3/\mu\text{l}$ ) in C, Y and YC groups, respectively. There were no significant

Table 4. The mean values of serum IgA, IgG and IgM levels counts in probiotic and yeast culture-supplemented lambs (Mean  $\pm$  SD).

Parameter	Group	Days of experiment						
		0	15	30	45	60	75	90
IgA, mg/dl	C	316.67 $\pm$ 49.33	340.00 $\pm$ 56.79	326.67 $\pm$ 83.27	301.67 $\pm$ 48.05	323.33 $\pm$ 49.33 <sup>b</sup>	300.00 $\pm$ 43.59 <sup>b</sup>	303.33 $\pm$ 41.63 <sup>b</sup>
	Y	316.33 $\pm$ 31.94 <sup>B</sup>	330.00 $\pm$ 60.83 <sup>B</sup>	301.67 $\pm$ 37.53 <sup>B</sup>	333.33 $\pm$ 40.41 <sup>B</sup>	373.33 $\pm$ 61.10 <sup>aA</sup>	380.00 $\pm$ 45.83 <sup>aA</sup>	386.67 $\pm$ 51.32 <sup>aA</sup>
	YC	330.00 $\pm$ 30.00 <sup>B</sup>	332.00 $\pm$ 39.40 <sup>B</sup>	313.33 $\pm$ 23.09 <sup>B</sup>	323.33 $\pm$ 58.59 <sup>B</sup>	376.67 $\pm$ 5.77 <sup>aA</sup>	383.33 $\pm$ 32.15 <sup>aA</sup>	380.33 $\pm$ 49.50 <sup>aA</sup>
IgG, mg/dl	C	1130.00 $\pm$ 301.16	1156.67 $\pm$ 184.48	1116.67 $\pm$ 20.82	1145.00 $\pm$ 96.57 <sup>b</sup>	1150.00 $\pm$ 112.69 <sup>b</sup>	1143.33 $\pm$ 73.71 <sup>b</sup>	1156.67 $\pm$ 51.32 <sup>b</sup>
	Y	1253.33 $\pm$ 260.8 <sup>B</sup>	1313.33 $\pm$ 45.09 <sup>B</sup>	1263.33 $\pm$ 132.0 <sup>B</sup>	1366.67 $\pm$ 115.47 <sup>aA</sup>	1380.00 $\pm$ 155.24 <sup>aA</sup>	1386.67 $\pm$ 15.04 <sup>aA</sup>	1420.00 $\pm$ 105.83 <sup>aA</sup>
	YC	1183.33 $\pm$ 202.1 <sup>B</sup>	1283.67 $\pm$ 183.22 <sup>B</sup>	1280.00 $\pm$ 72.11 <sup>B</sup>	1383.33 $\pm$ 37.86 <sup>aA</sup>	1333.33 $\pm$ 205.99 <sup>aA</sup>	1380.00 $\pm$ 43.59 <sup>aA</sup>	1566.67 $\pm$ 130.51 <sup>aA</sup>
IgM, mg/dl	C	185.00 $\pm$ 8.66	176.67 $\pm$ 5.77	175.00 $\pm$ 5.00	160.00 $\pm$ 17.32 <sup>b</sup>	163.33 $\pm$ 15.28 <sup>b</sup>	166.67 $\pm$ 63.5 <sup>b</sup>	173.33 $\pm$ 5.77 <sup>b</sup>
	Y	193.33 $\pm$ 15.28 <sup>B</sup>	196.67 $\pm$ 86.22 <sup>B</sup>	180.00 $\pm$ 10.00 <sup>B</sup>	213.67 $\pm$ 40.87 <sup>aB</sup>	219.67 $\pm$ 26.27 <sup>aA</sup>	218.67 $\pm$ 53.72 <sup>aA</sup>	250.00 $\pm$ 30.00 <sup>aA</sup>
	YC	187.00 $\pm$ 12.53 <sup>B</sup>	216.00 $\pm$ 65.02 <sup>B</sup>	217.00 $\pm$ 10.00 <sup>B</sup>	231.67 $\pm$ 27.54 <sup>aB</sup>	298.33 $\pm$ 7.64 <sup>aA</sup>	306.67 $\pm$ 11.55 <sup>aA</sup>	345.00 $\pm$ 49.24 <sup>aA</sup>

<sup>a,b</sup> Means with different superscripts within the same column are significantly different at  $p < 0.05$ .

<sup>A,B</sup> Means with different superscripts within the same row are significantly different at  $p < 0.05$ .

variations observed between the animal groups throughout the duration of the experiment. Regarding to neutrophils percentages in the blood, there were no significant changes ( $p > 0.05$ ) between the animal groups during the experimental period, except on the 30<sup>th</sup> day of experiment, on which a significant reduction ( $p < 0.05$ ) of the neutrophils percentage in YC group was observed in comparison to C group, and on the 60<sup>th</sup> day of experiment, when a significant reduction ( $p < 0.05$ ) of the neutrophils percentage in Y and YC groups was observed when compared with C group. Moreover, the lymphocytes percentages of the lambs supplemented with probiotic or yeast culture (group Y and YC) were significantly ( $p < 0.05$ ) higher from day 30 until the end of experiment comparing with day 0 and C group. However, there were no significant ( $p > 0.05$ ) differences in the percentages of monocytes and eosinophils between the lambs of all three groups during the experimental period.

Regarding the levels of serum total protein and albumin (Table 3), both total protein and albumin levels of the lambs in each group have increased gradually until they reached the peak at the 30<sup>th</sup> day of the experiment and then begun to decline. On day 90, a significant increase ( $p < 0.05$ ) in the level of total protein in YC group in comparison to other groups was observed. In the last four examinations, a significant decrease ( $p < 0.05$ ) of albumin levels in the C group comparing to the other groups was observed. Moreover, the level of serum globulin was reduced in the C group animals comparing to other groups during the entire experiment. In the Y group, the concentration of globulin increased gradually until the day 45, in the next two examinations it decreased, and finally globulin concentration reached a peak at the end of experiment. During the entire study in this group of animals, level of globulin was significantly ( $p < 0.05$ ) higher in comparison to day 0. The same tendency was observed in YC group. There were not

any significant changes in A/G ratio between the treatment groups and comparing with day 0 during the entire experiment.

The serum concentrations of IgA in lambs provided with probiotic and yeast culture (group Y and YC) revealed a significant ( $p < 0.05$ ) increase when compared with the control animals in the last three examinations (Table 4). On days 75 and 90, this difference was also significant ( $p < 0.05$ ) comparing with day 0. The same tendency was observed in the concentration of IgG. Serum concentration of IgM was significantly ( $p < 0.05$ ) higher in Y and YC groups compared to the control group, starting from 45<sup>th</sup> day until the end of experiment. In the Y group, in the last two examinations, this concentration was also significantly ( $p < 0.05$ ) higher than on day 0, whereas in YC group such situation was observed in the last three examinations.

## Discussion

The mean values of total leukocytic counts estimated in the current study were within the normal physiological ranges recorded by Njidda et al. (2014), indicating a good health of all lambs. In the light of the results of total leukocytic counts in probiotics-treated lambs and comparing these results with that of non-treated lambs, it seems that the use of probiotics (*S. cerevisiae*) or yeast culture causes no significant changes in the levels of total leukocytic counts in growing lambs. These findings are in a close agreement with the results presented by Sayed (2003), Milewski (2009), Milewski and Sobiech (2009), Dimova et al. (2013) and Dabiri et al. (2016), who reported no significant difference in the total leukocytic count in both probiotic supplemented and control lambs or kids. Analyzing the leucogram changes, a slight increase in the number of neutrophils in the control group was found in relation to the

experimental groups. Lambs receiving yeast supplementation showed a significantly higher number of lymphocytes in relation to the control group and to the day 0 of the experiment. These changes were intensified with the duration of the experiment. Similar results were observed by Morril et al. (1995) and Sarker et al. (2010) in calves supplemented with probiotics. In another study on calves (Dobicki et al. 2007), dried *Saccharomyces cerevisiae* brewing yeasts have been shown to cause significant changes in the ruminal microbial population (decrease in the number of protozoa and increase in the number of biologically active bacteria), resulting in increased bacterial protein flow and its better absorption. These mechanisms positively influenced calf growth rate and improved their immune status. The changes in the leukocyte population observed in our studies may be related to the beneficial effects of the yeast used (both in the form of live yeast and dried yeast culture) to improve the immunological status of the examined lambs. It is worth mentioning that over the time of the experiment, the levels of serum total protein, albumin, globulins and A/G ratio levels in all lambs of our experiment were within the normal physiological ranges recorded by Njidda et al. (2014). Similarly, Galip (2006), Abas et al. (2007), Dimova et al. (2013) and Soren et al. (2013) noticed no significant differences in the concentrations of total protein between control and probiotic or yeast culture supplemented lambs. In this study, a significant increase in the total protein content was observed at the end of experiment in lambs fed diet containing dried yeast. The obtained results may be a consequence of a better digestibility of the dietary protein through the enzymatic effect of protease, and alteration of amino acid profile of the digesta due to increasing the microbial protein synthesis. Our results were similar to those obtained by Hussein (2014) who found that the yeast culture supplementation has led to an increase of blood total protein in sheep. Moreover, Ragheb et al. (2003) found that concentration of total protein was higher in yeast culture (Lacto-Sacc)-treated calves than in the control ones.

The albumin concentration was statistically higher (in the second phase of the experiment) in the serum of lambs supplemented with either live yeast or dried yeast culture. This is confirmed with the results obtained by Hussein (2014) who reported significant higher albumin values in probiotic supplemented lambs. Moreover, Galip (2006), Abas et al. (2007), Dimova et al. (2013) and Soren et al. (2013) observed no change in both albumin and globulin levels in probiotic supplemented lambs. The increase in serum albumin content in lambs receiving yeast supplementation indicates that such additives stimulate liver function and conse-

quently improve the albumin synthesizing ability. In general, it is noted that globulin concentrations were significantly higher in both Y and YC groups in most of the samplings throughout the experiment. These results were supported by the findings of Hussein (2014), who reported significantly higher globulin values in probiotic supplemented lambs. Also, Roodposhti and Dabiri (2012) found that probiotic supplementation significantly increased plasma globulin concentrations in calves. The increase in globulins level in probiotic or yeast culture supplemented lambs may be attributed to the increase of net globulins amounts as a result of the increase in gamma globulins caused by *Kopffer cell* proliferation and the increase in number of plasma cells in the bone marrow (Ragheb et al. 2016). Buts et al. (1990) support our hypothesis as they demonstrated that oral administration of *Saccharomyces cerevisiae* to growing rats significantly increased IgA and secretory component of immunoglobulins.

The results concerning A/G ratio obtained in this study are in accordance with those reported by Khattab et al. (2003) in sheep, who reported a decrease in A/G ratio due to YC supplementation. However Mahrous and Abou-Ammou (2005) found that YC supplementation did not affect blood A/G ratio in sheep.

As far as we know, there is scarce number of available studies that discuss the effect of the use of probiotics on the levels of immunoglobulins concentrations in animals, particularly in sheep. Trebichavsky and Splichal (2006) reported that probiotics could enhance the immunity by promoting the antibodies, IgA and cytokines production. Our results are consistent with the results of Roodposhti and Dabiri (2012) who found that plasma IgG1 concentration tended to be higher in symbiotic and prebiotic-treated calves but the differences were not significant. Moreover, the results obtained by Pătrăscanu et al. (2011) may support our data as they reported that  $\gamma$ -globulin, IgM, IgG and lysozyme showed significantly increased values in probiotic supplemented sows, indicating the immune stimulatory action of probiotics. They added that the increase in the serum gamma globulin and lysozyme could be attributed to the probiotic effect on minimizing stress in animals. In the same context, Cetin et al. (2005) reported that probiotic supplementation has increased the IgG and IgM levels in turkeys. Also, Zhang and Kim, (2014) observed that probiotics containing *L. acidophilus*, *B. subtilis* and *C. butyricum* increased the serum levels of IgA and IgM in chickens. In contrast to our results, Maragkoudakis et al. (2010) reported that supplementation of dairy goats with *Lactobacillus plantarum* PCA 236 had no effects on blood IgG, IgM and IgA concentrations. Also, Morill et al. (1995) showed that probiotic supplementation in calves had

no effect on immunoglobulins. Riddell et al. (2010) noticed also that probiotic had no significant effect on plasma IgG1 concentration of pre-ruminant calves.

## Conclusion

The results obtained indicate that using of probiotic (*S. cerevisiae*) and yeast culture in lambs during the growing period increased the levels of immunoglobulins (IgA, IgG and IgM), and the effect of yeast culture seems to be better than that of the probiotic. Thus, the tested probiotic and yeast culture improve the immunological status of lambs.

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