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THE MODULATION OF PERFORMANCE, CHOLESTEROL CONTENT AND IMMUNE RESPONSE INDUCED BY *SACCHAROMYCES CEREVISIAE* IN LAYING HENS

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SUMMARY

This study was conducted to evaluate the effect of *Saccharomyces cerevisiae* supplementation of a commercial layer diet on the laying performance, blood lipid profile, liver and yolk cholesterol content, and immune response during 8-week period. A total of 180 Hy-line Brown laying hens of 40 wk of age were allocated to 4 dietary treatments, where every 45 hens were divided into three replicates. The basal diet was supplemented with *Saccharomyces cerevisiae* at the levels of 0.0, 0.2, 0.4, and 0.6%. Results showed that the highest egg number and egg weight were observed with 0.6% *Saccharomyces cerevisiae* supplement. Supplementing diets with *Saccharomyces cerevisiae* at all levels (0.2, 0.4, and 0.6%) significantly ($P<0.05$) increased egg mass, antibody production, cell-mediated immune response, and reduced feed consumption, plasma cholesterol, LDL-cholesterol levels and liver cholesterol concentration. Furthermore, hens fed diets supplemented with 0.4 and 0.6% *Saccharomyces cerevisiae* showed significant enhancement of feed conversion ratio, an increase of plasma HDL-cholesterol and a reduction of both yolk cholesterol concentration and Heterophil:lymphocyte (H/L) ratio. In conclusion, *Saccharomyces cerevisiae* could be used to enhance production performance and immune response, and control cholesterol content of plasma, yolk and liver.

Keywords: *laying hens, Saccharomyces cerevisiae, production performance, cholesterol, humoral trsponse, cell-mediated response*

INTRODUCTION

Table egg is considered one of the most important sources of animal protein, and it plays a key role in compensating food shortage in Egypt and other developing countries. Because of its chemical and nutritive characteristics, it is consumed either directly or indirectly as being an important ingredient in many recipes. However, according to the scientific research that reported high cholesterol content of the egg, which reaches 250 mg / egg (Kritchevsky and Kritchevsky, 2000; Simpopoulos, 2000), it is considered a threat to the public health, particularly health problems related to heart diseases and hardening of the arteries (Jeppesen, 2003). Subsequently, medical institutions are forced to call for rationalizing in the consumption of eggs to be no more than two eggs per week. Therefore, there has been a continuous effort done by the poultry industry for a resolution to reduce egg cholesterol concentrations so that a low-cholesterol egg will be available to those consumers who are in need of controlling their dietary cholesterol intake. The use of nutritional strategies to reduce egg cholesterol concentrations introduces itself as an attractive alternative. Another challenge faces commercial egg production industry is stress. Harsh environmental conditions results in stress that negatively affects physiological functions and hens productivity (Korver *et al*., 1998; Takahashi *et al*., 2000). Millions of hens are raised for eggs, and most spend their lives in battery cages, stacked tier upon tier in huge warehouses. Over the past few decades, commercial breeding programs have likely ignored the ability of the poultry flocks to cope with modern production environments in favor of production traits (Onbasilar and Aksoy, 2005). Environmental stress may depress the immune function of birds by impeding production of antibodies and effective cell-mediated immunity (Zulkifli *et al.*, 1994). The phagocytic potential of chicken macrophages is decreased during heat exposure (Miller and Qureshi, 1992). Guo *et al.* (1998) reported

that environmental stress resulted in restraint of the development of immune organs of broilers. One of the ways for elevating production performance and enhancing the immune response of chickens is to use effective nutritional additives in the feed (Al-Khalifa *et al.,* 2013).

Yeast culture products containing *Saccharomyces cerevisiae*, which are rich in enzymes, vitamins, and other nutrients, have many beneficial effects on animals such as growth rate, feed efficiency, egg production, and reproduction (Dawson, 1993). However, there are still conflict reports on the beneficial effect of culture supplementation in poultry diets. Several studies reported improved feed efficiency (Liu and Yoon, 2002 and Tangendjaja and Yoon, 2002), increased egg weight and decreased egg yolk cholesterol without affecting performance and egg traits (Yalcın *et al.,* 2008). Also, some other studies revealed that *Saccharomyces cerevisiae* increased egg production (Thayer *et al.,* 1978) or decreased egg production (Dizaji and Pirmohammadi, 2009) and improved internal egg quality (Miles and Bootwalla, 1991). In contrast, other studies described by Day *et al.* (1987) and Nursoy *et al.* (2004) reported no effect of dietary yeast culture on feed consumption, egg production, egg weight, and feed efficiency in laying hens.

Saccharomyces cerevisiae fermentation product showed an increase in immune function of broilers when fed for 42 d (Gao *et al*., 2008). Other studies have shown similar results on the broiler immune system when fed *S. cerevisiae* fermentation products (Al-Homidan and Fahmy, 2007; El-Husseiny *et al.*, 2008). It was hypothesized that a fermentation product derived from *S. cerevisiae* could modulate the immune system to allow the bird to effectively handle the stress of an intestinal pathogenic coccidian infection. Furthermore, the immune-regulatory effects of yeast cell wall on cell-mediated responses have been confirmed in several animal studies (Kogan *et al.*, 2008). However, the direct effect of *Saccharomyces cerevisiae* cells is not well understood in chickens.

The objective of this study was to evaluate the effects of *Saccharomyces cerevisiae* supplementation to the laying hen diets on egg production parameters, lipid profile, and immune response. We hypothesized that different level supplementations of *Saccharomyces cerevisiae* in poultry feeds may increase performance indices, modulate fat metabolism, reduce cholesterol concentration, and stimulate both of humoral and cell mediated immune response.

MATERIALS AND METHODS

Management and diets

The current study was conducted in the Poultry Research Farm, Faculty of Agriculture, Ain Shams University. A total of 180 Hy-line brown laying hens 40-wk of age were used in the current investigation. Hens were housed in 60 laying cages with 3 hens per cage in an open poultry house with a light regimen of 16L: 8D throughout the whole experimental period of the study. Four groups of 45 hens each (3 replicates of 15 hens per group) were randomly assigned to 4 dietary treatments. The basal diet (Table 1) was formulated to meet the recommendations of the National Research Council (NRC, 1994), and supplemented with 0, 2, 4 or 6 g/kg feed lyophilized *Saccharomyces cerevisiae* (*Sc*, strain BY4742, from C. De La Roche Saint-André; CNRS, Marseille, France) for 8 wk. All birds were reared under the same environmental, managerial and hygienic conditions. Feed and water were provided *ad libitum* during the entire experimental period.

Production parameters:

Egg production was recorded daily throughout the experimental period. Data from feed consumption, egg production, egg weight, and feed conversion were gathered weekly. For statistical analyses, data were averaged by replicate throughout the experimental period for each treatment group. Feed conversion ratio was calculated as gram feed consumed per gram egg produce.

Lipid profile parameters:

1- Plasma cholesterol and triglyceride analysis:

After 8 wk of yeast supplementation, all birds were fasted for 12 h and 18 blood samples from each group (6 samples per replicate) were collected via the brachial vein from each group using heparinized tubes. Plasma was isolated by centrifugation at 1,500 × *g* for 10 min at 4°C and stored at −20°C until analysis. Plasma total cholesterol (**TC**), triglyceride (**TG**), high-density lipoprotein cholesterol (HDL-cholesterol), and low-density lipoprotein cholesterol (HDL-cholesterol) were measured using an

enzymatic kit (Wako cholesterol 439-17501, Wako HDL-Cholesterol 431-52501, and Wako triglycerides 432-40201, respectively, Wako Pure Chemical Industries Ltd., Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-cholesterol) was calculated according to the formula of Friedewald *et al*. (1972): (LDL-chol.)= (Total chol.) − (HDL-chol.) − Triglyceride/5.

2- Liver and egg cholesterol analysis:

At the end of the experimental period, 30 eggs from each group (10 eggs per replicate) were collected for egg cholesterol analysis. One gram of each egg yolk was homogenized with 15 mL of chloroform-methanol 2:1 (by volume), and filtered as described by Elkin and Rogler, 1990. For liver cholesterol analysis, 9 laying hens from each group (3 hens per replicate) were decapitated and liver was collected, washed with saline, blotted dry on filter paper, and freezed –20°C. From each liver, 1 g was homogenized with 12-mL of chloroform-methanol 2:1 (by volume) and filtered directly into a 50-mL volumetric flask using a glass microfiber filter. Following re-homogenization and re-filtration, the liver filtrate was diluted to a final volume of 50-mL with chloroform-methanol 2:1 (by volume). Then, obtained samples were stored at –20°C. Cholesterol concentrations in egg yolk and liver were determined using the same reagent kit as those used for plasma analysis (Wako cholesterol 439-17501, Wako Pure Chemical Industries Ltd., Tokyo, Japan).

Table (1). Ingredients and nutrient composition (%) of basal diet.

ingredient	%
Yellow corn	56.55
Soybean meal (44%)	27.60
Wheat bran	1.00
soybean oil	3.00
Bone meal	3.00
Limestone	8.00
Salt (NaCl)	0.40
Vit. & min. mixture *	0.30
DL-Methionine	0.15
Total	100
Calculated nutrient composition**	
Metabolizable energy (Kcal/kg)	2800
Crude protein (%)	17.47
Calcium (%)	4.02
Available phosphorus (%)	0.52
Lysine (%)	0.95
Methionine (%)	0.42
Linoleic acid (%)	2.88

** Vitamin and mineral mixture at 0.3% of the diet supplies the following per Kg of the diet: vitamin A, 8,000 IU/kg; vitamin D, 1,500 IU/kg; riboflavin, 4 mg/kg; cobalamin, 10 µg/kg; vitamin E, 15 mg/kg; vitamin K, 2 mg/kg; choline, 500 mg/kg; niacin, 25 mg/kg; manganese, 60 mg/kg; zinc, 50 mg/kg.*
*** According to NRC (1994).*

Measurement of immune response

1. Total white blood cell (WBC) counts:

Total WBC counts were made by a hemocytometer slide and light microscopy using brilliant crystal blue stain (Haddad and Mashaly, 1990). Briefly, 490 µl of stain was mixed with 10 µl of blood sample and total white blood cells were determined.

2. The heterophil: lymphocyte (H/L) ratio:

Blood was sampled for H/L ratio when the birds were 19, 35, and 45 wk of age. A total of 12 hens per strain were randomly selected from each cage or floor pen. Two drops of blood were taken from the right brachial vein within a minute of catching the hen to avoid stress due to handling and were dropped separately onto the slide. Blood smears were made on each slide, air-dried, fixed with methyl alcohol, and stained with Giemsa stain (Humason, 1979). On each slide, heterophils and lymphocytes were counted until a total of 100 cells were reached. After averaging the cells of 2 slides, the ratio of heterophils to lymphocytes was calculated**.**

3. Cell-mediated immune response:

The PHA- wattle test involves subcutaneous injection of a mitogen (phytohemagglutinin, PHA) and measurement of wattle swelling as a surrogate of T-cell mediated Immunocompetence. The PHA is dissolved in Phosphate-Buffered Saline (PBS) and the resulting concomitant swelling is quantified at the site of injection over time. The resulting swelling, usually measured 24 h post-injection, is interpreted as an index of cell mediated Immunocompetence. Prior to injection, the injection site (wattle) should be marked. The thickness of the wattle was measure with micrometer. Then birds were injected intradermally in the wattle with 0.5 mg of PHA-P in 0.1 ml of PBS. Post-injection thickness was typically measured 24 h post-injection. Wattle swelling was calculated as the difference between the thickness of the wattle prior to and after injection of PHAP (Goto *et al*., 1978)

4. Humoral immune response:

Sheep Red Blood Cells (SRBC) were diluted to a 5% vol/vol suspension in 0.68% NaCl. Hens were intravenously injected with 1 ml of the 5% washed SRBC and blood samples are collected 7 days post-challenge; then, sera samples were prepared by centrifugation at room temperature (1000 rpm) and samples were stored at-20°C until tested. The test is performed in 96-well U-bottom plates. Sera samples were added to the first and second wells (25 µl). Then, 25 µl of 0.68% NaCl (prepared previously and kept in the fridges, pre-warmed before usage in the assay) were added to columns 2-11. Serial dilutions (1:2) were made from column 2-11. A negative control of phosphate buffer saline was used in column 12. Then, 25 µl of a 2% solution of sheep red blood cells were added to each well. The plates were vortexed gently for a few seconds and incubated overnight at room temperature. The titers were recorded as the column number of the last plasma dilution showing clear evidence of agglutination (clear ring). In other words, titers were expressed as log of the reciprocal of the highest dilution exhibiting visible agglutination.

Statistical analysis:

Data were subjected to a one-way analysis of variance with treatment effect using the General Linear Model (GLM) procedure of SAS User's Guide, 2001. The main factor was dried yeast supplementation levels. When significant differences among means existed, means were separated using Duncan's multiple range tests (Duncan, 1955).

RESULTS AND DISCUSSIONS

Layer performance:

Table (2) represents the effect of three levels of dietary yeast (2, 4, and 6 g/kg diet) on egg production, egg weight, egg mass, feed consumption and feed conversion ratio. Data showed that yeast supplementation at 6 g/kg diet significantly improved egg production by 2.22% compared to control group.

Table (2): Effect of yeast supplementation to laying hen diets on egg production parameters.

	Yeast level (%)			
Trait	0	0.2	0.4	0.6
Egg number/hen/8wk	$52.24^{b}\pm0.54$	$52.39^{b}\pm0.61$	$52.57^{b}\pm0.42$	$53.40^{a}\pm0.38$
Egg weight, (g)	$61.75^{b}\pm0.42$	$61.80^{b}\pm0.39$	$63.12^{a}\pm0.41$	$63.34^{a}\pm0.33$
Egg mass/8wk (g)	$3225.82^{b}\pm25.42$	$3243.26^{b}\pm31.24$	$3318.22^{a}\pm23.48$	$3382.36^{a}\pm31.65$
Feed consumption/8wk (g)	$7354.87^{a}\pm102.54$	$7132.48^{b}\pm98.75$	$7090.81^{b}\pm100.52$	$7060.68^{b}\pm97.96$
Feed conversion ratio	$2.28^{a}\pm0.07$	$2.20^{ab}\pm0.10$	$2.13^{b}\pm0.07$	$2.08^{b}\pm0.10$

$^{a, b}$ Means within the same row with different letters are significantly different ($P < 0.05$)

Further, providing dietary yeast at 0.4 and 0.6% significantly increased ($P < 0.05$) egg weight by 1.37 and 1.59g and improved egg mass by 2.9 and 4.8% respectively compared to control. Supplementing yeast at 2g/kg had no effect on these parameters compared to control. The improvement in egg number and egg mass due to yeast inclusion is in agreement with the results of Shivani *et al.* (2003); Shareef and Al-Dabbagh (2009) and Hassanein and Soliman (2010). They observed higher percentage of egg

production for hens fed yeast-supplemented diets than the control hens. The increment of egg production with 0.6% yeast level and improvement of egg weight, and egg mass with 0.4 or 0.6% yeast level may be attributed to the antagonistic effect of yeast against harmful enteric microflora which may cause mal-absorption of nutrients. So that, adding yeast may enhance digestion, absorption and save more nutrients for egg formation. Soliman (2003) attributed the best egg production of hens fed dietary yeast to the decreased proliferation of pathogenic bacteria. The number and weight of eggs were not affected by dietary yeast culture supplementation at 2 g/kg diet. Nursoy *et al.* (2004) stated that egg weight was not affected by adding yeast into diet.

Dietary yeast at the three levels, 0.2, 0.4, and 0.6% significantly reduced ($P < 0.05$) feed consumption by 3, 3.6, and 4% during the experimental period compared to control. Furthermore, The feed conversion ratio of hens fed diets supplemented with 0.4% or 0.6% yeast were found to be significantly ($P < 0.05$) enhanced than the feed conversion ratio of the control by 6.6% and 8.8% respectively during the same period. This result is in agreement with Liu and Yoon, (2002), who reported that yeast culture supplementation to the diet decreased feed intake ($P<0.05$) in laying hens. In addition**,** several researchers have reported that feed efficiency was improved ($P<0.05$) by yeast culture supplementation to diets of laying hens (Abou El-Ella *et al*., 1996) and broilers (Onifade *et al*., 1999). It has been hypothesized that improvement in feed efficiency in laying hens may partially be attributed to the establishment of an intestinal bacterial population that favored improved nutrient retention (Liu and Yoon, 2002). The improvement in feed conversion may be attributed to the improvement in nutrients absorption and utilization associated with adding yeast which reduces the proliferation of enteric harmful bacteria. In general, the improvements in hen's productivity, reduction in feed consumption, and enhancement in feed conversion ratio of yeast-fed hens were probably due to several mechanisms including: improving the energy utilization (Bradle and Savag, 1995), enhancing digestion coefficient of crude protein (Soliman, 2003), and stimulating the activity of both pancreatic chymotrypsin and pancreatic α-amylase (Mature *et al.,* 2010). These Pancreatic digestive enzymes proved to be very effective in the digestion of dietary starch, fat, and proteins and also closely related to productive performance of poultry.

Plasma lipid profile, liver, and yolk cholesterol:

The effects of different levels of *Saccharomyces cerevisiae* on plasma triglyceride, plasma total cholesterol, LDL-cholesterol, and HDL-cholesterol in laying hens are shown in table 2. Within the 0.40 and 0.60% *Saccharomyces cerevisiae* groups, there were not any significant differences in all the previously mentioned parameters. Plasma triglyceride was significantly reduced ($P<0.05$) by 19.7 and 17.2% when the hens were fed on diet supplemented with 0.4 and 0.6% yeast respectively compared to control group. In addition, administration of yeast at the levels of 0.20, 0.4 or 0.60% in the diet for 8 wk caused reduction in plasma cholesterol by 7.8, 6.8, and 6.5% and decreased plasma LDL-cholesterol by 12.8, 18.5, and 21.9% respectively compared to their control groups. On the other hand, Plasma HDL-cholesterol was significantly higher by 6.1 and 11% in laying hens fed diet supplemented with 0.4 and 0.6% yeast compared to the control group. Concerning egg yolk cholesterol, the results indicated that the yeast supplementation at the levels of 0.4 and 0.6% significantly reduced ($P<0.05$) egg yolk cholesterol by 18.5 and 20.7% compared to control group. With respect to liver cholesterol, data showed that yeast supplementation at all levels significantly decreased liver cholesterol compared to control group.

Table (3): Effect of yeast supplementation to laying hen diets on plasma, yolk and liver cholesterol

		Yeast level (%)			
Trait	N	0	0.2	0.4	0.6
Plasma triglyceride, mg/dl	18	$203.13^{a}\pm6.91$	$189.52^{a}\pm7.12$	$163.15^{b}\pm5.85$	$168.17^{b}\pm6.13$
Plasma Cholesterol, mg/dl	18	$131.39^{a}\pm4.54$	$121.10^{b}\pm4.12$	$122.46^{b}\pm3.65$	$122.84^{b}\pm3.17$
HDL-cholesterol, mg/dl	18	$61.45^{b}\pm1.88$	$60.12^{b}\pm1.75$	$65.45^{a}\pm1.82$	$68.23^{a}\pm1.63$
LDL-cholesterol, mg/dl	18	$69.94^{a}\pm1.76$	$60.98^{b}\pm1.32$	$57.01^{c}\pm1.74$	$54.61^{c}\pm1.45$
Yolk cholesterol, mg/g	30	$12.96^{a}\pm1.10$	$10.96^{ab}\pm0.95$	$10.56^{b}\pm0.78$	$10.27^{b}\pm0.89$
Liver cholesterol, mg/g	9	$4.40^{a}\pm0.24$	$3.82^{b}\pm0.21$	$3.56^{b}\pm0.19$	$3.52^{b}\pm0.27$

a, b, and c Means within the same row with different letters are significantly differed ($P < 0.05$)
N = number of samples/treatment

Cholesterol concentrations exhibited a marginal decrease in the yeast supplemented groups. The results of the present study are in agreement with those obtained by Panda *et al*. (2006), showing that a probiotic supplementation caused a reduction in the level of serum and yolk cholesterol. Probiotic supplementation may also depress the concentration of cholesterol in blood and yolk (Mohan *et al.*, 1995). In addition, a similar hypocholesterolemic effect was observed in chickens supplemented with

beta-fructans (Yusrizal and chen, 2003). Low cholesterol concentrations in the present study may be due to the incorporation of cholesterol into the cellular membrane of the organism by the probiotic supplementation; thus, cholesterol assimilation by yeast in turn reduces cholesterol absorption in the system (Gilliland *et al.,* 1985). The cholesterol-lowering effect may also be due to deconjugation of bile salts, which are less soluble at lower pH and, thus, less absorbed in the gastro-intestinal tract. This results in huge fecal excretion of bile acids, which in turn lowers cholesterol in the body pool, as cholesterol constitutes the precursor of bile salts (Klaver and Van der Meer, 1993). Krasowska *et al.* (2007) reported that *Saccharomyces* strains are able to remove cholesterol from the growth medium and that the baker's yeast *S. cerevisiae* seems to be the perfect organism for lowering cholesterol in the gastrointestinal tract. Probiotics may also influence the cholesterol blood levels by reducing cholesterol synthesis (Fukushima and Nakano, 1995). Concerning HDL-cholesterol, our data are in agreement with Panda *et al.* (2006), who reported that the probiotics increased the HDL levels at the expense of LDL. Also, products of bacterial fermentation, specifically short chain fatty acids, may inhibit cholesterol synthesis in the liver and/or mobilize plasma cholesterol to the liver (Rafter, 2002). High-density lipoproteins form a class of lipoproteins that vary somewhat in size (8 to 11 nm in diameter). The lipoproteins carry fatty acids and cholesterol from the body's tissue to the liver. They help to prevent a buildup of cholesterol by taking excess cholesterol away and are therefore referred to as "good" cholesterol.

Immunological parameters:

1- Total WBC and H/L ratio*:*

Data listed in Table (3) represent the effect of different levels of yeast as dietary supplementation in laying hens on different parameters of the immune response. These parameters involved total count of circulating WBCs, The H/L ratio, antibody titer as humoral immune response and cell-mediated immune response. For cell-mediated immune response, PHA test was used. The data showed that yeast dietary supplementation, at all levels, resulted in a significant increase in circulating WBC total count but it did not reach statistical significance. With respect of H/L ratio, the current results revealed that inclusion yeast in laying hen diets at the levels of .04 and .06% significantly decreased H/L ratio by 25 and 29% respectively compared to control group. This result could be due to the anti-inflammatory effect of *Saccharomyces cervisiae. Saccharomyces cervisiae* has been shown to decrease inflammation in animal model of chemically-induced colitis (Foligne et al., 2010), to reduce digestive discomfort and abdominal pain, and to exert *in vitro* antagonist effect against *E. coli* (Etienne-Mesmin *et al.,* 2010). In addition, viable *Saccharomyces cervisiae* decreased the apical secretions of pro-inflammatory cytokines specially Interleukin-6 and interleukin-8 (Sougioultzis *et al.*, 2006).

Table (4): Effect of yeast supplementation to laying hen diets on red and white blood cells

	Yeast level (%)			
Trait	0	0.2	0.4	0.6
Total WBC, (10^3/mm)	44.15±5.84	48.27±6.20	51.25±4.33	54.20±6.15
H/L ratio	0.62^{a}±0.08	0.50^{ab}±0.09	0.46^{b}±0.07	0.44^{b}±0.08
PHA response (mm)	0.31^{a}±0.03	0.49^{b}±0.06	0.55^{b}±0.08	0.58^{b}±0.05
Antibody titer,(Log^2)	6.57^{b}±0.48	7.80^{a}±0.61	8.4^{a}±0.75	8.36^{a}±0.84

$^{a, b \text{ and } c}$ Means within the same row with different letters are significantly differed (P < 0.05)
N = 9 samples/treatment

2- Humoral immune response:

For antibody titer, hens were injected intravenously with 5% washed sheep red blood cells and the humoral response was measured a week after. The effect of dietary yeast treatments on overall antibody response against Sheep Red Blood Cells (SRBC) is shown in table 3. Results showed that providing diet supplemented with *Saccharomyces cervisiae*, at all levels, induced stimulation of humoral immune response. Administration of *Saccharomyces cervisiae* at the levels of 0.20, 0.4 or 0.60% in the diet for 8 wk improved the antibodies production by 18.7, 27.8, and 27.2$ respectively compared to control group. This higher antibody titer in laying hens supplemented with yeast could be explained by the beneficial effects of supplementation in maintaining a physiological balance of immune-competent cells and therefore providing a healthy environment for the immune system (Yalcin *et al.,* 2010). Mohiti Asli *et al*.,(2007) observed greater antibody production against SRBCs in laying hens fed 1 g yeast / kg in the

diet compared with the control group ($P < 0.05$). Other researchers reported higher antibody responses in broiler breeders fed mannanoligosaccharide, yeast cell wall, (Stanley *et al.,* 2000).

3- Cell mediated response:

The effect of dietary black seed treatment on PHA response of white and brown Hy-Line hens is shown in table 3. The resulting concomitant swelling was quantified at the site of injection after 24 h. It can be concluded that adding yeast *Saccharomyces cervisiae* can enhance the productive performance of laying hens and may lead to the development of low-cholesterol chicken eggs as demanded by health-conscious. As to the differences in the cell-mediated immune response measured by the PHA response between the control group and dietary yeast treatments, results of the current study showed that using 0.2, 0.4, or 0.6% of *Saccharomyces cervisiae* in the diet Hy-Line hens increased the PHA response by at least 50% compared to control group indicating that, it enhanced the cell-mediated immune response. Those results are in agreement with Gomez-Verduzco *et al.,* (2009), who reported that dietary supplementation of 0.05% of yeast cell wall increases local mucosal IgA secretions, cellular and humoral immune responses, and reduces parasite excretion in feces of neonatal animals infected with coccidia. These results provide clear evidence of the potential of this dietary strategy as an anti-coccidial alternative. Specifically, this study reveals the potential of yeast cell wall dietary supplementation to enhance cell mediated immune responses, which is an important factor of protection against coccidial infections (Dzierszinski and Hunter, 2008). Furthermore, Cell-mediated immune responses are thought to be the most important factor of protection against coccidiosis. CD4+ and CD8+ T-cells populations limit coccidia replication in the intestinal tract (Innes and Vermeulen, 2006). The effects of T-cells are mediated by the cytokines that these cells release. Interferon-gamma and tumor necrosis factor-alpha limit oocyst production in either primary or secondary infections (Yun *et al.,* 2000). The immune-regulatory effects of yeast cell wall on cell-mediated responses have been confirmed in several animal studies (Kogan et al., 2008). Therefore, it is possible to hypothesize that the reduction in *Eimeria* spp. replication in the intestine was caused by the yeast cell wall-induction of T-cell responses Gomez-Verduzco *et al.,* (2009).

CONCLUSION

Dietary supplementation with *Saccharomyces cervisiae* could improve the productivity, modulate lipid profile, and enhance the immune status of laying hens. This outcome could have an important application in the poultry industry.

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تعديل بكتريا سكارومايسس سيرفيزا (الخميرة) للكفاءة الإنتاجية ومحتوى الكولسترول والإستجابة المناعية للدجاج البياض

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تم إجراء تلك الدراسة لتقييم تأثير تقديم بكتريا سكارومايسس فى علائق الدجاج البياض لمدة 8 أسابيع على الكفاءة الإنتاجية , وصورة الدهون فى الدم , ومحتوى الصفار والكبد من الكولسترول , ذلك بالإضافة إلى الإستجابة المناعية.

فى تلك التجربة تم إستخدام 180 طائر بياض بنى (هاى لاين) على عمر 40 إسبوع حيث تم تقسيمها إلى 4 معاملات غذائية , تحتوى كل معاملة على 45 طائر قسمت لتشكل 3 تكرارات. وتم خلط علائق الدجاج البياض بأربع مستويات من البكتريا (صفر – 0,2 – 0,4 – 0,6 %).

أشارت النتائج إلى أن أعلى معدل لوزن وإنتاج البيض كان فى المجموعة التى تغذت على عليقة تحتوى على بكتريا سكارومايسس بتركيز 0,6%. أيضاً لوحظ أن تقديم عليقة تحتوى على تلك البكتريا بتركيزات 0,2 – 0,4 – 0,6 % أدى إلى زيادة معنوية (عند مستوى 5%) فى كتلة البيض , وإنتاج الأجسام المضادة , والإستجابة المناعية الخلوية , ذلك بالإضافة إلى خفض إستهلاك العلف ومستوى الكولسترول الكلى فى الدم والكبد وكولسترول الدم من نوع LDL.

وعلاوة على ذلك فإن تقديم عليقة تحتوى على 0,4 و0,6 % من بكتريا السكارومايسس حسنت بشكل معنوى من معدل التحويل الغذائى وزادت من كولسترول الدم نوع HDL بالإضافة إلى خفض كل من كولسترول البيض ونسبة خلايا الهتروفيل إلى الخلايا الليمفاوية (H/L).

وتخلص الدراسة إلى أن استخدام بكتريا سكارومايسس سيرفيزا فى علائق الدجاج يمكن أن يحسن من الكفاءة الإنتاجية والإستجابة المناعية بالإضافة إلى التحكم فى مستوى الكولسترول فى الدم والصفار والكبد.