

Effect of addition of *Saccharomyces cerevisiae* to the diet of laying hens in physiological traits and qualities attributes of the product eggs

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Abstract: This study was conducted in one of the commercial farms of poultry in the Diwaniyah city for the period from 13/01/2014 until 13/03/2014 in order to study the effect of addition of dried *Saccharomyces cerevisiae* to the diet of laying hens in physiological traits and qualities of the product quality of the eggs by using 45 type egg-laying hens ESA Brown, aged 40 weeks and randomly distributed on the three treatments and each treatment consisted of three replicates and (five hens in one replicate). The treatments of the experiment as follows: T1 treatment represented the group of control that fed on the basal diet without any additives, T2 treatment represented the second group of birds that fed on the basal diet plus 0.2% of *Saccharomyces cerevisiae*, T3 treatment fed on basal diet plus 0.3% *Saccharomyces cerevisiae*.

The results of the experiment revealed to the presence of a significant increase ($P < 0.05$) in the Red Blood Cells (RBC), white blood cells (WBC) and the concentration of hemoglobin values (Hb) and percentage of Packed Cells Volume (PCV), and there is a significant increase in the percent between the Heterophils to Lymphocyte ratio, as well as we noted the same significant increase were happened in the concentration of the total protein, albumin, globulin, glucose, high-density lipoprotein and liver enzymes GOT and GPT as well as the results of the experiment showed to a significant decrease incidence ($P < 0.05$) in the concentration of cholesterol, tri glycerides, uric acid, low-density lipoprotein and ALP enzyme in T2 and T3 treatment compared with control group T1.

In the quality characteristics of the eggs we noted the presence of a significant improvement ($P < 0.05$) in egg weight, yolk weight, albumin weight, shell weight, thickness of the shell and the high-density lipoprotein of the egg yolk and we seen a significant reduction ($P < 0.05$) in egg yolk cholesterol and low-density lipoprotein in T2, T3 compared with T1.

We conclude from the results of this experiment that the addition of *Saccharomyces cerevisiae* to the diet of laying hens led to an improvement in the health status of birds through the improvement of the physiological traits and reduce in the blood and egg yolk cholesterol, and thus the production of suitable meat and eggs for human consumption and improved the qualities of produced eggs served the consumer in terms of health and economic development.

Key word: *Saccharomyces cerevisiae*, laying hens, physiological traits, egg qualities.

I. Introduction

Feeding is one of the basic elements in the poultry industry as it constituting 75-80% of the total cost of production and have a direct impact on productive process, one important ingredient is the feed additives as it is of great importance to improve the quality and quantity of production, Where used a lot of feed additives such as microorganisms, enzymes and hormones etc. For the purpose of increasing the production (1).

The poultry products are a basic foundation in the achievement of food security and consumption is one of the standards of modern civilization and progress of peoples including poultry meat to higher nutritional value compared with red meat (1).

Modern medical studies pointed for the the World Health Organization (WHO) and the Agency for Food and Drug Administration (FDA) showed the presence of the risk of wide use of antibiotics in poultry breeding life and took these risks threaten the health of the world's population, as the excessive use of antibiotics leads to the occurrence of many problems like the appearance of resistance for the of some strains of microorganisms against some types of antibiotics and the elimination of harmful bacteria and beneficial together (2).

The many negative effects of antibiotics led to gradually replace them by probiotic and prebiotic, So the researchers active in this field to treated poultry diets by the use of microorganisms such as yeasts and mainly dry *saccharomyces cerevisiae* (3).

Saccharomyces cerevisiae are yeast organisms that unicellular gram positive stain would be unable to adhesion to the intestinal wall, but it is capable of high consumption of oxygen and thus provides the presence of anaerobic conditions suitable for the growth and proliferation of lactobacilli (4).

Yeast belonging to the genus *Saccharomyces* means sugars and fungal type *cervevisiae* and participation it means to beer yeast(5) ,it show is oval with a white or creamy color and reproduce vegetatively by budding or fission and sexually by spores cystic(4,5) .

The yeast present freely in thegastrointestinal tract cavity and it is calledtransient microorganisms that means it does not stick in the epithelial cells, and sorting of enzymes to the intestineswhich works to increase the readiness of nutrients to feed as well as increase the percentage ofdigested protein in the gut of poultry (6).

the yeast have role in non-specialized immune stimulation as well as its role in activating the toxins act as a competitive reaction with some pathogenic microbes (7) .

It has been observed that the addition of probiotic component of *Saccharomyces cerevisiae* to broiler diet led to a significant improvement in the productive performance , low perdition ratio and improve traits of blood (7,8) .

Saccharomyces used as prebiotic because they contain mannanoligosaccharide,it is one of the components of the cell wall of yeast, where accounts for about 50% of the carbohydrate component of the cell wall and acts as a magnet for intestinal bacteria(6) ,It has been observed that the addition of thisyeast at 0.1% works to reduce fat and cholesterol in serum as an indicator of decline in broiler heat stress, with a high concentration of total protein and albumin serum and reducing triglycerides , uric acid and as in the fat of the abdomen(9,10) .So this study aimed to investigate the effect of adding *Saccharomyces cerevisiae* with two different concentration for laying hensdiet in physiological attributes and the quality of theproduct eggs.

II. Material and methods

This study was conducted in one of the fields of the commercial sector in theDiwaniya city for the period from 13/01/2014 until 13/03/2014 by using 45 type egg-laying hensESA Brown, aged 40 weeks and randomly distributed to threetreatmentsand each treatment consisted of three replicates (five hens in one replicate).The treatment of the experiment as follows:

T1: treatment represented thecontrolgroup thatfedonbasal diet without any additives.

T2: treatment represented the second group of birds that fedon the basal diet plus 0.2% of *Saccharomyces cerevisiae*(2 gm/kg feed stuff) .

T3: treatment fed onbasal diet plus 0.3% *Saccharomyces cerevisiae*(3gm/kg feed stuff) .

Table (1) illustrated the diet which used in feeding the birds and their chemical composition by chemical analysis according to the analysis feed materials listed in NRC for 1994.(11) .

Table (1) Proportions of components and materials used in the feed ration experiment

material	Rate of feed composition %
maize	45
barely	30
Soybean meal	11.5
Protein concentrate*	8
Limestone	5
salt	0.5
Calculated chemical analysis**	
%Crude protein	16.5
Metabolisable energykcal/kg	2754
%calcium	2.70
Available phosphorus %	0.57
Lysine	0.74
Methionine	0.31

* concentrated protein Golden(Jordanian) each kg contain 2500 kcal /kg digestible energy ,40% crude protein ,9% fat ,4.5 %crude fiber ,0.9% calcium ,2.4 %available phosphorus ,2.3 %lysin ,1.25 %methoinin +cystin , Vit A 100000 IU ,Vit B₁ 10 mg/kg ,Vit K₃ 20 mg/kg .

At the end of the experiment blood was collected randomly from six birds per treatment and as the collection of blood from the wing vein ,blood samples were divided into two sections put the first section in the tubes contain the coagulation inhibitor EDTA ,and put second in the tubes free from anticoagulant.

Use the first section of blood in the measurement of blood parameters were calculated as the number of red blood cells (RBC) by using an optical microscope according to the method mentioned by (12) ,as well as calculated the number of white blood cells(WBC) on a glass slide especially for the purposes of differential counting according to the method described in (12) .and then calculated ratio of hetrophils cells to lymphocytes by method referred to in (13) ,in addition to that we calculated concentration of hemoglobin (Hb) according to the method of (14) ,and calculate the values of Packed Cell Volum (PCV) By pipeline open lattice-minute parties according to the method mentioned of (14) .

The second section of the blood put in the tubes free from anticoagulant was used in the measurement of biochemical traits, as blood samples placed in the centrifuge 3000 r / min for 15 minutes, Where used kits manufactured by the Jordanian world company (Diamond) for medical reagents for measuring cholesterol mg / 100 ml according to the method mentioned of (15) and triglycerides mg / 100 according to method (16), total protein, albumin and globulin gm/100 m by method of (16), and glucose mg / 100 ml according to the method of (17), and uric acid mg / 100 ml by method mentioned in the (18).

Blood enzymes (GOT, GPT, ALP) were measured by using a kits supplied from the producing French company (bioMerieux) and according to (19), while estimated HDL according the method of (20) and LDL by method of (21).

As for the qualities of the product eggs quality have been taking the 6 eggs from each treatment were measured of interior and exterior characteristics of egg by method of (22), was measured weight of the yolk, the albumin and egg shell by sensitive balance and measure the thickness of the egg shell by Verne especially for it.

The chemical tests of the eggs were measured egg yolk cholesterol by method of (15) and measuring high-density lipoprotein (HDL) in the egg yolk according to the method (20) and low density lipoprotein (LDL) according to what is stated in (21).

As for the statistical analysis we used complete randomized design (CRD) to evaluate the effect of different treatments in studied traits and compared the significant differences between the averages of the treatments by using Duncan test, and used SPSS statistical program for data analysis (23).

III. Result and discussion

Table (2) showed the results of the effect of adding *Saccharomyces cerevisiae* to diet in physiological blood attributes of laying hens, Where recorded the second and third treatments significantly increased ($p < 0.05$) in the number of red blood cells (RBC) were reached to $(2.92, 2.80)10^6/\text{mm}^3$ compared with control group amounting to 2.29, we also noted a significant increase in the number of white blood cells (WBC) for the T2, T3 which amounted to $(15.35, 15.11)10^3/\text{mm}^3$ compared with control group amounting to (14.05) which fed on a diet free of any addition. As for the concentration of blood hemoglobin the T3 had recorded the significant increase in the concentration of hemoglobin which amounted to 7.83 gm/100 ml, followed by the T2 amounting to 7.65 compared with control group amounting to 6.42 gm/100 ml.

The third treatment recorded a rise significantly ($p < 0.05$) in the percentage of PCV which reached to 24.46 followed by 23.52% in T2 group compared to the control was 21.1%, we also noted a significant decrease ($p < 0.05$) in the proportion of the heterophils cells to lymphocytes in the T2, T3 amounting to 0.55 and 0.57, respectively, compared to the control group which reached to 0.91.

The improved characteristics of the blood physiological parameters caused by improvement in the health status of the birds and the effect of addition on readiness for some vital elements like proteins and vitamins. The results clearly indicate to the role of *Saccharomyces cerevisiae* in stimulate and promote the process of blood cells formation as a result of stimulated and revitalization of the work of the liver through excreted enzymes activator. (24).

Which in turn stimulated the kidneys to secrete the erythropoietin hormone which stimulus for the process of formation of the blood and this is the main reason behind the rise in the values of PCV Which depends mainly on the preparation of red blood cells also increase the number of red blood cells would contribute to an increase in hemoglobin concentration being a carried on red blood cells, This may be the reason to raise the number of white blood cells, so that one of the mechanisms of action of yeast and probiotic to promote public health through its work in promoting the development and production of white cells (25).

This may be the reason for the high number of red blood cells because of the increased efficiency of the Thyroxin hormone, it one of the organization hormones for metabolism, as it leads to increased hemoglobin concentration as well as increase of thyroxin lead to increase metabolic reactions in the body tissue which increases the need for tissue to oxygen. Thus increasing stimulated the process of manufacturing red blood cells to transport oxygen which necessary for the completion of these metabolic reactions (26).

Table (3) explained the effect of adding *Saccharomyces cerevisiae* in diet on some physiological traits of laying hens, including concentration of cholesterol, triglycerides, high and low density lipoproteins in serum.

the T3 recorded the a significant decrease ($P < 0.05$) in the concentration of serum cholesterol, which amounted to 176.53 mg / 100 ml followed by the T2 which amounted to 184.46 mg / 100 ml compared to the control group that reached to 201.3 mg / 100 ml.

The low concentration of serum cholesterol corresponded to the many of the studies, that *Saccharomyces cerevisiae* contain the glucan fiber which worked to lower cholesterol by several mechanisms, including its association with bile acids in the digestive tract, thus reduces bile acids which date back to the liver and thus stimulated the production of bile acids from cholesterol, as well as the glucan fiber fermented in the gut by bacteria and leads to the production of short-chain fatty acids like Butrate, propionate, acetate, These acids

are absorbed by the pyloric vein to the liver and inhibit the production of cholesterol in the liver, that these fibers remain for a time in the stomach and thus reduce the absorption of sugar, which helps to reduce insulin in the blood, which in turn leads to a lack of production of cholesterol in the liver. The presence of these non-soluble fiber reduces the absorption of fat, including cholesterol by increasing the viscosity of the intestines and these results were consistent with the (26).

We seen from Table(3) a significant decrease ($p < 0.05$) in the concentration of triglycerides for the T3 which stood at .97.93 mg/100 ml followed by a T2 group was 121.6 compared to control group which amounted to 182 mg/100 ml, and we noted a significant decrease ($p < 0.05$) of the concentration of low-density lipoprotein in the T3 which stood at 48.93 mg/100 ml followed by T2 51.56 compared to the control group was reached to 56.22, also T3 recorded a significant improvement ($p < 0.05$) in the concentration of high-density lipoprotein, which reached to 65.5 mg/100 ml followed by a T2 about to 44.68 compared to the control group 40.56 mg/100 ml.

It is believed that short-chain fatty acids such as acetic and butyric, propionic acid produced by consumption of fiber by selected microorganisms leads to a reduction in TG, LDL and increase HDL (27,28).

Table (4) also shows a significant decrease ($p < 0.05$) in the concentration of glucose in the blood serum for T3, T2 which stood at 185.04 and 185.32 mg/100 ml respectively compared to the control group 214.3, this is due to the positive effect of adding *Saccharomyces cerevisiae* to the diet or the role of glu can which located in the cell wall of yeast, as (29) pointed out that these fibers remain for a while in the stomach and thus reduce the absorption of sugar.

Table (4) also shows the presence of a significant decrease ($p < 0.05$) in the concentration of uric acid for the T2, T3 where amounted to 6.45 and 6.91 mg/100 ml respectively, compared to control group 7.18, that uric acid is the final product of purine metabolism and the process of demolition of protein and non-protein nitrogen in birds and *Saccharomyces cerevisiae* sort of extracellular enzymes which worked to increase the readiness of nutrients and increase the percentage of crude protein in the gastrointestinal tract of chickens (27).

The *Saccharomyces cerevisiae* activated microorganisms that produce the urease enzyme which analyzes the urea to ammonia in the gastrointestinal tract and improve growth (27).

Table (4) as well as showed the significant superiority ($p < 0.05$) of the T2, T3 in total protein concentration was 4.38, 4.10 gm/100 ml respectively compared to control 3.33 that due to the role of *Saccharomyces cerevisiae* to increase the activity of the liver to manufacture of proteins, as well as lower protein demolitions, with regard we noted a rise in serum albumin in the blood serum for the T3, T2 which amounted to 1.42 and 1.41 gm/100 ml, respectively, compared to control 1.21, and this because of the fact that albumin is the main protein in blood proteins has an important role in the stability of the body, it is a good store of amino acids and is a good indicator of the health and productive status of chicken, this is due to the role of the components of the cell wall in increasing the concentration of this protein and the rate of re-balancing of the body, the albumin are a major proteins which responsible for the stability of the body and maintain the natural balance (10,27).

We also noted the significant increase ($p < 0.05$) in the concentration of globulin for the T3 which reached to 2.9, followed by T2 about to 2.7 gm/100 ml compared to control group was 2.08 gm/100 ml, confirmed (9) that the reason for high serum proteins back to maximum benefit from intake food, especially protein.

The Table (5) shown the effect of *Saccharomyces cerevisiae* adding to the diet in the activity of chicken blood enzymes, the results of statistical analysis were obtained a significant increase in the concentration of GOT, GPT enzymes for the T3, T2 in GOT enzyme reaching 132.6 and 128.2 IU/L respectively, compared to the control group which amounted to 122.6 IU/L while the GPT enzyme (48.9, 44.5) for T3, T2 respectively compared to control group was 39.43 IU/L, while shown a significantly decreased in ALP enzyme in the T3, T2 where it reached about (29.1, 28.8) IU/L respectively compared to control 36.9 and this confirms that the yeast did not adversely affect the physiological performance for birds, but it was a positive role in making significant increase in GOT, GPT enzymes (28).

The table (6) shown the effect of adding *Saccharomyces cerevisiae* in the qualities of the product quality of the eggs where we noted a significant increase in the weight of the eggs for the of T3, T2 reaching about (55.74, 55.72) gm respectively compared to the control group

54.09 gm, These results were identical to the results of (30), He explained that the terms of *Saccharomyces cerevisiae* is involved in diverse microorganism which increase the readiness of nutrients with significantly impact in some melting of metals and that improve the chances of absorption and metabolism, which will provide the needs of the formation of the egg and that increases in the rate of egg weight, As well as the table explained a significant increase in the yolk weight for the T3, T2 it reached to (17.7, 17.27) gm respectively compared to the control group 16.68 gm, this is due to the beneficial bacteria and beneficial sugars present in the cell wall of *Saccharomyces cerevisiae* during the processes of electoral fortification and competitive exclusion which contributed to the slow speed of the passage of the mass food and increase the period of

survival and exposure to digestive enzymes, which act increased the chance processes of digestion and absorption and metabolism and improve the quality of the egg and yolk weight(3,30) .

As well as the table shown the existence of a significant increase in the albumin weight egg for the T3,T2 reached to(33.6,33.4)gm respectively compared to control group 32.39gm This is due to the impact of the rule of useful microorganisms in the oviduct and stimulated the production of albumin(31) .

As well as the table(6) shown increase in the eggs shell for T3 ,T2 amounted about (4.98,4.92)gm respectively compared to control group 4.90gm , it also increased the overall average thickness of the egg shells are significantly in T2 ,T3 which reached to(0.37 ,0.35)mm respectively compared to control group 0.31 ,the improvement in the rate of weight and thickness of the egg shell returns to enable Saccharomyces cerevisiae secretion of digestive enzymes Such as phytase enzyme which worked on the analysis of complex composite containing phosphoric acid, which makes it a negative charge attracts cations like Ca,P ,Cu ,Fe ,This also work to prevent the phytase enzyme linked to some of the proteins or by breaking and digested it and so increase the availability and readiness of nutrients, especially in the grain(31) .

As can be seen from Table(6) a significant decrease in the concentration of egg yolk cholesterol for T3 ,T2 which reached to(9.92 ,10.30)mg/gm yolk respectively compared to control group 11.69 .

We also note a significant decrease in the concentration of low-density lipoprotein for T3 ,T2 which reached to (52.26 ,54.39)mg/gm respectively compared to control group 60.26 ,We also note a significant rise in the concentration of high-density lipoprotein in T3 ,T2 which reached to (79.9 ,72.13) respectively compared to control group 64.20 as explained previously in effect of Saccharomyces cerevisiae on serum cholesterol and lipoproteins .

Table (2) effect the addition of Saccharomyces cerevisiae in blood parameter of laying hens

treatment parameter	Control (T1)	T2	T3
RBC 10 ⁶ /mm ³	b 2.29 ± 0.22	a 2.80 ± 0.05	a 2.92 ± 0.01
WBC 10 ³ /mm ³	c 14.05 ± 0.02	b 15.11 ± 0.04	a 15.35 ± 0.02
Hb gm/100 ml	c 6.42 ± 0.01	b 7.65 ± 0.03	a 7.83 ± 0.01
PCV %	c 21.1 ± 0.05	b 23.52 ± 0.16	a 24.64 ± 0.03
H/L	b 0.91 ± 0.02	a 0.57 ± 0.01	a 0.55 ± 0.01

T1: represented the control group that fed on basal diet with out any additives .

T2: represented the second group of birds that fed on the basal diet plus 0.2% of Saccharomyces cerevisiae.

T3: represented the third group fed on basal diet plus 0.3% Saccharomyces cerevisiae .

The numbers represent averages ± standard error .

Different letters indicate the presence of significant differences between the averages under probability level 5% .

Table (3) effect the addition of Saccharomyces cerevisiae in serum cholesterol and lipoproteins

treatment parameter	Control (T1)	T2	T3
Cholesterol Mg/100 ml	c 201.33 ± 0.88	b 184.46 ± 2.11	a 176.53 ± 0.36
Triglycerides Mg/100 ml	c 182 ± 1.45	b 121.66 ± 2.18	a 97.93 ± 0.76
HDL Mg/100 ml	c 40.56 ± 0.34	b 44.63 ± 0.37	a 65.50 ± 0.60
LDL Mg/100 ml	c 56.22 ± 0.53	b 51.56 ± 0.33	a 48.93 ± 0.21

T1: represented the control group that fed on basal diet with out any additives .

T2: represented the second group of birds that fed on the basal diet plus 0.2% of Saccharomyces cerevisiae.

T3: represented the third group fed on basal diet plus 0.3% Saccharomyces cerevisiae .

The numbers represent averages ± standard error .

Different letters indicate the presence of significant differences between the averages under probability level 5% .

Table (4) effect the addition of *Saccharomyces cerevisiae* in biochemical parameter of laying hens

treatment parameter	Control (T1)	T2	T3
Total protein gm/100 ml	c 3.33 ± 0.88	b 4.10 ± 0.05	a 4.38 ± 0.04
Albumin gm/100 ml	b 1.21 ± 0.01	a 1.41 ± 0.008	a 1.42 ± 0.01
Globulin gm/100 ml	c 2.08 ± 0.04	b 2.72 ± 0.01	a 2.9 ± 0.08
Glucose Mg/100 ml	b 214.33 ± 0.95	a 185.32 ± 0.22	a 185.04 ± 0.12
Uric acid Mg/100 ml	c 7.18 ± 0.008	b 6.91 ± 0.01	a 6.45 ± 0.02

T1: represented the control group that fed on basal diet with out any additives .

T2: represented the second group of birds that fed on the basal diet plus 0.2% of *Saccharomyces cerevisiae*.

T3: represented the third group fed on basal diet plus 0.3% *Saccharomyces cerevisiae* .

The numbers represent averages ± standard error .

Different letters indicate the presence of significant differences between the averages under probability level 5% .

Table (5) effect the addition of *Saccharomyces cerevisiae* in blood enzymes

treatment parameter	Control (T1)	T2	T3
GOT IU/L	c 122.62 ± 0.90	b 128.23 ± 0.54	a 132.60 ± 1.38
GPT IU/L	c 39.34 ± 0.11	b 44.50 ± 1.28	a 48.95 ± 0.127
ALP IU/L	b 36.95 ± 0.35	a 28.88 ± 0.33	a 29.16 ± 0.20

T1: represented the control group that fed on basal diet with out any additives .

T2: represented the second group of birds that fed on the basal diet plus 0.2% of *Saccharomyces cerevisiae*.

T3: represented the third group fed on basal diet plus 0.3% *Saccharomyces cerevisiae* .

The numbers represent averages ± standard error .

Different letters indicate the presence of significant differences between the averages under probability level 5% .

Table (6) effect the addition of *Saccharomyces cerevisiae* in Qualitative Characteristics and cholesterol yolk of product egg

treatment parameter	Control (T1)	T2	T3
Egg weight gm	b 54.09 ± 0.005	a 55.72 ± 0.037	a 55.74 ± 0.67
Yolk weight gm	c 16.68 ± 0.01	b 17.27 ± 0.01	a 17.70 ± 0.02
Albumin weight gm	c 32.39 ± 0.05	b 33.44 ± 0.02	a 33.62 ± 0.01
Shell weight gm	c 4.90 ± 0.005	b 4.92 ± 0.005	a 4.98 ± 0.003
Shell thickness mm	c 0.31 ± 0.005	a 0.37 ± 0.003	b 0.35 ± 0.005
Yolk cholesterol Mg/gm yolk	c 11.69 ± 0.31	b 10.30 ± 0.02	a 9.92 ± 0.008
Yolk LDL Mg/gm yolk	c 60.26 ± 0.58	b 54.36 ± 0.80	a 52.26 ± 0.27
Yolk HDL Mg/gm yolk	c 64.20 ± 2.12	b 72.13 ± 0.08	a 79.93 ± 0.88

T1: represented the control group that fed on basal diet with out any additives .

T2: represented the second group of birds that fed on the basal diet plus 0.2% of *Saccharomyces cerevisiae*.

T3: represented the third group fed on basal diet plus 0.3% *Saccharomyces cerevisiae* .

The numbers represent averages ± standard error .

Different letters indicate the presence of significant differences between the averages under probability level 5% .

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