Effect of Probiotic (*Saccharomyces cerevisiae*) Adding to Diets on Intestinal Microflora and Performance of Hy-Line Layers Hens

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Abstract: As experiment was conducted to evaluate the affect of adding various levels of a live yeast to laying hen diets on their laying and feeding performance, egg shell, egg components and some blood constituents, as well as the intestinal microflora make-up. This were studied to validate the mode of a live yeast action in improving laying hens performance. For this purpose 75 Hy line (W-36) white layers were sited from 70 to 79 week of age in individual cages and randomly distributed into five experimental groups of 15 layers each. The individual hen was represented as an experimental unit. The five experimental groups were fed on five graded levels of a live yeast as 0.0% (control), 0.4%, 0.8%, 1.2% and 1.6%. The main results indicated an increase in egg production percentage of layers fed with 0.4% and 0.8% a live yeast which recorded 83.4% and 80.6% respectively compared with 74% of control which was similar to the groups of layers fed 1.2% (74.9%) and 1.6% (74.6%). Average egg weight was not influenced by adding yeast into diets. Egg mass results were parallel to these of egg production where the values of 46.7, 51.0, 50.2, 48.3 and 46.1 g egg/hen/day were recorded for the group of birds fed with 0%, 0.4%, 0.8%, 1.2% and 1.6% a live yeast respectively. Egg albumen and egg yolk were affected significantly. There was a slight improvement in egg shell thickness and percentage. Feed intake values were approximately similar within the different treatments. Feed conversion ratios (g feed/g egg) of layers fed yeast levels of 0.4% (2.08) and 0.8% (2.07) were better than the control group (2.27). Blood total protein levels of birds fed 0.4% (3.82), 0.8% (3.65) and 1.2% (3.97) yeast were lower than the control (4.16), while the value of 1.6% yeast (4.16) was slightly higher than control. Blood albumen levels were parallel to those of blood protein while blood globulin values were not affected. Blood cholesterol levels of layers fed yeast-supplemented diets were lower than the control. Blood total lipids were not affected by treatments. Ileal content pH of layers fed 0.8% and 1.2% yeast levels was lower than the control. Microbiological examination of ileal content indicated an obvious reduction in bacterial total count. While Lactobacilli bacterial count was increased. There were reductions in bacterial strains of *Escherichia coli (E.coli), Klebsiella sp., Staphylococcus sp., Micrococcus sp., Campylobacter sp., and Closterdium perfringers* of layers fed various yeast levels. The results of this study suggest adding live yeast at 0.4% or 0.8% into laying hen diets can enhance the productive performance and nutrients utilization via the inhibitory effect of yeast against pathogenic bacteria. [Journal of American Science. 2010;6(11):159-169]. (ISSN: 1545-1003).

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1. Introduction

Microorganisms used as probiotics in animal mutrition: Probiotics are live microorganisms that, when administered through the digestive tract, have a positive impact on the host's health. Microorganisms used in animal feed are mainly bacterial strains belonging to different genera, e.g. *Lactobacillus, Enterococcus*, *Pediococcus* and *Bacillus.* Other probiotics are microscopic fungi, including *Saccharomyces* yeasts. Some probiotic microorganisms are normal residents in the digestive tract, while others are not (Guillot, 2009). Different mechanisms of probiotic action have been suggested,

but most are only hypothetical. The positive effect can result either from a direct nutritional effect of the probiotic, or a "health" effect, with probiotics acting as bioregulators of the intestinal microflora and reinforcing the host's natural defences (Fuller, 1977; Fuller, 2001).

Kabir (2004) indicated that the gut microflora forms with its host animal a complex ecosystem and microbial interactions ensure the stability of the ecosystem and the health of the host. In some cases the gut microflora is unbalanced and the biological defences against pathogenic agents less effective. The positive effect observed can be the result of either a direct nutritional effect, similar to the effect obtained with antibiotics, or a "health" or sanitary effect, where the probiotic act as a bioregulator of the gut microflora and reinforces the natural defences.

The different mechanisms of action suggested are: (i) nutritional effect include: (1) Reduction of metabolic

reactions that produces toxic substances (2) Stimulation of indigenous enzymes (3) Production of vitamins or antimicrobial substances.

(ii) Sanitary effect include (1) Increase in colonization resistance. (2) Stimulation of the immune response. Some experiments have demonstrated in vitro the effects of strains of *Saccharomyces cerevisiae* on the activity of anaerobic rumen microorganisms. The addition of S. cerevisiae live cells to cultures of some cellulolytic fungal species stimulated zoospores germination and cellulose degradation. The addition of yeasts stimulates also the growth of some anaerobic bacteria, including the cellulolytic and the lactic acid utilising bacteria (Chaucheyras et al., 1995; Yoon and Stern, 1996).

Kizerwtter and Binek, (2009) reported that probiotics have reduced the incidence and duration of diseases. Probiotic strains have been shown to inhibit pathogenic bacteria both in vitro and in vivo through several different mechanisms. The mode of action of probiotics in poultry includes: (i) maintaining normal intestinal microflora by competitive exclusion and antagonism (ii) altering metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production (iii) improving feed intake and digestion iv) stimulating the immune system (Apata 2008; Kabir, 2009).

Kabir et al. (2005) attempted to evaluate the effect of probiotics with regard to clearing bacterial infections and regulating intestinal flora by determining the total viable count (TVC) and total *lactobacillus* count (TLC) of the crop and cecum samples of probiotics and conventional fed groups at the 2nd, 4th and 6th week of age. Their result revealed competitive antagonism. The result of their study also evidenced that probiotic organisms inhibited some nonbeneficial pathogens by occupying intestinal wall space. They also demonstrated that broilers fed with probiotics had a tendency to display pronounced intestinal histological changes such as active impetus in cell mitosis and increased nuclear size of cells, than the controls. Recently, Mountzouris et al. (2007) demonstrated that probiotic species belonging to *lactobacillus, Strepococcus, Bacillus, Bifidobacterium, Enterococcus, Aspergillus, Candida,* and *Saccharomyces* have a potential effect on modulation of intestinal microflora and pathogen inhibition.

A few years ago active living yeast, has been documented as probiotic feed additive for poultry, due to its improvement effect on performance characteristics. Including a live yeast into laying hen diets improved egg production percentage (Kim et al., 2002 and Shivani et al., 2003), and egg weight (Han et al., 1999; Park et al., 2001 and Park et al., 2002). Dumanovski (2000); Sharma et al. (2001); Kim et al. (2002) and Kabir (2009) reported that, adding a live yeast into laying hens diet improved feed intake and feed conversion ratio.

Inclusions of yeast into laying hen diets enhanced egg shell breaking strength (Park et al., 2002), and reduced soft or broken eggs (Park et al., 2001).

In Egypt, a very few studies have been conducted to investigate the effect of feeding yeast on performance of laying hens. Soliman (2003) studied the effect of supplementing a constant level of live yeast into laying hens diets, he observed an improvement in average egg weight, feed conversion values and nutrients utilization. The mode of beneficial action of yeast can be attributed to its antagonistic bacteria and altering gut microflora make up Line et al., 1998; Wakwak et al. (2003) and Kabir 2009) observed a sharp reduction in bacterial total count of ileum content, due to supplementing yeast into Japanese quail diets. In contrast ileal content of lactobacilli bacteria increased significantly due to adding yeast into laying hen diets *(*Kim et al., 2002; Hossain et al., 2005). Adding yeast to poultry diets leads to reduced bacterial counts of *E.coli and Closterdium perfringers* (Park et al., 2002; Nava et al., 2005), *Salmonella and Campylobacter (*Line et al., 1998). In this concern the research is still lacking under Egyptian conditions.

the objective of this study aimed to investigate the effect of enriching Hy line (W-36) laying hen diets with various levels of active a live yeast on their laying and feeding performance, egg shell, egg components and some blood constituents. As well as ileal bacterial make-up will be studied to validate the mode of yeast action in improving performance of laying hens.

This study provides a summary of the use of probiotic (Saccharomyces cerevisiae) for prevention of bacterial diseases in poultry as well as demonstrating the potential role of probiotics in the growth performance and immune response of poultry.

2. Materials and Methods

This study was carried out at (Layer Nutrition Research Unit), Faculty of Agriculture, Ain Shams University.

It was conducted using 75 Hy-Line (W-36) white layers which were randomly sited from 70 to 79 week of age in individual battery cages located in open sided laying house. The hens were randomly

distributed into five treatment groups of 15 layers each. The individual hen was represented as experimental unit. For nine weeks experimental period the hens were fed on a basal diet supplemented with five graded levels of active live yeast *Saccharomyces cerevisia* (produced by Starch, Yeast and Clean Co., Alex.) as 0.0% (control) 0.4%, 0.8%, 1.2% and 1.6%.

The basal diet was formulated (Table 1) to meet all nutrient requirements of laying hens according to (Hy-Line 2000) management guide. Feed was provided ad lib in an individual feeders and water was supplied through automatic nipples. Lighting hours were 17 hours per day. Egg weight in grams was recorded daily for each hen throughout the experimental period. Average egg weight, egg production percentage and average egg mass (g/hen/day) were calculated for each hen and treatment group. Feed consumption in grams per hen was recorded weekly and average feed consumption per treatment group was calculated. Feed conversion ratio was calculated as gram feed consumed per gram egg produce (g. feed/ g. egg). Body weight gain was calculated for each hen and treatment group by subtracting individual body weight of hen at 70 weeks from that at 79 weeks of age. Egg component percentages were assessed by using 12 eggs per treatment represent 6 hens as two consecutive eggs per hen. For this purpose, egg was individually weighted, broken, yolk and albumin was separated weighed and related as percentage to whole egg weight. Egg shell with membrane were cleaned, dried, weighed and related as percentage e to the whole egg.

Table (1): Composition and calculated analysis of experimental diet.

Feed Ingredient	Percentage (%)
Yellow corn	59.93
Soybean meal (48%)	24.23
Corn gluten meal	2.0
Calcium carbonate	9.16
di-calcium phosphate	1.84
Oil	2.0
Common salt	0.364
Methionine	0.076
Premix*	0.4
Total	**100**
Calculated analysis:	
ME (kcal/kg)	2806
Protein (%)	17.39
Calcium (%)	3.97
Av. Phosphorus (%)	0.465
Meth. + Cyst (%)	0.66
Lysine (%)	0.86

*: Vitamins and minerals Premix: each 1 kg supplied the following per kilogram of diet; vit. A: 12000 lu, vit. D3: 3000 lu, vit. E.: 12 mg.
vit. B12 0.02 mg, vit. B1 1 mg, Choline chloride 0.16 mg, Copper 3 mg, Iron 30 mg.
Manganese 40 mg, Zinc 45 mg and Selenium 3 mg according to NRC (1994).

Egg shell thickness (millimeter) was determined using a micrometer. Initial and final body weights of layers were recorded and average body weight gain was calculated.

Blood Analysis and Microbiological Examination:

At the end of the experiment five hens per experimental group were slaughtered, blood samples were collected and centrifuged for 15 minutes. Plasma total protein was determined according to Biuret method (Henery, 1964), albumin according to Doumas et al. (1971). Plasma globulin was calculated by subtracting albumin from total protein. Then albumin to globulin ratio was calculated. Plasma total lipid was determined according to Knight et al. (1972) and total cholesterol according to Watson (1960).

For microbial experimentation, ileal content samples were collected by pressing the outer wall of cut ileal to push its content into clean, sterile glass bottle. The pH value of ileum content were determined using pH meter. Microbiological experimentation procedure was done as follows: One gram of ileal content was adjustely weighed and transferred into test tube containing 9 ml of 0.1 sterile peptone the samples were mixed well and serial dilutions were prepared.

Cultivation and Enumeration of Bacteria:

Bacterial total count was examined with nutrient agar medium composed of (per liter) yeast extract 2.5 g trypton 5 g, glucose 1 g, agar 15 g and distilled water up to one liter (Swanson et al., 1992).

Lactobacilli bacteria was counted with M.R.S. agar medium which is composed of casein peptone 10 g meat extract 10 g, yeast extract 5 g, glucose 20 g, tween 80 1 g, K_2hpo_4 2 g, sodium acetate 5 g, diammonium citrate 2 g, $Mnso_4$ 0.2 g and distilled water up to 1 liter (Laner and Kandier, 1980)..

Coliforms bacteria were counted by using MacConkey agar medium that is composed as pancreatic digest of gelatin 17 g, pancreatic digest of casein 1.5 g, peptic of animal tissue 1.5 g, lactose 10 g, bile salts 1.5 g, sodium chloride 5 g, neutral red 0.03 g, crystal violet 0.001 g, agar 3.5 g, and distilled water up to 1 liter (Oxoid, 1992).

Campylobacter strains were grown in stationary cultures in 5 ml of Rosef broth without antibiotics for 48 hours in a microaerobic atmosphere created by using BBL gas pak plus anaerobic system envelopes without the palladium catalyst. Rosef broth contains (per liter) peptone 10g, lablemco (oxid) 8 g, yeast extract 1 g, Nacl 5 g, rezasurin solution (0.025% wt/vol) 1.6 g (Ryan and Ray, 2004).

Colstridium perfringers were grown in a stationary culture in an anaerobic atmosphere and subsequently diluted in sterile Rosef broth or sterile saline to concentrations of 10^6 to 10^8 CFU per ml, then PCR procedure was used for examination (Baumgart et al., 2007).

Klebsiella and Proteus gram negative *Entero-bacteria* were grown in MacConkey agar medium and eosin/methylene blue agar medium composed (per liter) of peptone 10 g, lactose 5 g, dipotassium phosphate 2 g, eosin Y 0.4 g, methylene blue 0.065 g, and agar 13.5 g (Oxoid, 1992).

Staphylococcus sp. and Micrococcus sp. gram positive bacteria was grown in nutrient agar medium, MacConekay agar medium and *Staphylococcus* medium (No. 110) that composed (per liter) yeast extract 2.5 g, tryptone 10 g, glateene 30 g, lactose 2 g, D/manitol 10 g, Nacl 75 g, dipotassium phosphate 5 g, agar 15 g, pH 7 ± 0.02 (Mathews et al., 1997).

Statistical Analysis:

Statistical analysis was carried out using statistical program SAS (1988). Ducan's multiple tests was used to separate means.

3. Results and Discussion

Shareef and Dabbagh (2009) reported that Saccharomyces cervisiae supplementation of broilers, to the level of 1, 1.5 and 2%, were significantly, increase the body weight gain, feed consumption and feed conversion efficiency. The beneficial effect of *Saccharomyces cerevisiae* is attributed to the fact that it is a naturally rich source of proteins, minerals and B-complex vitamins.

It is well known that yeast culture, and its cell wall extract containing 1,3-1,6 D-glucan and Mannan oligosaccharide are the important natural growth promoters for modern livestock and poultry production (Van Leeuwen et al., 2005a). The advantages of these promoters over the traditional antibiotic growth promoters are 1) no withdrawal time, 2) no residual effect, and 3) no causes of microbial mutation *(*Gibson and Roberfroid, 2008). *Saccharomyces cerevisiae* is considered as one of the live microorganisms probiotic that, when administered through the digestive tract, have a positive impact on the hosts health through its direct nutritional effect. Field reports (Banday and Risam, 2002) have suggested that probiotic supplementation improved performance of broilers. The different mechanisms of probiotic action suggested are; nutritional effect by regulation of metabolic reactions that produces toxic substances; stimulation of endogenous enzymes and by production of vitamins or antimicrobial substances. Moreover, Saccharomyces cerevisiae could act as bioregulalor of the intestinal micro flora and reinforcing the host natural defenses, through the sanitary effect by increasing the colonization resistance and stimulation of the immune response (Line et al., 1998). These effects were largely reflected by using mannan Oligosaccharide, the naturally derived extract from the cell wall of *Saccharomyces cerevisiae.* This oligosaccharide content is approxi-mately50% of the carbohydrate fraction and improved body weight gain in broiler chickens and that this effect can be attributed to the trophic effect of this product on the intestinal mucosa, because it increases villus height, particularly during the first 7 days of the chickens life (Santin et al., 2001).

Oligosaccharides used to control pathogenic scours of all kinds in livestock caused by *Salmonella*, and *E.coli* etc (Laegreid and Bauer, 2004). Mannan-oligosaccharides are thought- to block the attachment of pathogenic bacteria to the animal's intestine and colonization that may result in disease, while acting as a nutrient to other beneficial bacteria. It is also thought to stimulate the animal's immune system, thereby further reducing the risk of disease (Firon and Ofek, 1983). Oyofo et al. (1989) observed that the adherence of *Salmonella typhimurium* to enterocytes of the small intestine of chicks, in vitro, was inhibited in the presence of mannose. Later, they found that inclusion of mannose in the drinking water of chicks reduced *S. typhimurium* colonization of the cecum.

Saccharomyces cerevisiae Probiotic supplementation has been shown to reduce the cholesterol concentration were reported in egg yolk by (Abdulrahim et al., 1996) and serum in chicken (Mohan et al., 1996). Recent report suggested that feeding of chicory beta fructans an oligosaccharide, a prebiotic, reduced the serum cholesterol and abdominal fat of broiler chicken (Yusrizal, 2003). Gilliland et al. (1985) suggested that the Prebiotic supplementation could have enhanced the lactobacilli count. Similar results have been reported by others *(Mohan, 1996).*

Laying Performance:

Egg production percentage of laying hens fed 0.4% (83.4%) and 0.8% (80.6%) live yeast was higher than the control value (74%) which was approximately similar to those fed with 1.2% (74.9%), 1.6% (74.6%) yeast in their diets. The differences between egg production percentages lacked significance (Table 2).

Table (2): Effect of feeding different yeast levels on laying performance and egg components.

Item	Yeast Level				
	0.0%	**0.4%**	**0.8%**	**1.2%**	**1.6%**
Egg production	74.0	83.4	80.6	74.9	74.6
Av. Egg weight (g)	63.1	61.2	62.7	64.5	61.8
Egg mass (g egg/hen/day)	46.7	51.0	50.2	48.3	46.1
Egg component					
Egg yolk (%)	27.3	28.1	28.8	27.6	27.7
Egg albumin (%)	63.7	62.6	61.7	63.1	62.9
Egg shell (%)	9.00	9.33	9.45	9.39	9.39
Egg shell thickness (mm)	0.396^{b}	0.425^{ab}	0.426^{a}	0.416^{ab}	0.420^{ab}

a, b: Means with different superscripts are significantly different ($P<0.05$).

The improvement in egg production due to low level of yeast inclusion is in agreement with the result of Kim et al., (2002); Shivani et al. (2003); Shareef and Al-Dabbagh (2009) who observed higher percentage of egg production for hens fed yeast-supplemented diets than the control hens.

Average egg weight was not influenced significantly by adding yeast into diets. Nursoy et al.

(2004) stated that, egg weight was not affected by adding yeast into diet. The improvement in egg production reflected on egg mass (g egg/hen/day) values which increased from 46.7 (control) to 51.0 and 50.2 by adding 0.4% and 0.8% yeast level respectively, while the high levels of yeast (1.2% and 1.6%) declined egg mass value to be 48.3 and 46.1 respectively.

The increment in egg production and egg mass with 0.4% and 0.8% 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 saving more nutrients for egg formation. Soliman (2003) attributed the best hen day egg production of hens fed dietary yeast to the decrease proliferation of pathogenic bacteria. The high inclusion of yeast level has an adverse effect on nutrient digestibility (Romashko, 1999). Thereby, laying performance was not improved due to adding of 1.2% or 1.6% live yeast into diet.

Feeding Performance and Body Weight Gain:

Feed intake values of different treated groups were approximately similar and lacked significance. *Kim et al. (2002)* stated that, feed intake values were not statistically different among yeast feeding groups and control.

Feed conversion ratios (g feed/g egg) of birds fed with 0.4% (2.08) and 0.8% (2.07) dietary yeast were better than that of control (2.22), while 1.2% (2.24) and 1.6% (2.25) yeast levels did not show any improvement compared to the control. Park et al. (2002); Soliman (2003) and Zhang et al., (2005)

observed an improvement in feed conversion ratio of laying hens fed yeast supplemented diets.

The slight improvement in feed conversion inherent with low inclusion levels of yeast (0.4% or 0.8%) may be attributed to the improvement in nutrients absorption and utilization associated with adding yeast which reduces the proliferation of enteric harmful bacteria that responsible of mal-absorption (Table 3). Bradle and Savag (1995) observed an improvement in energy utilization due to feeding dietary yeast. Soliman (2003) reported that, supplementation of yeast into laying hen diets significantly improved digestion coefficient of crude protein.

Body weight gain values of layers fed different yeast levels were not significantly higher than control (Table 3). Sharma et al. (2001) stated that, the weight gain of egg type chicken fed yeast supplemented diet was higher than those fed control diet.

Table (3): Effect of feeding various live yeast levels on feeding performance and body weight gain.

Item	Yeast Level				
	0.0%	**0.4%**	**0.8%**	**1.2%**	**1.6%**
Feed intake (g/hen/day)	104.00	105.7	105.00	108.3	103.6
Feed conversion (g feed/g egg)	2.22	2.08	2.07	2.24	2.25
Initial body weight (g)	1475	1444	1480	1478	1481
Final body-weight (g)	1497	1494	1540	1555	1552
Body weight gain (g)	22	50	60	76.8	71.6

Non-significant differences.

Egg Component:

Incorporating of live yeast into laying hen diets did not influence egg albumin or egg yolk percentages and the difference; among treatments lacked significance (Table 2). Nursoy et al. (2004) did not find any affect on egg albumin or egg yolk of laying hens fed yeast-supplemented diet.

However, egg shell percentage and egg shell thickness values were improved due to feeding various yeast levels, especially at 0.8%, when compared to the control group (Table 2).

The improvement in egg shell percentage and egg shell thickness may be attributed to the enhancement of calcium absorption and retention associated with adding yeast into the diet (Bradly and Savage, 1995). Park et al. (2001) reported that, hens fed diets with yeast produced less soft shell and broken egg than control.

Blood Constituents:

Blood total protein values of birds fed on 0.4% (3.82), 0.8% (3.65), and 1.2% yeast (3.97) were lower than the control (4.16) (Table 4). However, the level of 1.6% yeast (4.33) was slightly higher than control. Similar results were recorded for blood albumin. There was no effect on blood globulins due to adding yeast to the diet.

The results of blood protein did not agree with those obtained by Wakwak et al. (2003), who did not find any effect on blood protein or albumin due to adding yeast into growing quail diets.

The lower values of blood proteins of birds fed on 0.4%, 0.8% and 1.2% yeast than the control may be attributed to the inhibitory effect of yeast against harmful intestinal microflora because harmful enteric bacteria secretes inflammatory agents lead to increase protein synthesis in liver and accordingly increased blood content of protein. Klasing and Austic (1984) observed an increase in protein synthesis in liver of chickens infected with *Escherichia coli* bacteria. Similar explanation can be introduced for the higher blood protein value of layers fed 1.6% dietary yeast, that the high inclusion of active live yeast may induce an inflammation in the small intestine wall causing increase in blood protein level.

Blood cholesterol levels of layers fed yeast supplemented diets were lower than the control (Table 4). Victor et al. (1993) and Endo et al., (1999) found that cholesterol content was lower with inclusion of yeast into broiler chicks' diets. Blood total lipid was not affected by adding yeast into diets.

Table (4): Effect of feeding various live yeast levels on blood constituents.

Item	Yeast Level				
	0.0%	**0.4%**	**0.8%**	**1.2%**	**1.6%**

Total protein (g/dL)	4.16[a]	3.82[ab]	3.65[b]	3.97[ab]	4.33[a]
Albumin (g/dL)	2.23[a]	1.83[b]	1.80[b]	2.08[ab]	2.36[a]
Globulin (g/dL)	1.93	1.99	1.87	1.89	1.97
Alb./Glob.	1.16	0.92	0.97	0.91	0.84
Cholesterol (g/dL)	161.5[a]	149[ab]	133.7[b]	158.2[ab]	149[ab]
Total lipid (mg/ dL)	418.0	395.0	396.2	437.7	423.0

a, b: Means with different superscripts are significantly different (P<0.05).

Ileal pH and Intestinal Bacteria:

Ileal content pH was not affected by adding active yeast into laying hens diets (Table 5). However, there were a reduction in digesta pH of layers fed yeast level of 0.8% and 1.2% which recorded 6.00 and 6.31 respectively against 6.58 for control. Dawson et al. (1990) and Gibson and Roberfroid, (2008) observed a reduction in ruminal pH value of steers fed active yeast.

There was an effect yeast on bacterial total count which was sharply reduced when supplemented yeast level increased. The most reduction was recorded for the birds fed 1.6% live yeast (Table 5 and Fig. 1).

Table (5): Effect of feeding active yeast levels on pH value of ileal content and intestinal bacteria make-up.

Microbial Strains	**Yeast Level**				
	0.0%	**0.4%**	**0.8%**	**1.2%**	**1.6%**
Ileal content pH	6.58	6.88	6.00	6.31	6.58
Log 10 cfu./mg					
Bacterial total count	15	12.5	12.7	10.1	5.4
Escherichia coli	7.0	2.5	3.5	2.5	2.25
Lactobacilli sp.	6.0	4.25	15.1	10.0	8.5
Klebsiella sp.	1	1	N.d	1	1
Staphylococcus sp.	3	1	2	1	1
Proteus sp.	2	1	2	1	1
Micrococcus sp.	2	N.d	3	N.d	N.d
Combylobacter sp.	4	N.d	3	2	N.d
Closterdium perfringers	3	N.d	2	1	N.d

N.d: Non-dectable.

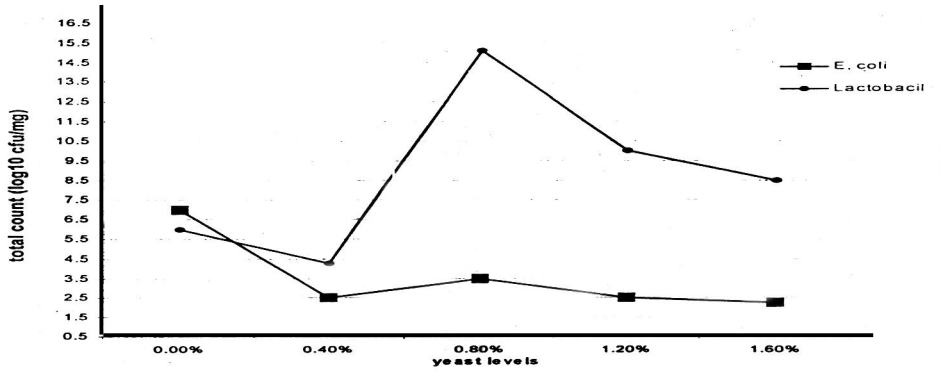

Figure (1): Effect of yeast level on total count of E.coli and lactobacilli sp..

The inhibitory effect of yeast on intestinal microflora had been established by Line et al. (1998); Wakwak et al. (2003) and Nava et al., (2005), who reported that, yeast has a reduction effect against pathogenic gut microflora.

Count of *Lactobacilli* bacteria increased due to adding active live yeast at 0.8%, 1.2% and 1.6% into laying hens diets. This result confirms those of Kim et al. (2002), who added *Pichia farinose* yeast strain into laying hens' diets and Park et al. (2002) and Kabir (2009), who included *Saccharomyces cervisiae* into broiler diets. Their results indicated an increase in viable count of ileal lactobacilli's due to adding live yeast.

The viable counts of Lactobacilli are inversely related to the pH value of ileal digesta (Table 5), where the reduction in pH values is associated with increasing Lactobacilli count. This may confirm that Lactobacilli bacterial grow well in slightly acidic media (Fuller, 2001).

Lactobacilli bacteria secrete lactic acid which reduces digesta pH so the reduction in pH value may be due to direct action of intestinal bacilli bacteria or to indirect effect of yeast on increasing intestinal bacilli bacteria. Live yeast enriched diet led to a sharply reduction in pathogenic bacterial strains of *E.coli* and *Campylobacter sp.* These strains usually cause mild to moderate gastroenteritis, diarrhea and mal-absorption of nutrients in chickens.

The current results are in agreement with those of Park et al. (2002), who stated that the counts of *closterdium perfringer* and E.coli bacteria were lower due to adding *Sacchatomyces cervisia* yeast into broiler chicks' diets. The antagonistic effect of live yeast against intestinal microflora was elucidated by Line et al. (1998) and Laegreid and Bauery (2004) who stated that, several harmful pathogenic bacteria have been shown to exhibit a binding specific for the sugar mannose. A live yeast cells contain mannose in their wall. This mannose in the cell wall may cause the yeast to act as a decoy for the attachment of pathogens. Because yeast has been demonstrated not to permanently colonize animals, the yeast and any yeast-bound pathogens pass out in the bird excretion and bacterial colonization is diminished.

Kabir et al., (2004) reported that probiotic microorganisms, once established in the gut, may produce substances with bactericidal or bacteriostatic properties (bacteriocins) such as lactoferrin, lysozyme, hydrogen peroxide as well as several organic acids. These substances have a detrimental impact on harmful bacteria, which is primarily due to a lowering of the gut pH. A decrease in PH may partially offset the low secretion of hydrochloric acid in the stomach. In addition, competition for energy and nutrients between probiotic and other bacteria may result in a suppression of pathogenic species. Numerous factors such as animal to animal variation, strain of yeast, and experimental procedures have contributed to the variation in results of yeast culture studies. However, the digestive advantages of enhanced nutrient digestibility, cecal fermentation and subsequent production parameters provide justification for nutritionists to continue to research yeast culture supplementation.

4. Conclusion:

It can be concluded that adding live yeast *Saccharomyces cervisiae* can enhance the productive performance of laying hens and nutrients utilization via the inhibitory effect of yeast against pathogenic bacteria which may cause mild enteritis and mal-absorption of nutrients.

Probiotics constitutes now an important aspect of applied biotechnological research and therefore as opposed to antibiotics and chemotherapeutic agents can be employed for growth promotion in poultry. Scientists now are triggering effort to establish the delicate symbiotic relationship of poultry with their bacteria, especially in the digestive tract, where they are very important to the well being of man and poultry (Kabir, 2009). Since probiotics do not result in the development and spread of microbial resistance, they offer immense potential to become an alternative to antibiotics. The present study reveals that probiotics could be successfully used as nutritional tools in poultry feeds for promotion of growth, modulation of intestinal microflora and pathogen inhibition, immunomodulation and promoting meat quality of poultry.

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