

APPRAISAL OF BACILLUS SUBTILIS AND SACCHAROMYCES CEREVISIAE SUPPLEMENTATION TO LAYER JAPANESE QUAIL DIET ON PRODUCTIVITY, BIOCHEMICAL PARAMETERS, AND DIGESTIVE ENZYMES

GAMAL M. MEHANI¹ MARWA A. FWAZ²; REHAM NABIL³; AHMED S. OSMAN⁴; MOHAMED E. GHONIME⁵ AND MOHAMED A. MOUSA²

¹ Nutrition and Clinical Nutrition Department, Faculty of Veterinary Medicine, Minya University

² Nutrition and Clinical Nutrition Department, Faculty of Veterinary Medicine, Sohag University.

³ Nutrition and Clinical Nutrition Department, Faculty of Veterinary Medicine, Sadat City University.

⁴ Biochemistry Department, Faculty of Veterinary Medicine, Sohag University.

⁵ Animal and Poultry Production Department, Faculty of Agriculture and Natural Resources, Aswan University.

Received: 3 March 2024; **Accepted:** 31 March 2024

ABSTRACT

The current work aimed to study the impacts of supplementation of *Bacillus subtilis* (BS) and *Saccharomyces cerevisiae* (SC) on egg production of Japanese quail, blood biochemical parameters, and the specific activity of some digestive enzymes (trypsin, chymotrypsin, and amylase). A total of 150 female Japanese quail were randomly divided into three groups. First group (control group) had the basal diet contained the recommended requirements; where the 2nd group fed on the basal diet enriched with BS (1×10^8 CFU/kg feed); and the third group, the basal diet enriched with SC (3×10^8 CFU/kg feed). The group supplemented with BS showed a significant improvement in egg production compared to the other groups ($P \leq 0.05$). There was a significant decreased in serum cholesterol, LDL, and increased in HDL in the BS and SC supplemented groups. In the BS and SC-enriched groups, the serum levels of urea and creatinine were significantly decreased in supplemented groups ($P \leq 0.05$) than the control group. Serum total protein, albumin, and globulin levels were not affected. Levels of liver function markers (AST and ALT) were decreased ($P \leq 0.05$) in the second group. BS improved the activity of digestive enzymes, particularly α -amylase. In conclusion, adding BS and SC to the layer quail diets has a potential effect on egg production, digestion, and general health.

Keywords: Japanese quail, *Bacillus subtilis*, *Saccharomyces cerevisiae*, egg production, biochemical parameters, and digestive enzymes.

Abbreviations: BS: *Bacillus subtilis*; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CFU: colony-forming unit; HDL: high-density lipoprotein; LDL: low-density lipoprotein; SC: *Saccharomyces cerevisiae*.

Corresponding author: Marwa A. Fwaz

E-mail address: marwaahmedfwaz@gmail.com

Present address: Nutrition and Clinical Nutrition Department, Faculty of Veterinary Medicine, Sohag University.

INTRODUCTION

The Coturnix Coturnix japonica, sometimes known as the Japanese quail, is a native of Asia, Europe, and northern Africa. As a result of the Japanese quail's rapid growth and production cycle and high disease resistance their production has been characterized as being highly productive with economically viable (Alkan *et al.*, 2010).

As antibiotic residues in poultry products are a crucial problem and the emergence of antimicrobial resistance, using of probiotics and prebiotics in the poultry production is urged (Patterson and Burkholder 2003). Probiotic was defined as microorganism that exists in nature and supports the host health when given in sufficient doses, according to WHO/FAO (2001). Probiotics are live cultures of microorganism (bacteria, fungi, and yeast) that improve animal's health and nutritional status via enriching GIT flora growth (Zhang and Kim, 2014)).

Probiotics as feed additives are now used in the diets of livestock to support general health, growth, production, and reproduction of livestock. Using Bacillus subtilis as a feed ingredient in poultry diets has approved by Taiwan (The Council of Agriculture, 2015).

Bacillus subtilis is a spore-forming bacteria that can withstand harsh environmental conditions and is resistant to thermal, alkalis, and acids, and it has a rapid multiply ability in GIT. Consequently, Bacillus subtilis possesses properties that make it an alternative choice to other growth promoters. Since Bacillus subtilis growth need an aerobic condition, it absorbs the free oxygen in the GIT, promoting and developing intestinal function (Ramlucken *et al.*, 2020). It produces an exogenous digestive enzyme, resulting in improved digestive efficiency of nutrients (Abd El-Moneim and Sabic, 2019). It has been demonstrated by Hatab *et al.*, (2016) that feeding BS to laying hens could greatly increase egg yield and

quality. Forte *et al.* (2016) reported a modification in the microorganism eco-system of the gut in layer hens fed BS.

Among all yeast species, *saccharomyces cerevisiae* (SC) is the most common one used in animal production (Dama and Singh, 2010). Due to it is rich in protein, polysaccharides, nucleotides, trace elements, vitamins, and growth factors. Bin *et al.* (2019) found that the addition of SC improved the production efficiency, promotes GIT development, regulated the gut microbiota eco-system, improved meat quality and general health condition. Besides, SC improved the digestive enzyme activity and reduced the pH of GIT that decreased the pathogenic microbe's presence via lactic acid production (Yang *et al.*, 2008).

The main objective of the current work was to appraise the SC and BS supplementation in laying quail diet, on the egg production and quality, digestibility, digestive enzymes activity, and the capacity of antioxidant enzymes.

MATERIALS AND METHODS

The study was conducted at the Nutrition and Clinical Nutrition Research Center, Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt. The ethical approval number is VUSC-025-1-23 of the faculty of veterinary medicine, at Sadat City University.

Japanese quail

One hundred and fifty (150) 35-day-old female Japanese quails (*Coturnix coturnix japonica*) were obtained from a local commercial company for quail production, Sohag Governorate.

Feeding

The diet of laying quails was formulated from the available ingredients, Corn, soybean meal, and corn gluten meal, besides feed additives. Crude protein was 20% as a minimum and ME was 2900 Kcal/Kg for the

quail production period according to NRC (1994) for quails. Mash diet was prepared and allowed for birds ad libitum throughout

experimental period. The general health condition was observed regularly.

Table 1: Chemical composition and metabolizable energy value of feed ingredients used in experimental diet.

Feedstuff	Dry matter (%)	Crude protein (%)	Ether extract (%)	ME (Kcal/Kg)	Crude fiber (%)	Ash (%)
Egyptian Yellow corn	89	8	3.8	3350	2.2	1.5
Soyabean meal (48%)	90	48	1	2440	3.9	6.4
Corn gluten meal	90	62	2.5	3720	1.3	3.3

Metabolizable energy (ME) from NRC (1994).

Table 2: Physical composition and proximate analysis of the laying quail diet (as-fed basis).

Ingredients	%
Corn	62.00
Soybean meal; 48% CP	29.00
Corn gluten meal	1.50
Limestone	5.50
Di-calcium phosphate	1.15
NaCl	0.30
DL-Met	0.18
L-Lys HCL	0.06
Premix (Vitamin-Mineral)	0.30
Proximate analysis (Analyzed)	
DM%	86.49
CP%	19.87
CF%	2.60
Ash%	2.88
EE%	2.82
ME, Kcal/kg (Estimated)	2974.4
Lysine (Estimated)	1.08
Methionine (Estimated)	0.49
Premix provides each kg of diet with; Vit.A, 12000 IU; Vit.E, 10 IU; Vit.k3, 3mg; Vit.D3, 2200 ICU; Vit.B1, 2.2 mg; Vit. B2, 10 mg; Ca pantothenate, 10 mg; niacin, 20 mg; Vit.B6, 1.5 mg; Choline, 500 mg; Vit.B12, 10µg; Folic acid, 1 mg; Biotin, 50µg; Mn, 55mg; Zn, 50mg; Fe, 30mg; Cu, 10mg; Se, 0.1 mg. Solvida company product (min.mix, SOLVIDA COMPANY EGYPT.	

Experimental groups:

One hundred and fifty, (35-day-old) female Japanese quails, were divided into three groups (50 birds per each). The control group received a basal diet (Table 1) formulated to cover the laying quail requirements according to NRC (1994) of

quail), without probiotics, 1st group (BS group) received the basal diet plus *Bacillus subtilis*, (CLOSTAT® HC SP dry, Kemin Industries Inc) 1×10^5 CFU/kg feed; The 2nd (SC group) fed on the basal diet with *Saccharomyces cerevisiae*,

(Actisaf Sc 47; Phileo by Lesaffre, France) 10^8 CFU/kg feed.

Experimental design and measurement:

Four weeks feeding trial was started at 35 days of age. Eggs from each group were collected and weighed daily, and egg pH was determined. The slaughter was carried out after the 2nd and the 4th week of the beginning of the experiment. Serum samples and duodenum tissue were taken and prepared from three birds for biochemical analysis and digestive enzyme activity evaluation.

Proximate analysis of feed ingredients and experimental diet:

The feed ingredients and diet were analyzed using the standard analysis methods of the AOAC, (2012). A hot air oven at 105°C, was used to determine the dry matter; the Micro-Kjeldahl method, was carried out to determine the crude protein; a muffle furnace at 660°C, was used to detect the ash content; The crude fiber was determined by an automated fiber analyzer (Ankom 2000).

Productive Performance

The productive performance parameters (egg production, egg weight, % eggshell, and pH of whole egg) were measured every day, throughout the experimental period. The average egg production rate (egg /hen per day) was calculated daily to determine the laying percentage.

Biochemical parameters determination

During slaughtering three blood samples per group were collected in sterile tubes without anticoagulant (plain tubes), after the blood clot, samples were centrifuged for 15 minutes at 4000 rpm at room temperature until clear serum was

obtained. The prepared serum was saved at -80°C till biochemical parameters analysis.

The serum was used for the analysis of total protein (TP) (Gornal *et al.*, 1949), albumin, globulin (Dumas, 1975), creatinine (Larsen, 1972), urea (Fawcett and Scott, 1960), AST, ALT (Reitman and Frankel, 1957), triglycerides (Fassati and Prencipe, 1982), cholesterol (Richmond, 1973), high-density lipoprotein (HDL) (Lopez-Virella *et al.*, 1977), low-density lipoprotein (LDL) (Wieland and Seidel, 1983). Biochemical analyses were measured by using commercial kits purchased from the bio-diagnostic company using a spectrometer device (PG Instruments Ltd).

Digestive enzymes activity assay:

During the work, three quails were randomly selected from each group and slaughtered, where the duodenum tissue was immediately collected after evisceration. The tissue samples were diluted 10× with monobasic and dibasic phosphate, then homogenized via a tissue homogenizer, and centrifuged at 10000 rpm for 30 min at 4°C. The supernatants were stored at -80°C for further analysis. Tissue total protein, and enzyme activities (trypsin, chymotrypsin, and Amylase) were determined.

Tissue protein was estimated by Folin Reaction (Lowry *et al.*, 1951) since the tissue protein is estimated by reading the absorbance, at 750 nm, against a standard curve of a known protein solution (Bovine Serum Albumin-BSA- solution). Trypsin activity was determined according to Torrissen *et al.* (1994); chymotrypsin activity was determined according to Rungruangsak-Torrissen and Sundby

(2000); and α -amylase activity was determined according to Miller (1959) by using a spectrometer.

Statistical Analysis:

The result values were expressed as mean \pm standard error of triplicate samples. The statistical analysis was carried out by one-way ANOVA method, whereas Duncan's multiple range test used for post hoc test, with the IBM SPSS statistical package (version 22, SPSS Inc., Chicago, IL, USA). The statistically significant value was considered at $p < 0.05$.

RESULTS

Egg production:

In the current study, the laying quail diet enriched with BS had a significant ($P \leq 0.05$) impact on egg production from the second week. At the end of the trial, the egg production was (0.84 ± 0.04 egg/hen/day) in the BS group, where it was (0.717 ± 0.034 and 0.649 ± 0.032) in the control and SC groups respectively (Table 3).

There was an insignificant increase in eggshell percentage in the BS supplemented group in the second week ($15.72 \pm 0.68\%$ of the egg weight), while it was 15.6 ± 0.61 and $14.66 \pm 0.49\%$ in the SC and control groups respectively (Table 3).

The diet enriched with BS had a positive time-dependent effect on egg production from the 2nd week of production, whereas the SC addition increased egg production in the 2nd and 3rd weeks then this effect was diminished in the 4th week (Table 3). Both supplements had an insignificant improvement in egg-shell weight

percentage. There was a significant increase ($P \leq 0.05$) in egg weight in the BS group (12.09 ± 0.1) followed by the SC group (11.901 ± 0.085) than the control group which came last (10.78 ± 0.185). However, there was no difference in egg pH among all groups (Table 4).

Biochemical parameters:

the BS and SC supplemented groups showed a decrease in the triglyceride, 280.85 ± 5.19 and 262.8 ± 3.6 mg/dl, respectively in comparison with the control group, 337.49 ± 4.62 mg/dl also a significant improvement ($P \leq 0.05$) in cholesterol, it was recorded as 221.86 ± 17.46 , 260.71 ± 13.72 and 335.62 ± 9.64 mg/dl, for BS, SC, and Control group, respectively. A significant ($P \leq 0.05$) increase in HDL of the BS group, (52.01 ± 3.6 mg/dl) than both control and SC groups (35.11 ± 3.26 and 36.4 ± 2.2 mg/dl, respectively). LDL was lower in the BS group (140.94 ± 3.2 mg/dl) when compared with control and SC groups (230.665 ± 4.08 and 165.49 ± 4.68 mg/dl respectively) (Table 5).

Serum total protein and albumin not affected with the inclusion of BS and SC (Table 5). We found that serum total protein was (7.33 ± 0.96 , 8.24 ± 1.19 , and 8.25 ± 0.37 g/dl) and albumin was (4.28 ± 0.53 , 5.26 ± 1.1 , and 5.51 ± 0.22 g/dl) in control, BS and SC groups respectively. In line with our findings, Hatab *et al.* (2016) and Abdel Baset *et al.* (2020) both reported that the addition of probiotics had no effects on serum albumin and total protein (Table 6).

Uric acid and creatinine are indicators of renal function. *Bacillus subtilis*, as all probiotic microorganisms could utilize,

urea, and uric acid as nutrients for growth (Dev *et al.*, 2020). From our findings, serum urea and creatinine levels were significantly decreased in the BS and SC groups (Table 7), where urea values were (21.45 ± 1.13 and 31.05 ± 2.92 , respectively) at the end of the trial. In the same pattern, the creatinine levels were noticed (0.76 ± 0.052 and 0.82 ± 0.04) in BS and SC groups, respectively.

In (Table 8), Serum ALT level revealed that BS supplementation had no significant effect on ALT as it was (38.18 ± 1.88) whereas the control group was (41.56 ± 2.78 mg/dl), but there was a significant effect of SC supplementation

on ALT (54.58 ± 1.94). We recorded a significant decrease ($P \leq 0.05$) in AST level in the BS group (20.87 ± 1.78 mg/dl), and there was no significant difference among control and SC groups where it was 27.7 ± 1.29 and 28.77 ± 1.63 respectively.

Digestive enzyme activities:

In our investigation, the SC group showed a significant increase in trypsin, α -amylase, and chymotrypsin activity during the whole period of the experiment. On the other hand, BS supplementation improved these enzymes' activity, but it is time-dependent ($P \leq 0.05$) (Table 9).

Table 3: Egg production of laying quails supplemented with dietary BS and SC:

Criteria	Control	Bacillus subtilis	Saccharomyces cerevisiae	P value
Egg number (%)				
1st week	0.25 ± 0.05	0.24 ± 0.09	0.25 ± 0.09	0.99
2nd week	0.56 ± 0.05^b	0.72 ± 0.04^a	0.63 ± 0.03^b	0.168
3rd week	0.626 ± 0.03^b	0.74 ± 0.03^a	0.635 ± 0.03^b	0.036
4th week	0.717 ± 0.034^b	0.84 ± 0.04^a	0.649 ± 0.032^c	0.009
Eggshell (%)				
1st week	18.81 ± 0.71^{ab}	18.82 ± 0.78^a	17.46 ± 0.54^b	0.059
2nd week	15.55 ± 0.35	16.03 ± 0.63	15.81 ± 0.46	0.819
3rd week	15.016 ± 0.38^b	17.34 ± 1.41^a	15.62 ± 0.22^a	0.03
4th week	14.66 ± 0.49	15.72 ± 0.68	15.6 ± 0.61	0.41
pH				
1st week	6.7 ± 0.98	6.83 ± 0.98	7.7 ± 0.15	0.95
2nd week	7.46 ± 0.15^b	7.66 ± 0.15^b	8.07 ± 0.21^a	0.51
3rd week	7.95 ± 0.28^{ab}	7.86 ± 0.17^{ab}	8.42 ± 0.22^a	0.83
4th week	7.63 ± 0.25	7.63 ± 0.13	7.12 ± 0.21	0.24

Data are expressed as Mean \pm SE.

Averages in the same row with different superscripts are significantly different ($P \leq 0.05$).

Table 4: Egg weight (g) of laying quails supplemented with dietary BS and SC.

Criteria	Control		Bacillus Subtilis		Saccharomyces cerevisiae		P value
	Fresh	after 10 days	Fresh	after 10 days	Fresh	after 10 days	
1st week	7.89±0.9 ^b	7.32±1.02 ^b	11.29±0.63 ^a	10.26±0.33 ^a	10.15±0.42 ^b	9.38±0.43 ^b	0.00041
2nd week	10.279±0.24 ^b	10.021±0.32 ^b	11.064±0.11 ^{ab}	10.865±0.12 ^{ab}	11.74±0.24 ^a	11.41±0.28 ^a	<0.01
3rd week	10.89±0.18 ^b	10.76±0.168 ^b	11.73±0.2 ^a	11.55±0.22 ^a	11.87±0.13 ^a	11.7±0.125 ^a	<0.01
4th week	10.78±0.185 ^c	10.73±0.18 ^c	12.092±0.10 ^a	11.96±0.12 ^a	11.901±0.085 ^{bc}	11.82±0.06 ^{bc}	<0.01

Data are expressed as Mean ± SE.

Averages in the same row with different superscripts are significantly different ($P \leq 0.05$).

Table 5: Lipid profile of laying quails supplemented with BS and SC.

Criteria	Control	Bacillus subtilis	Saccharomyces cerevisiae	P value
Triglyceride (mg/dl)				
At 46 days	260.24±5.45 ^a	252.51±11.15 ^b	232.72±10.70 ^c	0.0001
At 64 days	337.49±4.62	280.85±5.19	262.80±3.60	0.023
Cholesterol, (mg/dl)				
At 46 days	347±11 ^a	242.59±23.2 ^b	228.83±9.04 ^b	0.003
At 64 days	335.62±9.64 ^a	221.86±17.46 ^b	260.71±13.72 ^b	0.003
HDL, (mg/dl)				
At 46 days	40.27±4.8	47.53±2.91	35.49±1.72	0.15
At 64 days	35.11±3.26 ^b	52.01±3.6 ^a	36.40±2.20 ^b	0.011
LDL, (mg/dl)				
At 46 days	231.73±3.34 ^a	149±5.72 ^b	157.61±6.90 ^b	<0.01
At 64 days	230.66±4.08 ^a	140.94±3.26 ^b	165.49±4.68 ^b	0.001

Data are expressed as Mean ± SE.

Averages in the same row with different superscripts are significantly different ($P \leq 0.05$).

Table 6: Protein profile of laying quails supplemented with BS and SC:

Criteria	Control	Bacillus subtilis	Saccharomyces cerevisiae	P value
Total protein, (g/dl)				
At 46 days	7.43±0.61 ^c	8.20±0.40 ^b	8.84±0.30 ^a	<0.01
At 64 days	7.33±0.96 ^b	8.24±1.19 ^a	8.25±0.37 ^a	0.02
Albumin, (g/dl)				
At 46 days	5.32±0.62	6.93±0.87	6.19±0.43	0.308
At 64 days	4.28±0.54	5.26±1.1	5.51±0.22	0.490
Globulin, (g/dl)				
At 46 days	1.10±0.12	1.26±0.48	0.65±0.20	0.400
At 64 days	3.05±0.45	3.64±0.68	3.74±0.29	0.600

Data are expressed as Mean ± SE.

Averages in the same row with different superscripts are significantly different ($P \leq 0.05$).

Table 7: Kidney function of laying quails supplemented with BS and SC:

Criteria	Control	Bacillus subtilis	Saccharomyces cerevisiae	P value
Urea, (mg/dl)				
At 46 days	52.33±2.34 ^a	16.65±2.78 ^c	30.72±2.08 ^b	<0.01
At 64 days	44.44±2.52 ^a	21.45±1.13 ^c	31.05±2.92 ^b	0.001
Creatinine, (mg/dl)				
At 46 days	1.03±0.16	0.76±0.05	0.82±0.04	0.227
At 64 days	1.00±0.020 ^a	0.80±0.01 ^b	0.82±0.06 ^b	0.07

Data are expressed as Mean ± SE.

Averages in the same row with different superscripts are significantly different ($P \leq 0.05$).

Table 8: Liver enzymes of laying quails supplemented with BS and SC:

Criteria	Control	Bacillus subtilis	Saccharomyces cerevisiae	P value
ALT, (U/L)				
At 46 days	33.75±2.61	40.01±2.06	45.92±2.69	0.117
At 64 days	41.56±2.78	38.18±1.88	40.58±1.94	0.189
AST, (U/L)				
At 46 days	29.77±1.46 ^a	22.196±1.67 ^b	27.11±1.75 ^a	0.009
At 64 days	27.70±1.29 ^a	20.87±1.78 ^b	25.77±1.63 ^a	0.015

Data are expressed as Mean ± SE.

Averages in the same row with different superscripts are significantly different ($P \leq 0.05$).

Table 9: Digestive enzymes activity of laying quails supplemented with BS and SC.

Criteria	Control	Bacillus subtilis	Saccharomyces cerevisiae	p value
Trypsin (units/g wet weight)				
At 46 days	12.91±0.53 ^c	15.07±0.31 ^b	17.41±0.43 ^a	0.001
At 64 days	15.09±0.19 ^b	18.05±0.32 ^a	18.86±0.41 ^a	0.0004
Chymotrypsin (units/g wet weight)				
At 46 days	15.18±0.64 ^b	15.6±0.23 ^b	18.83±0.25 ^a	0.002
At 64 days	15.39±0.79 ^b	18.57±0.61 ^a	19.04±0.65 ^a	0.019
α -amylase (units/g wet weight)				
At 46 days	796.25±17.99 ^b	1157.97±55.67 ^a	1151.57±140.86 ^a	0.044
At 64 days	709.24±41.47 ^b	1323.24±69.72 ^a	1250.26±72.67 ^a	0.001

Data are expressed as Mean ± SE.

Averages in the same row with different superscripts are significantly different ($P \leq 0.05$).

DISCUSSION

In concurrence with different outcomes, Ayasan *et al.* (2006) found that laying

Japanese quail diets enriched with probiotics had a significant ($P \leq 0.05$) increase in egg production and weight. They explained the egg weight increase to the vital action of

probiotics at higher doses (10^{10} CFU/g). However, Onol *et al.* (2003) found that there were no beneficial results of probiotic addition on the egg production and weight of laying quails. Also, Tsai *et al.* (2023) noticed no significant difference in egg weight and shell weight of laying hens in response to BS addition.

In concurrence with our results, Dizaji and Pirmohammadi (2009) found that SC dietary supplementation did not substantially improve the production performance of laying hens from 46 to 55 weeks of age. Al-Homidan (2007) concluded that SC addition to laying hen diets significantly improves egg production.

Dietary BS supplementation has been shown to increase hens' ability to produce eggs, which has been associated to a decrease in the pH of the gastrointestinal tract and an inhibition of bacterial enzyme activity. (Neijat *et al.*, 2019).

Biochemical parameters:

In agreement with our findings, Dehkohne *et al.* (2021) found a significant decrease in the serum triglycerides in rats that were supplemented with BS. Also, these results are supported by Rajput *et al.* (2013) result, who reported positive effects of BS addition on the serum triglyceride in broilers. In contrast to these results, Ding *et al.* (2020) demonstrated that diets supplemented with BS had higher plasma triglycerides and higher LDL.

Mohamed *et al.* (2022) noted that broiler diet enriched with BS reduced serum triglyceride, cholesterol, and LDL. They explain that as a potential method of BS in the digestion of cholesterol. Cholesterol is the precursor of bile acids production, the more the bile salt produced the lower the serum cholesterol detected (Guo and Zhang 2010).

In agreement with our findings of biochemical parameters, Galip and Seyidoglu, (2012) noted that rabbits fed diet

supplemented with SC for 56 days significantly decreased serum cholesterol. The reduction of serum cholesterol level might be attributed to its integration forming the cellular membrane of the SC or mitigating cholesterol synthesis (Krasowska *et al.*, 2007). Additionally, probiotics converted cholesterol in the GIT to coprostanol, that excreted with the feces (Sivamaruthi *et al.*, 2019), also it could inhibited the cholesterogenesis enzyme and reduced the activity of hydroxy-methyl-glutaryl coenzyme-A, which is responsible in cholesterol synthesis (Shokryazdan *et al.*, 2017).

In contrast to our findings, Yazhini *et al.* (2018) findings showed a significant ($P \leq 0.05$) improvement in serum protein with probiotic addition, where they explain that as a result of BS ability to suppress pathogenic microorganisms' growth, and consequently improved the efficiency of protein utilization via lowering protein breakdown into nitrogen besides increasing nutrient absorption due to its effects of increase height of villi and width of the crypts (Abd El-Moneim *et al.*, 2019).

Following our finding, Hatab *et al.* (2016) reported that feeding probiotics reduced uric acid, whereas Stropfova *et al.* (2006) observed no impact with the addition of probiotics on serum uric acid. Also, Seyidoglu and Galip (2014) reported that rabbit serum urea and creatinine were not affected by the addition of SC. In contrast to these findings, Tonekabon, (2013) showed an increased in serum uric acid with the addition of the probiotics.

Our findings were supported by Dehkohne *et al.* (2021) and Abdel Baset *et al.* (2020) who found that probiotics significantly decrease liver enzymes. Dietary BS supplementation reduced liver enzymes as it has a hepatoprotective action, which results in better health and non-pathological metabolism of the hepatic and cardiac tissues (Aluwong *et al.*, 2013). According to Abu-El-

Ella *et al.* (2014), who reported a significant increase ($P \leq 0.05$) in AST and ALT in response to yeast supplementation, which is consistent with our findings.

Digestive enzyme activities:

The necessity of digestive enzymes is to breaking down food into smaller and absorbable components. It has a direct impact on nutrient digestion efficiency, growth, and egg production (Pi *et al.*, 2014). Probiotics have the ability to produce digestive enzymes including amylases, lipases, and proteases, reflecting their contribution to the digestion process (Adel *et al.*, 2016).

At the end of the experiment, there was a significant improvement ($P \leq 0.05$) in trypsin, chymotrypsin, and α -amylase activities in the supplemented groups. In consistent with our findings, El-Bab *et al.* (2022) found that SC addition enhanced the digestive enzymes secretion and improved nutrient digestibility. Chandra and Liddle, (2009) documented that a content of SC of β -glucan increased the cholecystokinin production from enteroendocrine cells, which stimulating pancreatic juice secretion. In contrast to theses finding, He *et al.* (2009), recorded no improvement in digestion and digestive enzyme activity.

According to Gadde *et al.* (2017), BS produced endogenous enzymes and aids in the excretion of external enzymes. In agreement with our results, Ding *et al.* (2020) showed an improvement of α amylase activity with supplementation of BS. These findings are in agreement with Huo *et al.* (2017) who suggested the BS addition to the diet to increase the protease and α -amylase activity. Other research of Borda-Molina *et al.* (2018) concluded a correlation between an improvement of the GIT eco-system (increased Bifidobacterium and Lactobacillus) and improved digestion with the addition of BS in chicken diets.

Finally, we found a significant improvement in layer quail production, biochemical parameters, hepatic biomarkers, and renal function markers with the addition of BS and SC, besides improved digestion. These probiotics have many modes of action, such as enzyme production (Ding *et al.* 2020), acid production, increased height of villi, immune modulation, increased mucin secretion, and decreased the pathogenic microorganism's growth (Patterson and Burkholder, 2003).

It can be concluded that BS supplementation was very effective during the growth and production stage, as it improved digestion efficiency and immunity of the bird, that was noticed during our work and the high egg production that was recorded, while the use of SC could be more effective in the growth stage and it need more investigation to determine its role in growth and production.

REFERENCES

- Abd El-Moneim, A.E. and Sabic, E.M. (2019): Beneficial effect of feeding olive pulp and *Aspergillus awamori* on productive performance, egg quality, serum/yolk cholesterol and oxidative status in laying Japanese quails, *Journal of Animal and Feed Sciences*, 28, 52–61.
- Abd El-Moneim, E.A.; El-Wardany, I.; Abu-Taleb, A.M.; Wakwak, M.M.; Ebeid, T.A. and Saleh, A.A. (2019): Assessment of in ovo administration of *Bifidobacterium bifidum* and *Bifidobacterium longum* on performance, ileal histomorphometry, blood hematological, and biochemical parameters of broilers, *Probiotics and Antimicrobial Proteins*, In Press.
- Abdel Baset, S.; Ashour, E.A.; Abd El-Hack, M.E. and El-Mekkawy, M.M. (2020): Effect of different levels of pomegranate peel powder and probiotic supplementation on growth, carcass traits, blood serum metabolites,

- antioxidant status and meat quality of broilers. *Anim. Biotechnol.* 1:1–11.
- Abu-El-Ella, A.A.; El-Malky, O.M. and Zeedan, Kh. I.I. (2014):* Studies on using biogen-zinc on productive and reproductive performance of ruminants: 1- Physiological responses of Damascus goats to diet supplementation with biogen-zinc. *Egyptian J. Sheep & goats Sci.*, 9 (3): 29-48.
- Adel, M.; Lazado, C.C.; Safari, R.; Yeganeh, S. and Zorriehzahra, M.J. (2016):* Aqualase-sup®, a yeast-based in-feed probiotic, modulates intestinal microbiota, immunity and growth of rainbow trout (*Oncorhynchus mykiss*) *Aquac. Res.* 48: 1815–1826.
- Al-Homidan, I.H. (2007):* The effect of dried yeast (*Saccharomyces cerevisiae*) supplementation on growth, performance, carcass chemical analysis, immunity, ileum villi heights and bacterial counts of broiler chickens. *Egyptian Poultry Science* 27: 613-623.
- Alkan, S.; Karabag, K.; Galic, A.; Karsli, T. and Balcioglu, M.S. (2010):* Determination of body weight and some carcass traits in Japanese quails (*Coturnix coturnix japonica*) of different lines. *Kafkas Univ Vet Fak Derg*; 16:277-80.
- Aluwong, T.; Hassan, F.; Dzenda, T.; Mohammed, K.A.W.U. and Joseph, A. Y.O. (2013):* Effect of different levels of supplemental yeast on body weight, thyroid hormone metabolism and lipid profile of broiler chickens. *Journal of Veterinary Medical Science*, 75(3), 291-298.
- AOAC. (2012):* Official Methods of Analysis, 19th ed.; Association of Official Analytical Chemists: Arlington, VA, USA.
- Ayasan, T.; Ozcan, B.D.; Baylan, M. and Canogullari, S. (2006):* The Effects of dietary inclusion of probiotic protexin on egg yield parameters of japanese quails (*Coturnix coturnix Japonica*). *International Journal of Poultry Science*;5(8):776-779.
- Bin, D.; Shuang, X.; Lu, L. and Xi, C. (2019):* Research progress on application of *Saccharomyces cerevisiae* in animal production. *Feed Res.* 7, 114–116.
- Borda-Molina, D.; Seifert, J. and Camarinha-Silva, A. (2018):* Current perspectives of the chicken gastrointestinal tract and its microbiome *Comput. Struct. Biotechnol. J.*, 16 pp. 131-139.
- Chandra, R. and Liddle, R.A. (2009):* Neural and hormonal regulation of pancreatic secretion. *Curr. Opin. Gastroenterol.* 25:441–446.
- Dehkohneh, A.; Khalili, A.; Ardalani, O.; Mohsenzadeh, A.; Sabati, H., and Jafari, P. (2021):* Impacts of *Bacillus subtilis* JQ61816 on lipid panel and expression of genes involved in cholesterol metabolism in hypercholesterolemic rats. *Journal of Current Biomedical Reports*, 2(4), 176-184.
- Dev, K.; Mir, N.A.; Biswas, A.; Kannoujia, J.; Begum, J.; Kant, R.; Mandal, A. (2020):* Dietary synbiotic supplementation improves the growth performance, body antioxidant pool, serum biochemistry, meat quality, and lipid oxidative stability in broiler chickens. *Anim. Nutr.* 6, 325–332.
- Dama, K. and Singh, S.D. (2010):* Probiotics improving poultry health and production: An overview. *Poult. Punch.* 26, 41.
- Ding, B.; Wu, J.; Li, X.; Zhang, C.; Men, X. and Xu, Z. (2020):* Effects of *Bacillus subtilis* on growth performance, serum parameters, digestive enzyme, intestinal morphology, and colonic microbiota in piglets. *AMB Express*, 10, 1-10.
- Dizaji, S.B. and Pirmohammadi, R. (2009):* Effect of *Saccharomyces cerevisiae* and bioplus 2B on performance of laying hens. *International Journal of Agriculture and Biology*, 11, 495-497.

- Doumas, B.T. (1975):* Standards for total serum protein assays—a collaborative study. *Clinical chemistry*, 21(8), 1159-1166.
- El-Bab, A.F.F.; Saghir, S.A.M.; El-Naser, I. A.A.; El-Kheir, S.M.M.A.; Abdel-Kader, M.F.; Alruhaimi, R.S.; Alqhtani, H.A.; Mahmoud, A.M.; Naiel, M.A.E. and El-Raghi, A.A. (2022):* The Effect of Dietary *Saccharomyces cerevisiae* on Growth Performance, Oxidative Status, and Immune Response of Sea Bream (*Sparus aurata*). *Life* (Basel, Switzerland), 12(7), 1013.
- Fassati, P. and Prencipe, L. (1982):* Triglyceride enzymatic colorimetric method. *J. of Clin. Chem.*, (28), 2077.
- FAO (2001):* The state of food insecurity in the world. Rome.
- Fawcett, J. and Scott, J. (1960):* A rapid and precise method for the determination of urea. *Journal of clinical pathology*, 13(2), 156-159.
- Forte, C.; Moscati, L. and Acuti, G. (2016):* Effects of dietary *Lactobacillus acidophilus* and *Bacillus subtilis* on laying performance, egg quality, blood biochemistry and immune response of organic laying hens. *J Anim Physiol Anim Nutr* 100.5: 977-987.
- Gadde, U.; Kim, W.H.; Oh, S.T. and Lillehoj, H.S. (2017):* Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: A review. *Anim. Health Res. Rev* 18, 26–45.
- Galip, N. and Seyidoolu, N. (2012):* Effect of yeast culture on serum lipid and meat lipid values of rabbits. *Journal of animal and Veterinary Advances*, 11(22), 4115-4120.
- Gornal, A.C.; Bardawill, C.J. and David, M.M. (1949):* *J. Biol. Chem.* 177: 751
- Guo, C.; Zhang, L. (2010):* Cholesterol-lowering effects of probiotics A review. *Wei Sheng Wu Xue Bao.* 50: 1590–1599.
- Hatab, M.H.; Elsayed, M.A.; Ibrahim, N.S. (2016):* Effect of some biological supplementation on productive performance, physiological and immunological response of layer chicks. *J. Radiat. Res. Appl. Sci*, 9, 185–192.
- He, S.; Zhou, Z.; Liu, Y.; Shi, P.; Yao, B.; Ringø, E. and Yoon, I. (2009):* Effects of dietary *Saccharomyces cerevisiae* fermentation product (DVAQUA®) on growth performance, intestinal autochthonous bacterial community and non-specific immunity of hybrid tilapia (*Oreochromis niloticus* ♀×*O. aureus* ♂) cultured in cages. *Aquaculture*. 294:99–107.
- Huo, S.Y.; Deng, Z.L.Y and Jiang, J.P. (2017):* Effects of antibiotics substitution agent Z-27 on intestinal digestive enzymes, nutrients and growth performance of weaned piglet. *J Henan Agric Sci* 46:125–129 (in Chinese).
- Krasowska, A.; Kubik, A.; Prescha, A. and Lukaszewicz, M. (2007):* Assimilation of omega 3 and omega 6 fatty acids and removing of cholesterol from environment by *Saccharomyces cerevisiae* and *Saccharomyces boulardii* strains. *Journal of Biotechnology*, 131(2), S63-S64.
- Larsen, K. (1972):* Creatinine assay by a reaction-kinetic principle. *Clinica chimica acta*, 41, 209-217.
- Lopez-Virella, M.F.; Stone, P.; Ellis, S. and Colwell, J.A. (1977):* Cholesterol determination in high-density lipoproteins separated by three different methods. *Clinical chemistry*, 23(5), 882-884.
- Lowry, O.H.; Rosenbrough, N.J.; Farr, A.L. and Randall, R.J. (1951):* “Protein measurement with the Folin Phenol Reagent”, *J Biol Chem* 193, pp. 265-275.
- Miller, G.L. (1959):* Use of dinitrosalicylic acid reagent for determination of

- reducing sugar. *Analytical Chemistry* 31(3): 426-428.
- Mohamed, T.M.; Sun, W.; Bumbie, G.Z.; Dosoky, W.M.; Rao, Z. Hu P, Wu, L. and Tang, Z. (2022): Effect of Dietary Supplementation of *Bacillus subtilis* on Growth Performance, Organ Weight, Digestive Enzyme Activities, and Serum Biochemical Indices in Broiler. *Animals (Basel)*. 2022 Jun 16; 12(12): 1558.
- National Research Council. (1994): Nutrient requirements of poultry. Nutrient requirements of ring-necked Pheasants, Japanese quail, and Bobwhite quail. 9th Rev. Washington, DC, USA: National Academy Press.
- Neijat, M.; Shirley, R.B.; Barton, J. and Thiery, P. (2019): Effect of dietary supplementation of *Bacillus subtilis* DSM29784 on hen performance, egg quality indices, and apparent retention of dietary components in laying hens from 19 to 48 weeks of age *Poult. Sci.*, 98, pp. 5622-5635.
- Onol, A.G.; Sari, M.; Oguz, F.K.; Gulcan, B. and Erbas, G. (2003): Sürekli scak stresinde bulunan yumurtlama donemindeki bildircinlerin rasyonlarına probiotik katkisinin bazi verim ve kan parametreleri üzerine etkisi. *Türk Veterinerlik ve Hayvancilik Dergisi*; 27: 1397-1402.
- Patterson, J.A. and Burkholder, K.M. (2003): Application of prebiotics and probiotics in poultry production. *Poultry science*, 82(4), 627-631.
- Pi, D.G.; Liu, Y.L.; Shi, H.F.; Li, S. and Odle, J. (2014): Dietary supplementation of aspartate enhances intestinal integrity and energy status in weaning piglets after lipopolysaccharide challenge. *J Nutr Biochem* 25:456-462.
- Rajput, I.R.; Li, Y.L.; Xu, X.; Huang, Y.; Zhi, W.C.; Yu, D.Y. and Li, W. (2013): Supplementary effects of *Saccharomyces Boulardii* and *Bacillus subtilis* B10 on digestive enzyme activities, antioxidation capacity and blood homeostasis in broiler. *Int J Agric Biol* 15:1560-8530.
- Ramlucken, U.; Laloo, R.; Roets, Y.; Moonsamy, G. and Jansen, V. (2020): Advantages of *Bacillus* based probiotics in poultry production. *Livestock Science*. 241. 104215. 10.1016/j.livsci.104215.
- Reitman, A. and Frankel, S. (1957): *Amer J clin Path.*, 28: 56.
- Richmond, W. (1973): Use of cholesterol oxidase for assay of total and free cholesterol in serum by continuous-flow analysis. *Clinical chemistry*, 22(10), 1579-1588.
- Rungruangsak-Torrissen, K. and Sundby, A. (2000): Protease activities, plasma free amino acids and insulin at different ages of Atlantic salmon (*Salmo salar* L.) with genetically different trypsin isozymes. *Fish Physiol. Biochem.*, 22, 337-347.
- Seyidođlu, N. and Galip, N. (2014): Effects of *Saccharomyces cerevisiae* and *Spirulina platensis* on growth performances and biochemical parameters in rabbits. *Journal of the Faculty of Veterinary Medicine, Kafkas University*, 20, 331-336.
- Shokryazdan, P.; Faseleh Jahromi, M.; Liang, J.B.; Ramasamy, K.; Sieo, C.C. and Ho, Y.W. (2017): Effects of a *Lactobacillus salivarius* mixture on performance, intestinal health and serum lipids of broiler chickens. *PLoS ONE*. 12: e0175959.
- Sivamaruthi, B.S.; Kesika, P.; Chaiyasut, C. (2019): A mini-review of human studies on cholesterol-lowering properties of probiotics. *Sci. Pharm.* 87:26.
- Strompfova, V.; Marci řnáková, M.; Simonová, M.; Gancarříková, S.; Jonecová, Z.; Sciranková, L.; Koscová, J.; Buleca, V.; Cobanová, K.; Lauková, A. (2006): *Enterococcus faecium* EK13—An enterocin A-producing strain with probiotic

- character and its effect in piglets. *Anaerobe*, 12, 242–248.
- The Council of Agriculture*, (2015): Executive Yuan. Feed additives allowed to provide for livestock, poultry and aquatic animals. Agriculture and Animal Husbandry No. 1040043326A Announcement.
- Tonekabon, I.* (2013): Studies on *Bacillus subtilis*, as potential probiotics, on the biochemical parameters of rainbow trout, *Oncorhynchus mykiss* (Walbaum) to challenge infections. *Adv. Stud. Biol*, 5, 37–50.
- Torrissen, K.R.; Lied, E. and Espe, M.* (1994): Differences in digestion and absorption of dietary protein in Atlantic salmon (*Salmo salar*) with genetically different trypsin isozymes. *J. Fish Biol.*, 45, 1087– 1104
- Tsai, M.Y.; Shih, B.L.; Liaw, R.B.; Chen, W.T.; Lee, T.Y.; Hung, H.W.; Hung, K.H. and Lin, Y.F.* (2023): Effect of dietary supplementation of *Bacillus subtilis* TLRI 211-1 on laying performance, egg quality and blood characteristics of Leghorn layers. *Animal bioscience*, 36(4), 609–618.
- Wieland, H. and Seidel, D.J.* (1983): lipid Res.24, 904.
- World Health Organization.* (2001): Message from, Dr Hussein A. Gezairy, Regional Director, WHO Eastern Mediterranean Region, to the joint AFC/WHO/FAO intercountry workshop on emerging food safety issues and consumer protection, Amman, Jordan, 28-30 October 2001.
- Yang, Y.; Iji, P.A.; Kocher, A.; Mikkelsen, L.L. and Choct, M.* (2008): Effects of dietary mannanoligosaccharides on growth performance, nutrient digestibility and gut development of broilers given different cereal-based diets.
- Yazhini, P.; Visha, P.; Selvaraj, P.; Vasanthakumar, P. and Chandran, V.* (2018): Dietary encapsulated probiotic effect on broiler serum biochemical parameters. *Vet World*. Sep; 11(9): 1344-1348.
- Zhang, Z.F. and Kim, I.H.* (2014): Effects of multi-strain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta odor contents in broilers. *Poultry Science*; 93:364-70

تقييم أضافة العصوية الرقيقة و الخميرة لعليقة السمان الياباني البياض علي معدل إنتاج البيض والقياسات الكيميائية الحيوية والانزيمات الهاضمة

جمال محمد مهني ، مروة أحمد فواز ، ريهام نبيل ، أحمد السيد عصمان ، محمد السيد غنيم ،
محمد عبد الله موسى

Email: marwaahmedfواز@gmail.com Assiut University web-site: www.aun.edu.eg

الهدف من الدراسة هو معرفة تأثير تغذية بعض العناصر الحيوية، مثل العصوية الرقيقة والخميرة علي معدل إنتاج البيض، ومؤشرات الدم الكيميائية، والنشاط النوعي لبعض الانزيمات الهضمية، مثل التربسين، والكموتريسين، والألفا أميليز في السمان الياباني البياض. تم تقسيم عدد ١٥٠ من إناث السمان الياباني إلى ثلاث مجموعات بصورة عشوائية. في المجموعة الأولى (المجموعة الضابطة) تم تغذية الطيور علي العليقة الضابطة الأساسية التي تحتوي علي المتطلبات الغذائية الموصي بها بينما غذيت طيور المجموعة الثانية علي العليقة الضابطة الأساسية مضافا إليها ($10^8 \times 1$) وحدة من مستوطنة العصوية الرقيقة لكل كيلو جرام علف) بينما تلقت المجموعة الثالثة العليقة الأساسية بالإضافة إلي ($10^8 \times 3$) وحدة من مستوطنة الخميرة لكل كيلو جرام علف). وأظهرت المجموعة المكتملة بالعصوية الرقيقة تحسناً كبيراً ($P \leq 0,05$) في إنتاج البيض بالمقارنة مع المجموعة الضابطة والمجموعة المكتملة بالخميرة. أظهرت المجموعة الثانية زيادة كبيرة في الدهون الثلاثية بينما شهدت تحسناً كبيراً في كوليسترول الدم ، البروتين الدهني منخفض الكثافة و البروتين الدهني عالي الكثافة. عند إضافة العصوية الرقيقة والخميرة، انخفضت مستويات اليوريا والكرياتينين انخفاضاً كبيراً ($P \leq 0,05$) بالمقارنة مع المجموعة الضابطة. كان المجموع الكلي للبروتينات والألبومين والجلوبيولين متشابهة في جميع المجموعات. وقد انخفضت مستويات انزيمات الكبد في الدم انخفاضاً كبيراً ($P \leq 0,05$) في المجموعة الثانية وقد حسنت إضافة العصوية الرقيقة علي نشاط الإنزيمات الهضمية، خصوصاً الألفا اميليز. خلصت الدراسة إلي أن استخدام العصوية الرقيقة والخميرة في علائق السمان الياباني البياض أدي إلي زيادة إنتاج البيض وتحسين الهضم والصحة العامة للطيور.