

**ORIGINAL RESEARCH**



**THE IMPACT OF PROBIOTIC AND PREBIOTIC ON EGG PRODUCTION, EGG QUALITY AND RETENTION OF NUTRIENTS IN LAYING HENS REARED UNDER DESERT CONDITIONS IN EGYPT**

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**ABSTRACT**

Poultry egg producers incur distressing financial loss during summer season in Egypt due to exposure of laying hens to heat stress resulting in alteration of nutritional behavior and physiological functions culminating in production loss and reduction of revenue from sale due to quality deterioration of eggs. However, some feed additives, such as, probiotics and prebiotics can prevent heat stress aiding to restore egg productivity and quality characteristics of eggs in laying hens during summer season. The current study was conducted on 80 H&N Brown Nick laying hens during summer months (July-October) in the year 2018 for 14 weeks from 42 to 56 weeks of age. The average of maximum ambient temperature during the study period was  $34.1 \pm 0.3.4$  °C, RH% was  $80.1 \pm 7.9$  % and THI was  $30.1 \pm 3.0$ . The hens were grouped (n=20) into four groups and were fed on probiotic (T1: 1kg Bio-plus 2B<sup>®</sup> / ton of feed), prebiotic (T2: 1kg Techno Mos<sup>®</sup> / ton of feed) and Synbiotic (T3: 1kg Bio-plus 2B<sup>®</sup> +1kgTechno Mos<sup>®</sup> / ton of feed) supplemented diets. One group served as the control (C). The characteristics included in the study were Hen-day egg production (%), egg weight (g), daily egg mass (g), feed consumption (g/hen/day), feed conversion ratio (feed Intake/egg mass), initial and final live body weights (g), body weight changes (g), egg and shell quality characteristics, and retention of nutrients. The results revealed that dietary probiotic, prebiotic or their blend exhibited non-significant ( $P > 0.05$ ) decrease in egg production, egg mass and feed consumption versus the control, whereas egg weight and feed conversion ratio were not significantly different ( $P > 0.05$ ) among the treatments. Body weight changes were slightly increased by probiotic treatment compared to the others. There was a significant increase ( $P < 0.05$ ) in shell weight due to prebiotic (T2) and synbiotic (T3) treatments. Also, the synbiotic treatment showed a significant increase ( $P < 0.05$ ) in yolk (%) and significant decrease ( $P < 0.05$ ) in albumen (%) and haugh unit ( $P < 0.01$ ), while yolk/albumen ratio was significantly increased ( $P < 0.05$ ) with all treatments compared with the control. The feed intake (g), excretion (g) and retention (%) of nutrients (DM, OM, CP, EE), the utilization of gross energy (kcal/kg) and apparent metabolizable energy, i.e., AME (kcal/kg) did not differ ( $P > 0.05$ ) among the treatment groups. It is concluded that dietary supplementation of probiotic (Bio-plus2B<sup>®</sup>), prebiotic (Techno Mos<sup>®</sup>) or their combination (synbiotic) did not positively affect the productive performance of laying hens during hot summer season, but yolk / albumen ratio was increased ( $P < 0.05$ ) in the treatment groups over the control group indicating better nutritional quality of eggs. Also, the haugh units of eggs in the treatment groups (Range: 75-87) were of AA grade ( $\geq 72$  HU) according to the gradation of United States Department of Agriculture (USDA) indicating better protein quality and desirable freshness of eggs, despite high ambient temperature ( $34.1 \pm 3.4$  °C) during summer in Sinai desert of Egypt.

**KEY WORDS**

Brown Nick Hens, Egg production, Egg quality, Prebiotic, Probiotic, Synbiotic, Summer season

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## INTRODUCTION

Poultry producers are subjected to great financial losses due to exposure of hens to hot waves in summer season. The high ambient temperature during summer season is considered as one of the most important stressors affecting egg production (Lara and Rostagno, 2013). Also, it may alter the nutritional behavior and physiological functions of poultry and cause negative effects on their performance and production (Lara and Rostagno, 2013). Heat stressed laying flocks often lay eggs with thinner shells (Lin et al., 2004). There are some feed additives that can alleviate the effect of high ambient temperature on the productivity and egg quality characteristics of laying hens. Several reports have confirmed that dietary supplementation of probiotics and prebiotics could reduce heat intensity on birds (Männer and Wang, 1991; Zulkifli et al., 2000). It has been shown that probiotic addition is more beneficial during stressful conditions (Jin et al., 1997).

Probiotics and prebiotics addition to the diet of laying hens have been found to improve egg production, egg weight, shell quality and feed conversion ratio (Abdelqader et al., 2013a; Youssef et al., 2013; Chung et al., 2015; Abdelqader, 2017) due to diminution of heat stress in birds (Männer and Wang, 1991; Zulkifli et al., 2000). Deng et al. (2012) have reported that *Bacillus licheniformis* addition to the diet of heat stressed laying hens increased egg production and feed intake. *Bacillus subtilis* and inulin individually or in combination have been found to significantly improve egg production and egg shell quality (Abdelqader et al., 2013b).

This work aimed to evaluate the effects of adding probiotic (Bio-plus2B<sup>®</sup>) and prebiotic (Techno Mos<sup>®</sup>) individually or together (Synbiotic) on egg production, egg quality, shell quality and retention of nutrients in Brown Nick laying hens in summer season in Egypt.

## MATERIALS AND METHODS

**Management of hens and the experimental rations:** The experiment was conducted in south Sinai experimental research station (Ras-Suder City) of the Desert Research Center, during the summer season (July-October 2018) in Egypt. Eighty H&N Brown Nick hens of 42 weeks of age were reared under summer conditions and were randomly allocated in 40 cages, each treatment had 10 cages. Each cage was allotted to 2 hens. The experimental treatments constituted as follows: C (control), T1 (Probiotic: 1kg Bio-plus2B<sup>®</sup> / ton feed), T2 (Prebiotic: 1kg Techno Mos<sup>®</sup> / ton feed), and T3 (Synbiotic: 1kg Bio-plus2B<sup>®</sup> +1kg Techno Mos<sup>®</sup> / ton feed). The hens were housed in wire cages of triple deck batteries. The birds were continuously exposed to 16 hours of light in a day. The experimental diet (Table 1) was formulated according to NRC (1994) and was iso-nitrogenous (18% CP) and iso-caloric (2800Kcal ME/Kg). Feed and fresh water were continuously available to the birds. The probiotic feed additive constituted, Bio plus 2B<sup>®</sup> (*Bacillus licheniformis* CH 200/DSM 5749 1.6 x10<sup>9</sup> CFU/g and *Bacillus subtilis* CH 201/DSM 5750 1.6 x10<sup>9</sup>). The prebiotic feed additive constituted, Techno Mos<sup>®</sup> (Mannan oligosaccharides and 1, 3 B-glucan that is derived from the cell wall of the yeast *Saccharomyces cerevisiae*).

Table1: Composition and calculated values of the experimental diet.

Ingredients	%	Ingredients	%	Calculated Values	
1. Yellow corn	58.05	7. Wheat bran	3.00	CP (%)	18.1
2. Soybean-meal 44%	20.50	8. Lysine	0.03	ME (Kcal/kg)	2808
3. Corn-gluten meal 60%	6.00	9. DL-Methionine	0.10	Calcium (%)	3.71
4. Sunflower oil	1.50	10. Salt	0.30	Avail P (%)	0.39
5. Limestone	8.67	11. Vit&MinPremix*	0.30	DL-Met (%)	0.42
6. Di-Calcium phosphate	1.55	<b>Total</b>	<b>100</b>	L-Lysine (%)	0.81

\*Vitamins and minerals premix: Each 2.5 kg contain: Vit A 1000000 IU, Vit D3 200000 IU, Vit E 10 g, Vit K 1000mg, Vit B12 10mg, Vit B1 1000mg, Vit B2 5000mg, Vit B6 1.5g, Pantothenic acid 10g, Niacin 30g, Folic acid 1g, Biotin 50mg, Iron 30g, Manganese 70g, Choline chloride 10g, Copper 4g, Zinc 50g, Selenium 100mg and Iodine 300mg.

**External climatic conditions:** The average of outdoor ambient temperature ( $29.1 \pm 2.9^\circ\text{C}$ ) and relative humidity ( $57.6 \pm 5.7\%$ ) were obtained from the Egyptian General Meteorological Authority (Table 2). A temperature-humidity index ( $30.1 \pm 3.0$ ) was calculated according to Zulovich and DeShazer (1990) with the following formula:  $\text{THI} = 0.60T_{\text{db}} + 0.40T_{\text{wb}}$ , where,  $T_{\text{db}}$  = dry-bulb temperature (maximum temperature),  $T_{\text{wb}}$  = wet-bulb temperature (minimum temperature)

Table 2: Outdoor ambient temperature ( $T^\circ\text{C}$ ), relative humidity (RH %) and temperature-humidity index (THI) during the experimental period.

Months	Temperature ( $^\circ\text{C}$ )			Relative Humidity (%)			THI
	Max	Min	Average	Max	Min	Average	
July	$35.8 \pm 7.5$	$24.9 \pm 5.2$	$30.5 \pm 6.4$	$79.8 \pm 16.6$	$29.2 \pm 6.1$	$55.1 \pm 11.5$	$31.4 \pm 6.5$
August	$35.3 \pm 6.3$	$25.8 \pm 4.6$	$30.1 \pm 5.4$	$82.1 \pm 14.7$	$33.3 \pm 6.0$	$58.9 \pm 10.6$	$31.5 \pm 5.7$
September	$33.9 \pm 6.2$	$22.3 \pm 4.1$	$28.8 \pm 5.3$	$80.0 \pm 14.6$	$29.6 \pm 5.4$	$57.1 \pm 10.4$	$29.3 \pm 5.3$
October	$30.3 \pm 7.1$	$22.3 \pm 5.3$	$25.9 \pm 6.1$	$77.1 \pm 18.2$	$37.7 \pm 8.9$	$59.4 \pm 14.0$	$27.1 \pm 6.4$
Mean ( $\mu$ )	$34.1 \pm 3.4$	$24.0 \pm 2.4$	$29.1 \pm 2.9$	$80.1 \pm 7.9$	$32.1 \pm 3.2$	$57.6 \pm 5.7$	$30.1 \pm 3.0$

Courtesy: Egyptian General Meteorological Authority

**Experimental measurements:** The individual live body weights of the hens were recorded at 42 and 56 weeks of age. The body weight changes were calculated as the difference between the initial and final body weights. Feed intake was recorded weekly. Egg mass (g/hen/day) was calculated by using egg weight and egg number. Feed conversion ratio (g feed intake/g egg mass) was calculated as the amount of feed consumed divided by egg mass.

Ten eggs were taken from each treatment at the end of the experiment for measuring egg quality traits (egg weight, egg shape index, egg albumen (%), egg yolk (%) and haugh unit) and shell quality traits (shell weight, shell percent, shell thickness, shell density, shell surface area and shell weight per unit of surface area (SWUSA). The percentages of albumen, yolk and shell were calculated as their weights relative to egg weight (Carter, 1968). Egg dimensions (mm) were measured by using a digital caliper to calculate the egg shape index (Panda, 1996).

Egg shape index = (Egg width / Egg length) x 100

Haugh unit was calculated according to Haugh (1937):

Haugh unit = 100 x log (H+7.57-1.7 x W<sup>0.37</sup>)

Where, H = Albumen height, W = Egg weight

Shell thickness (ST) was measured without membrane using micrometer.

Shell surface area (SA) and shell weight per unit of surface area (SWUSA) were calculated by the following equations (Nordstrom and Qusterhout, 1982).

SA (cm<sup>2</sup>) = 3.9782 × EW<sup>0.7056</sup>

SWUSA (mg/cm<sup>2</sup>) = SW (mg) /SA (cm<sup>2</sup>)

Where, 3.9782 = constant factor, EW = egg weight (g), SW= shell weight (g).

Shell density (SD) in g / cm<sup>3</sup> was estimated by the following equation (Curtis et al., 1985):

SD = SW (g) / SA (cm<sup>2</sup>) × ST (cm).

**Digestibility trial:** At the end of the experiment, the digestibility trial was undertaken to study the effect of adding probiotic, prebiotic and synbiotic on the retention of nutrients. Fresh excreta samples were taken every 24 h from 3 hens for each treatment during the last three consecutive days of the experiment. Feed intake was recorded. Feeds and manure samples were dried at 65°C till stabilization of weight and conserved for further analysis. The gross energy content of the feed and dried excreta was determined by completely combusting in a bomb calorimeter. The apparent metabolizable energy, AME (kcal/kg) was determined by the following equation (Nadeem et al., 2005).

AME (kcal / kg) = (GE intake - GE excretion) x 1000 / Feed intake (g)

Where, AME= Apparent Metabolizable Energy (kcal / kg).

GE= Gross energy (kcal)

**Statistical analysis:**The obtained data was statistically analysed by using General Linear Model (GLM) procedures (SAS program, 2002) using simple one-way analysis of variance according to the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where,  $\mu$  = General mean,  $T_i$  = Random effect of treatment ( $i = 1, 2, 3, 4$ ) and  $e_{ij}$  = Random error. Duncan (1955) test was conducted to test statistical differences among treatment means.

## RESULTS AND DISCUSSIONS

**Productive performance:** Non-significant ( $P > 0.05$ ) decrease was observed in egg production (%), egg mass (g) and feed consumption by the experimental treatment groups (T1, T2 and T3) compared to the control treatment (C), and are presented in Table 3.

**Table 3: Productive performance of laying hens (42-56 weeks of age) in Control (C), and Probiotic (T1), Prebiotic (T2) and Synbiotic (T3) treated groups.**

Item	C	T1	T2	T3	SE	Sig.
Hen-day egg production (%)	86.02	73.78	76.53	80.92	4.539	ns
Egg weight (g)	60.16	60.89	61.60	60.22	0.544	ns
Daily egg mass (g)	51.86	47.02	47.31	48.88	3.074	ns
Feed consumption (g/hen/day)	92.53	85.01	85.01	86.44	4.474	ns
Feed conversion ratio (FI/EM)	1.80	1.91	1.85	1.79	0.098	ns
Initial body weight (g)	1580	1578	1605	1561	23.05	ns
Final body weight (g)	1767.5	1835	1820	1772.5	33.65	ns
Body weight changes (g)	187.5	257	215	211.5	36.44	ns
SE = Stander error, Sig = Significant, ns = not significant						

The reduction in the previous parameters could be due to the variations in the gut microflora and the effect of the hot environmental conditions during the summer season. Miles et al. (1981) had investigated the effect of feeding *Lactobacillus acidophilus* culture on the productivity of laying hens under different climatic and geographical locations and found a significant increase in egg production at one location, a numerical improvement in the second location and no difference at the third location.

It is reported that the reduction of feed intake substantially contributes to impaired egg production during hyperthermia (Suk and Washburn, 1995). Moreover, the feed kept at room temperature (22 °C) began to show a decline in the microbial cell counts of the organisms and their viability after 5 days of storage (Armstrong et al., 2016). It is also reported that using 1g and 2g probiotic / kg feed have caused serious damages to the absorptive area of digestive system (Mahdavi et al., 2005).

There were non-significant differences among treatments for egg weight, feed conversion ratio, initial and final body weights, whereas body weight changes numerically increased (37.06 %) in probiotic treated group compared to the control group (Table 3).

There were also non-significant reduction in egg production, egg mass, and feed intake due to Bio-plus 2B addition (0.4, 1 and 2g / kg) in laying hens diet (Mahdavi et al., 2005). Likewise, the egg production was not affected by adding a product containing *Lactobacillus* and *Bacillus* (Davis and Anderson, 2002), *Lactobacillus* cultures (Kalavathy et al., 2009), a mixture of bacteria and fungi in laying hens diets (Balevi et al., 2001) and Bio-Mos in broiler breeder diet (Berry and Lui, 2000).

Probiotics in laying hens diet did not have significant effect on egg weight (Nahashon et al., 1996; Chen and Chen, 2003) and also probiotic did not affect egg production and egg mass, but the significant effect was observed on egg weight (Daneshyar et al., 2007). On the other hand, Nahashon et al. (1996) and Sheoran et al. (2017) found no significant effect on hen body weight and gain in response to dietary probiotic and prebiotic supplementation compared to the control. Likewise, Nahashon et al. (1994), Mohan et al. (1995), Salem et al. (2012), Elnagar (2013) and Fathi et al. (2018) showed that feed conversion ratio was not affected by probiotics.

Non-significant decrease in feed intake was observed in the groups fed a diet supplemented with *Bacillus subtilis* compared to the control group (Fathi et al., 2018). In contrast, egg production and feed intake were lower with the control group of laying hens, exposed to 34 °C than the other group that received 1kg *Bacillus licheniformis* / ton feed and exposed to 34°C, but there were non- significant differences between the control group and the group that received 0.1 kg *Bacillus licheniformis*/ton feed (Deng et al., 2012).

A significant decrease was observed in the egg production with *Saccharomyces cerevisiae* at the levels of 3 and 6g / kg diet (Elnagar, 2013). Dietary *Bacillus subtilis* (1 g / kg diet), inulin (1 g / kg diet) or their blend in laying hens diet significantly improved egg production, egg weight and egg mass compared with the control (Abdelqader et al., 2013a, Abdelqader et al., 2013b; Abdelqader, 2017). Likewise, the egg production was increased by the supplementation of *Bacillus licheniformis* and *Bacillus subtilis* (Kurtoglu et al., 2004) or using probiotic and mannan oligosaccharides in laying hens diets (Güçlü, 2011). A significant improvement in egg production was observed in hens fed a mixed culture of *Lactobacillus* (Tortuero and Fernández, 1995), *Lactobacillus acidophilus* (Haddadin et al., 1996), or *Bacillus subtilis* (Xu et al., 2006).

The laying hens were given diets containing *Lactobacillus* microorganisms and prebiotic feed supplement have been found to gain more body weight (Nahashon et al., 1994; Bozkurt et al., 2011). Moreover, body weight and weight gain in broilers were significantly increased by synbiotic treatments (Bio-plus 2B® plus Techno Mos®) compared to the control (Salem et al., 2012). On the other hand, the feed intake was increased by *Lactobacillus* culture in broilers, which were exposed to 36°C (Zulkifli et al., 2000). Also, *Saccharomyces cerevisiae* significantly decreased feed consumption (Elnagar, 2013). Several reports have confirmed that the inclusion of probiotic in the diet has been found to improve feed conversion ratio (Panda et al., 2008; Youssef et al., 2013; Chung et al., 2015).

These controversial results might be related to the dosage of probiotics and prebiotics, bacterial concentration and viability, species of probiotic, types of prebiotics, breed of birds, age of birds, the stage of production, climatic conditions, the degree of pollution and bacteria's tolerance of climatic conditions.

**Egg quality and Shell quality:** Egg quality and shell quality traits are depicted in Tables-4&5.

**Table 4: Egg quality of laying hens (42-56 weeks of age) in Control (C), and Probiotic (T1), Prebiotic (T2) and Synbiotic (T3) treated groups.**

Item	C	T1	T2	T3	SE	Sig.
Egg weight (g)	58.60	60.02	63.84	60.71	1.480	ns
Egg shape index	78.48	78.08	79.39	78.12	0.809	ns
Yolk (%)	27.36 <sup>b</sup>	28.66 <sup>ab</sup>	28.34 <sup>b</sup>	31.85 <sup>a</sup>	1.131	*
Yolk index	43.84	42.31	42.96	45.89	1.145	ns
Albumen (%)	58.63 <sup>a</sup>	57.20 <sup>ab</sup>	58.03 <sup>a</sup>	53.87 <sup>b</sup>	1.235	*
Yolk/Albumen ratio	47.19 <sup>c</sup>	50.26 <sup>b</sup>	49.46 <sup>b</sup>	60.45 <sup>a</sup>	3.394	*
Haugh unit	85.08 <sup>a</sup>	87.42 <sup>a</sup>	85.56 <sup>a</sup>	74.92 <sup>b</sup>	2.483	**

a,b,c: Means within the same row showing different letters are significantly different. SE=Stander error, Sig=Significant, ns=not significant, \* = (P<0.05), \*\* = (P<0.01).

**Table 5: Shell quality traits of laying hens (42-56 weeks of age) in Control (C), and Probiotic (T1), Prebiotic (T2) and Synbiotic (T3) treated groups.**

Item	C	T1	T2	T3	SE	Sig.
Shell weight (g)	6.74 <sup>b</sup>	6.74 <sup>b</sup>	7.27 <sup>a</sup>	7.34 <sup>a</sup>	0.147	*
Shell (%)	11.51	11.29	11.42	11.93	0.237	ns
Shell thickness (mm)	0.559	0.544	0.506	0.599	0.043	ns
Shell surface area (cm <sup>2</sup> )	70.32	71.46	74.65	72.08	1.227	ns
SWUSA (mg/cm <sup>2</sup> )	95.87	94.49	97.43	100.37	1.745	ns
Shell density (g/cm <sup>3</sup> )	0.183	0.183	0.198	0.175	0.013	ns

a,b: Means within the same row showing different letters are significantly different. SE=Stander error, Sig=Significant, ns=not significant, \* = (P<0.05).

There were non-significant ( $P>0.05$ ) differences among the treatments (control, probiotic, prebiotic and synbiotic) with regard to the egg weight, egg shape index and yolk index (Table 4). However, yolk (%) was significantly increased ( $P<0.05$ ) due to the synbiotic treatment (T3), whereas albumen (%) and haugh unit were significantly decreased ( $P<0.05$  and  $P<0.01$ , respectively) by the same treatment (T3). Yolk / Albumen ratio was significantly increased ( $P<0.05$ ) with all treatments compared with the control (Table 4). It decreases according to rise in storage temperature and storage time (Eisen et al., 1962; Samli et al., 2005) and is usually higher ( $P<0.05$ ) in summer than in spring season (Moula et al., 2003).

Shell quality (Table 5) between the treatment groups in respect of all traits were non-significant ( $P>0.05$ ) except a significant ( $P<0.05$ ) increase in shell weight in prebiotic (T2) and synbiotic (T3) treatment groups (Table 5). Similar results were reported earlier (Sheoran et al., 2017) in respect of shell weight, while egg shape index was not significantly affected in case of hens treated with prebiotic (mannan oligosaccharides). Likewise, dietary probiotic inclusion did not significantly affect the egg quality (Mahdavi et al., 2005; Mohebbifar et al., 2013).

Haugh unit is a measure of protein quality and freshness of egg (Rath et al., 2015). The eggs are graded in descending order based on its desirability as AA (72 or more), A (71-60), and B (59-31) by the United States Department of Agriculture (USDA) based on Haugh unit (Haugh, 1937). Scores of 90 and above are considered excellent, 70 is acceptable, and buyers generally reject eggs that score below 60.

Further, haugh unit and egg shape were not affected by dietary groups fed on *Bacillus subtilis* (Fathi et al. 2018). Also, the different levels of *saccharomyces cerevisiae* did not have a significant effect on haugh unit, albumen (%) and egg shape index, but yolk (%) was improved (Elnagar, 2013). On the contrary, 1 g *Bacillus subtilis* / kg diet exhibited the maximum increase in shell weight and shell thickness compared to the other treatments (Abdelqader et al., 2013a). Likewise, dietary supplementation with *Bacillus subtilis* at 400 ppm level (Fathi et al. 2018) and *saccharomyces cerevisiae* at the levels of 3g or 6g / kg diet (Elnagar, 2013) and at different levels of probiotics (*Lactobacillus fermentum*, *Bacillus* spp. and *Saccharomyces cerevisiae*) significantly increased shell thickness compared to non-treated laying hens (Sheoran et al., 2017).

The significant increase in shell weight with prebiotic (T2) and synbiotic (T3) treatments could be due to the low intestine pH which results from the generation of short chain fatty acids (SCFAs) and hydrogen peroxide by probiotic bacteria and the stimulating effect of the proliferation and maintenance of beneficial bacteria leading to the increase in SCFAs production. This helps to assimilate more calcium (Ashmead et al., 1985), since Ca and P salts require a very low pH to be solubilized and this acidic environment facilitates the ionization of minerals which is essential for absorption.

**Retention of nutrients:** The non-significant ( $P>0.05$ ) effect of probiotic, prebiotic and synbiotic on DM intake (g), DM excretion (g), DM retention (%), OM intake (g), OM excretion (g), OM retention (%), CP intake (g), CP excretion (g), CP retention (%), EE intake (g), EE excretion (g), and EE retention (%) are shown in Table 6.



Table 6: Intake (g), excretion (g) and retention (%) of nutrients of laying hens (42-56 weeks of age) in Control (C), Probiotic (T1), Prebiotic (T2) and Synbiotic (T3) groups.

Item	C	T1	T2	T3	SE	Sig.
DM intake (g)	79.18	97.54	99.46	97.95	11.51	ns
DM excretion (g)	29.62	35.62	32.89	35.91	4.19	ns
DM retention (%)	63.42	62.53	66.82	62.01	4.04	ns
OM intake (g)	62.12	76.53	78.04	76.85	9.03	ns
OM excretion (g)	21.62	27.20	25.77	25.10	2.86	ns
OM retention (%)	65.63	63.62	66.88	65.89	3.85	ns
CP intake (g)	14.17	17.47	17.80	17.50	2.06	ns
CP excretion (g)	8.20	10.20	10.47	10.17	1.33	ns
CP retention (%)	42.80	38.90	40.87	38.87	9.01	ns
EE intake (g)	2.61	3.22	3.28	3.23	0.38	ns
EE excretion (g)	0.99	1.74	1.15	1.28	0.34	ns
EE retention (%)	64.18	43.46	65.44	59.99	10.33	ns
SE=Stander error, Sig=Significant, ns=not significant						

Table 7: Utilization of gross energy (GE) and apparent metabolizable energy (AME) of laying hens (42-56 weeks of age) in Control (C), Probiotic (T1), Prebiotic (T2) and Synbiotic (T3) groups.

Item	C	T1	T2	T3	SE	Sig.
GE intake (kcal)	320.00	394.23	401.93	395.83	46.53	ns
GE excretion (kcal)	87.80	113.03	120.93	112.23	10.32	ns
GE retention (kcal)	232.17	281.20	281.00	283.60	41.58	ns
AME %	72.80	70.37	69.83	70.50	3.36	ns
AME (kcal/kg)	2942.20	2843.60	2821.00	2849.60	136.17	ns
GE= Gross energy, AME= Apparent metabolizable energy, SE=Stander error, Sig=Significant, ns=not significant.						

It is revealed that there were non-significant ( $P>0.05$ ) differences in gross energy intake (kcal), excretion (kcal) and retention (kcal), and apparent metabolizable energy, AME (% and kcal / kg) among the treatment groups (Table 7). However, the control treatment showed a numerical increase in the apparent metabolizable energy (% and kcal/kg) compared to the other treatments. This result followed the same results of feed intake and egg production. The beneficial protective probiotic function may have a nutrient and energy cost for the host because live microbes have nutrient requirements for their growth and proliferation. This could in turn explain the fact that in this study, a probiotic inclusion had no effect on energy metabolizability (Mikulski et al., 2012).

Similar results were observed earlier by Rodriguez et al. (2012) and Salem (2012) who reported that the effect of probiotic and / or prebiotic on nutrient utilization was not significant in comparison with the control. On the contrary, Park et al. (2016) found that *Enterococcus faecium* addition in ISA brown laying hens diets increased the retention of nutrients and decreased the excretion, leading to improve the nutrients digestibility. Likewise, the inclusion of *Mannan oligosaccharides* (MOS) into the diet of laying hens significantly increased the digestibility coefficient of DM, CP and ether extract (Jahanian and Ashnagar, 2015), while Chito-oligosaccharide improved DM and nitrogen digestibility (Meng et al., 2010).

**Weather conditions:** Table 2 showed the outdoor ambient temperature ( $T^{\circ}\text{C}$ ), relative humidity (RH %) and temperature-humidity index (THI  $^{\circ}\text{C}$ ) during the whole period of the experiment. The temperature-humidity index (THI) is used to predict and estimate the danger of hot climate. On the other hand, it is a useful way to evaluate the birds' productivity responses to the climatic changes. Dikmen and Hansen (2009) have indicated that THI is considered as a good measurement for the ambient environmental factors which affects the bird's body temperature. Purswell et al. (2012) have proved that birds' performance significantly decreased when THI exceeded approximately  $21^{\circ}\text{C}$ .

### CONCLUSION

The current research revealed that addition of Bio-plus2B<sup>®</sup> (probiotic) and Techno Mos<sup>®</sup> (prebiotic) individually or together (synbiotic) in diets of laying hens reared under hot climatic conditions did not significantly increase egg production (%), and improve feed conversion ratio, but significantly improved some egg quality traits and shell weight. We recommend that if the egg producers need to reach the maximum rate of egg production by using probiotic and prebiotic in laying hens diets during summer season, they have to preserve the feed with these products in low temperature place for keeping the viability and vitality of these bacteria at the maximum limit.

### REFERENCES

- Abdelqader, A et al. 2013a. Effects of dietary probiotic inclusion on performance, eggshell quality, cecal microflora composition, and tibia traits of laying hens in the late phase of production. *Tropical Animal Health and Production*, 45 (4), 1017-1024.
- Abdelqader, A et al. 2013b. Effects of dietary *Bacillus subtilis* and inulin supplementation on performance, eggshell quality, intestinal morphology and microflora composition of laying hens in the late phase of production. *Animal Feed Science and Technology*, 179 (1-4), 103–111.

- Abdelqader, A. 2017. Use of dietary probiotics to improve laying hen performance: Egg Innovations and Strategies for Improvements, Chapter: 27, pp. 283-295. DOI: 10.1016/B978-0-12-800879-9.00027-5.
- Armstrong, OD et al. 2016. Effect of different storage temperature on the viabilities change of probiotics in the fish feed. International Journal of Biochemistry and Biotechnology, 5 (4), 697-701.
- A.O.A.C. 2005. Official Methods of Analysis of AOAC International, 18<sup>th</sup> ed. AOAC, Gaithersburg, MD, USA.
- Ashmead, HD et al. 1985. Intestinal absorption of metal ions and chelates (Springfield, IL, Charles C. Thomas Publishers).
- Balevi, T et al. 2001. Effect of dietary probiotic on performance and humoral immune response in layer hens. British Poultry Science, 42, 456-461.
- Berry, W.D; Lui, P. 2000. Egg production, egg shell quality and bone parameters in broiler breeder hen receiving Bio-Mos<sup>®</sup> and egg shell 49. Poultry Science, 79 (Suppl.1), 124.
- Bozkurt, M et al. 2011. Performance of layer or broiler breeder hens varies in response to different probiotic preparations. Italian Journal of Animal Science, 10 (31), 162-169.
- Carter, T.C. 1968. The hen egg: A mathematical model with three parameters. British Poultry Science, 9, 165-171.
- Chen, Y.C; Chen, T.C. 2003. Effects of commercial probiotic or prebiotic supplementation on production, size and quality of hens egg. Poultry Science, 82 (Suppl. 1), 330 (Abstr).
- Chung, SH et al. 2015. Effects of multi strain probiotics on egg production and quality in laying hens fed diets containing food waste product. International Journal of Poultry Science, 14, 19-22.
- Curtis, PA et al. 1985. A comparison of selected quality characteristics of brown and white shell eggs. 1-Shell quality. Poultry Science, 64, 297-301.
- Daneshyar, M et al. 2007. The effect of probiotic supplementation on productive traits, egg quality and plasma cholesterol of broiler breeder hens. 16th European Symposium on Poultry Nutrition, p: 503-506.
- Davis, G.S; Anderson, K.E. 2002. The effects of feeding the direct-fed microbial, primalac, on growth parameters and egg production in Single Comb White Leghorn hens. Poultry Science, 81 (6), 755-759.
- Deng, W et al. 2012. The probiotic *Bacillus licheniformis* ameliorates heat stress-induced impairment of egg production, gut morphology and intestinal mucosal immunity in laying hens. Poultry Science, 91 (31), 577-582.
- Dikmen, S.; Hansen, P.J. 2009. Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environment? Journal of Dairy Science, 92 (1), 109-116.
- Duncan, D.B. 1955. Multiple range and multiple F-test. Biometrics, 11, 1-42.
- Eisen, EJ et al. 1962. The Haugh unit as a measure of egg albumen quality. Poultry Science, 41, 1461-1468.

- Elnagar, SHM. 2013. Effect of dried yeast (*Saccharomyces cerevisiae*) supplementation as feed additive to laying hen diet on egg production, egg quality, carcass traits and blood constituents. Egyptian Journal of Animal Production, 50 (2), 111-115.
- Fathi, M et al. 2018. Effects of dietary probiotic (*Bacillus subtilis*) supplementation on productive performance, immune response and egg quality characteristics in laying hens under high ambient temperature. Italian Journal of Animal Science, 17 (3), 804-814.
- Güçlü, B.K. 2011. Effects of probiotic and prebiotic (Mannan oligosaccharide) supplementation on performance, egg quality and hatchability in quail breeders. Ankara Üniv Vet FakDerg, 58, 27-32.
- Haddadin, MSY et al. 1996. The effects of *Lactobacillus acidophilus* on the production and chemical composition of hen's eggs. Poultry Science, 75 (4), 491-494.
- Haugh, R.R. 1937. The Haugh unit for measuring egg quality. U.S. Egg Poultry Magazine, 43, 522-555.
- Jahanian, R.; Ashnagar, M. 2015. Effect of dietary supplementation of mannan-oligosaccharides on performance, blood metabolites, ileal nutrient digestibility, and gut microflora in *Escherichia coli*-challenged laying hens. Poultry Science, 94 (9), 2165–2172.
- Jin, LZ et al. 1997. Probiotics in poultry: modes of action. World's Poultry Science Journal, 53 (4), 351-368.
- Kalavathy, R et al. 2009. Effects of *Lactobacillus* cultures on performance of laying hens, and total cholesterol, lipid and fatty acid composition of egg yolk. Journal of the Science of Food and Agriculture, 89 (3), 482-486.
- Kurtoglu, V et al. 2004. Effect of probiotic supplementation on laying hen diets on yield performance and serum and egg yolk cholesterol. Food Additives and Contaminants, 21 (9), 817–823.
- Lara, L.J.; Rostagno, M.H. 2013. Impact of heat stress on poultry production. Animals, 3 (2), 356–369.
- Li, X et al. 2007. Effect of fructooligosaccharides and antibiotics on laying performance of chickens and cholesterol content of egg yolk. British Poultry Science, 48 (2), 185-189.
- Lin, H et al. 2004. New approach of testing the effect of heat stress on egg shell quality: Mechanical and material properties of egg shell and membrane. British Poultry Science, 45, 476-482.
- Meng, QW et al. 2010. Effects of chito-oligosaccharide supplementation on egg production, nutrient digestibility, egg quality and blood profiles in laying hens. Asian Australian Journal of Animal Science, 23 (11), 1476-1481.
- Mahdavi, AH et al. 2005. Effect of probiotic supplements on egg quality and laying hen's performance. International Journal of Poultry Science, 4 (7), 488-492.
- Männer, K.; Wang, K. 1991. Effectiveness of zinc bacitracin on production traits and energy metabolism of heat-stressed hens compared with hens kept under moderate temperature. Poultry Science, 70 (10), 2139-2147.

- Mikulski, D et al. 2012. Effects of dietary probiotic (*Pediococcus acidilactici*) supplementation on performance, nutrient digestibility, egg traits, egg yolk cholesterol, and fatty acid profile in laying hens. *Poultry Science*, 91 (10), 2691–2700.
- Miles, RD et al. 1981. Effects of a living nonfreeze-dried *Lactobacillus acidophilus* culture on performance, egg quality, and gut microflora in commercial layers. *Poultry Science*, 60 (5), 993-1004.
- Mohan, B et al. 1995. Effect of probiotic supplementation on serum/yolk cholesterol and on egg shell thickness in layers. *British Poultry Science*, 36 (5), 799-803.
- Mohebbifar, A et al. 2013. Effects of commercial prebiotics and probiotics on performance of laying hens, egg traits and some blood parameters. *Annual Review & Research in Biology*, 3 (4), 921-934.
- Moula, N et al. 2003. Quality assessment of marketed eggs in Bassekabylie (Algeria). *Brazilian Journal of Poultry Science*, 15 (4), 395-400.
- Nadeem, MA et al. 2005. True metabolizable energy values of poultry feedstuffs in Pakistan. *International Journal of Agriculture & Biology*, 7 (6), 990-994.
- Nahashon, SN et al. 1994. Performance of Single Comb White Leghorn layers fed corn-soybean and barley-corn-soybean meal diets supplemented with a direct-fed microbial. *Poultry Science*, 73 (11), 1712-1723.
- Nahashon, SN et al. 1996. Performance of Single Comb White Leghorn fed a diet supplemented with a live microbial during the growth and egg laying phases. *Animal Feed Science and Technology*, 57 (1-2), 25-38.
- Nordstrom, J.O.; Qusterhout, L.E., 1982. Estimation of shell weight and shell thickness from egg specific gravity and egg weight. *Poultry Science*, 61 (10), 1991-1995.
- NRC, 1994. National Research Council. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.
- Panda, AK et al. 2008. Effect of probiotic (*Lactobacillus sporogenes*) feeding on egg production and quality, yolk cholesterol and humoral immune response of white leghorn layer breeders. *Journal of the Science of Food and Agriculture*, 88, 43-47.
- Panda, P.C. 1996. Shape and textbook on egg and poultry technology, 1<sup>st</sup> edition, New Delhi, India.
- Park, JW et al. 2016. Effect of dietary supplementation with a probiotic (*Enterococcus faecium*) on production performance, excreta microflora, ammonia emission, and nutrient utilization in ISA brown laying hens. *Poultry Science*, 95 (12), 2829-2835.
- Purswell, JL et al. 2012. Effect of temperature-humidity index on live performance in broiler chickens grown from 49 to 63 days of age. Ninth International Livestock Environment Symposium, Sponsored by ASABE, Valencia Conference Centre, Valencia, Spain
- Rath, PK et al. 2015. Evaluation of different egg quality traits and interpretation of their mode of inheritance in White Leghorns. *Veterinary World*, 8 (4), 449-452.

Rodriguez, ML et al. 2012. Wheat-and barley-based diets with or without additives influence broiler chicken performance, nutrient digestibility and intestinal microflora. Journal of the Science of Food and Agriculture, 92 (1), 184-190.

Salem M. Fayza. 2012. Comparative study of using probiotics and prebiotics in feeding broiler chicks reared in cages. PhD thesis in Poultry Nutrition, Department of Poultry Production, Faculty of Agriculture, Ain Shams University.

Salem, M. Fayza et al. 2012. Effects of probiotic and prebiotic supplementation on growth performance and blood metabolites in broiler chicks reared in batteries. Egyptian Journal of Nutrition and Feeds, 15 (2), 335-349.

Samli, HE et al. 2005. Effects of storage time and temperature on egg quality in old laying hens. Journal of Applied Poultry Research, 14, 548-533.

SAS 2002. Statistical analysis systems user's guide: Version 8.0. Cary: SAS Institute.

Sheoran, N et al. 2017. Effect of dietary inclusion of probiotics and prebiotics on external egg quality traits in White Leghorn layers. The pharma Innovation Journal, 6 (11), 8-13.

Suk, Y.O.; Washburn, K.W. 1995. Effects of environment on growth, efficiency of feed utilization, carcass fatness and their association. Poultry Science, 74 (2), 285-296.

Xu, CL et al. 2006. Effect of a dried *Bacillus subtilis* culture on egg quality. Poultry Science, 85 (2), 364-368.

Youssef, AW et al. 2013. Effect of probiotics, prebiotic and organic acids on layer performance and egg quality. Asian Journal of Poultry Science, 7, 65–74.

Zulkifli, I et al. 2000. Growth performance and immune response of two commercial broiler strains fed diets containing *Lactobacillus* cultures and oxytetracycline under heat stress conditions. British Poultry Science, 41 (5), 593–597.

Zulovich, J.M.; DeShazer, J.A. 1990. Estimating egg production declines at high environmental temperatures and humidity. ASAE Paper No. 90-4021, American Society of Agricultural Engineers, St. Joseph, MI, USA.

### UNDERTAKING

It is certified that the research paper '**THE IMPACT OF PROBIOTIC AND PREBIOTIC ON EGG PRODUCTION, EGG QUALITY AND RETENTION OF NUTRIENTS IN LAYING HENS REARED UNDER DESERT CONDITIONS IN EGYPT**' is an original research work carried out by the authors in Animal and Poultry Nutrition Department, Desert Research Center, El-Mataria, Cairo, Egypt. It has neither been published nor contemplated for publication elsewhere.

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