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Effect of Probiotics, Prebiotics and Organic Acids on Layer Performance and Egg Quality

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ABSTRACT
This study was conducted to evaluate the effect of various commercial feed additives on performance and egg quality of laying hens. These additives included probiotics (Protexin® and Clostat®), symbiotic (Diamond®) and organic acids (Galliacid®). A number of 180 HL Brown hens (27 wks of age) were divided into 5 treatment groups (6 replicates of 6 birds, each). Groups were assigned to 5 experimental diets: a basal diet of no additive (control), the basal diet supplemented with either 0.01% Protexin®, 0.05% Clostat®, 0.06% Diamond® or 0.06% Galliacid®. Data of layer performance and egg quality were obtained during 12 weeks experimental period. Supplementation of probiotics or symbiotic recorded higher ($p>0.05$) egg production than the control but organic acids supplementation significantly ($p<0.05$) increased egg production by 9.94%. Egg weight slightly improved ($p>0.05$) by dietary treatments. Supplementation of probiotics, symbiotic and organic acids significantly ($p<0.01$) increased egg mass. The best egg mass value was recorded for birds fed diet supplemented with organic acids. Feed conversion ratio improved ($p>0.05$) by dietary treatments. Adding probiotics, symbiotic or organic acids did not significantly affect shape index, yolk index, yolk %, SWUSA, Haugh unit or specific gravity. Addition of probiotics or organic acids showed significant ($p<0.05$) increase in shell thickness and yolk color. It could be concluded that these additives caused improvement in performance and egg quality of laying hen. More studies are needed to explain the effects of different sources and levels of these additives on performance and egg quality of laying hens.

Key words: Layers, feed additives, performance, egg quality

INTRODUCTION
Different feed additives such as probiotics, prebiotics, symbiotic and organic acids had been introduced as alternative feeding strategies to enhance productive performance of laying hens. Probiotics are naturally occurring microorganisms include bacteria, fungi or yeast. Different bacterial strains such as. *Lactobacillus, Enterococcus, Pediococcus* and *Bacillus* are used to prepare the commercial probiotics. The non-digestible feeds that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number, of bacteria in the colon are defined as prebiotics (Gibson and Roberfroid, 1995). Symbiotic or synbiotic is the combination of probiotics and prebiotics (Awad *et al.*, 2009; Oviedo-Rondon, 2009). In this composition, probiotics bacteria are produced and mixed with a substrate that serves as its protector and feeder, improving the survival and establishment of beneficial bacteria in the digestive tract (Trachoo *et al.*, 2008). The interaction between probiotics and prebiotics can favor the probiotics adaptation to the

prebiotics substrate and enhancing the effect of both. Organic Acids (OA) including lactic, citric, formic and fumaric acids or there different salts are known to control harmful microorganisms in digestive and respiratory organs of poultry (Park *et al.*, 2009). The most important role of organic acids is to reduce pH in the stomach and gut (Yesilbag and Colpan, 2006) and improve some immune responses of birds (Abdel-Fattah *et al.*, 2008).

Addition of probiotics to layer diets has been found to improve performance and some egg quality traits (Abdulrahim *et al.,* 1996; Jin *et al.*, 1997; Kurtoglu *et al.*, 2004; Li *et al.*, 2006). Significant increase on egg mass in ISA-Brown and Leghorn laying hens fed diet included probiotics had been reported by Panda *et al.* (2003) and Yoruk *et al.* (2004). On the other hand, the results of Mutus *et al.* (2006), Ramasamy *et al.* (2009) and Zarei *et al.* (2011) showed that inclusion of probiotics had no significant effect on egg production and egg mass.

Supplementing live yeast, as a probiotics, improved egg production percentage of laying hens (Kim *et al.*, 2002; Katoch *et al.*, 2003), egg weight and egg shell breaking strength (Park *et al.*, 2002), nutrients utilization (Soliman, 2003) and improved feed intake and feed conversion ratio (Kabir, 2009).

A positive effect of the prebiotics on some eggshell quality parameters in laying hens had been reported by Swiatkiewicz *et al.* (2010). This is probably connected with the stimulation of mineral availability. Scholz-Ahrens *et al.* (2007) reported that the mechanism of the positive effect of prebiotics on mineral utilization can be attributed to the high solubility of minerals because of the increased production of short-chain fatty acids.

Organic acids improved performance and eggshell quality of layers and old broiler breeders (Park *et al.*, 2002; Yesilbag and Colpan, 2006; Sengor *et al.*, 2007; Soltan, 2008). However, the results of using such different feed additives in layer diets are inconsistent.

Therefore, the objective of this study was to further investigate the effect of adding commercial products of probiotic, symbiotic (pre/probiotic) and organic acids to laying hen diets on performance and egg quality.

MATERIALS AND METHODS

Birds, diets and management: A total number of one hundred and eighty 27 week old HL Brown layers were randomly distributed into 5 groups of 36 layers each (6 replicates of 6 layers each) and randomly allocated in batteries of two tire system. Each of the 5 groups was randomly assigned to be fed 1 of 5 experimental diets. The diets were formulated to meet the nutrient requirements of laying hens according to the NRC (1994) recommendation. Two commercial products of probiotics (Protexin®, Clostat®), one commercial product of symbiotic (Diamond®) and one commercial mixture of organic acids (Galliacid®) were used in this study. Protexin® is a commercial probiotics preparation containing a mixture of microorganisms, produced by Novartis limited, International, UK. Clostat® is a commercial probiotic preparation containing a unique strain of *Bacillus subtilis* that has antimicrobial property produced by Kemin Industries, Ink. USA. Diamond® is a symbiotic containing a fermentation metabolites of Direct-fed yeast cells, *Saccharomyces cerevisiae*, produced by Diamond V Mills. Inc. Yeast grown on a media of processed grain by-products, roughage products, cane molasses, malt and corn syrup. Galliacid® consists of a mixture of fumaric acid, calcium formate, calcium propionate, potassium sorbate and hydrogenated vegetable oil, produced by Jefo Company, Saint- Hyacinthe Canada. These organic acids are coated and protected (microencapsulated) by a matrix of fatty acids.

Table 1: Formulation and nutrient composition of the basal diet

Ingredients	%
Yellow corn	63.00
Soybean meal (44%)	16.00
Wheat bran	3.00
Protein concentrate*	10.00
Limestone	7.00
Bone	1.00
Total	100.00
Calculated composition %**	
Crude protein	17.25
ME (kcal kg^{-1})	2770.00
Lysine	0.94
Methionine	0.38
Methionine and cystine	0.68
Calcium	3.60
Available P	0.40

*Protein concentrate contains: 45% CP, 1.80% CF, 1.63% EE, 5.84% Ca, 2.92% AP, 1.35% Methionine, 2.11% Methionine and Cystine, 2.70% Lysine, 1.27% sodium, Metabolisable energy 2656 kcal kg^{-1} and each 1 kg of this concentrate contains: Vit A, 100000 I.U; Vit D_3, 33000 I.U; Vit E, 100 mg; Vit K_3, 25 mg; Vit B_1, 10 mg; Vit B_2, 50 mg; Vit B_6, 15 mg; Vit B_{12}, 200 µg; Niacin, 400 mg; Pantothenic acid, 100 mg; Folic acid, 10 mg; Choline chloride, 8323 mg; Biotin, 500 µg; Copper, 50 mg; Iodine, 3 mg; Iron, 450 mg; Manganese, 800 mg; Zinc, 600 mg and Selenium, 1 mg; Cobalt , 1 mg; Antioxidant, 7.5 mg. **Calculated based on feed composition Tables of NRC (1994)

The formulation and nutrient composition of the basal diet are shown in Table 1. This diet was fed either with no additives (control diet) or supplemented with the studied additives as follows: 0.01% Protexin, 0.05% Clostat, 0.06% Diamond or 0.06% Galliacid. Such levels of supplementation were adopted according to the recommended levels of the producers. Birds were fed to the experimental diets for a 12 week period, beginning at the age of 27 week and continuing to 39 week old. During the experimental period feed and water were allowed for *ad libitum* consumption. A photoperiod of 16 h was maintained throughout the experimental period.

Sample collection and analytical procedure

Performance: Hens were weighed individually at the beginning and at the end of the experiment and change on their body weight were calculated. During the experimental period egg number and egg weight were recorded daily for 84 days. Egg mass was calculated as average egg weight multiplying by egg number. Egg production (%) was calculated as total egg number/the number of laying hens. Feed consumption was recorded and feed conversion ratio (total feed intake/total egg mass) was calculated every 14 day intervals.

Egg quality: Egg quality traits were measured according to Stino *et al.* (1982) and El-Wardany *et al.* (1994). These included shape index (egg width/egg length×100) using vernier caliper, egg shell thickness measured by membrane in mm, yolk weight (to the nearest 0.1 g), yolk % (yolk weight/egg weight×100), yolk index (yolk height/yolk diameter×100) and yolk color (using Roche yolk color fan). Haugh unit was calculated using the equation:

$$\text{Haugh unit} = 100 \log (H+7.57-1.7\ W^{0.37})$$

where, H is albumen height, W is egg weight (Haugh, 1937). Shell Weight per Unit of Surface Area (SWUSA) was calculated by dividing shell weight (plus adhering membranes "mg") by egg surface area (ESA in cm^2). ESA was calculated according to Carter (1975) using the following equation:

$$ESA = 3.9782 \times \text{egg weight}^{0.7056}\ (g)$$

Specific gravity of eggs was determined using the saline flotation method of Hempe *et al.* (1998). Salt solutions were made in incremental concentrations of 0.005 in the range from 0.1065-0.1120 g L^{-1}.

Statistical analysis: Data were statistically analyzed for analysis of variance (one way) using the General Liner Model of SAS (2000). Significant differences among treatment means were tested by Duncan's multiple rang test (Duncan, 1955).

RESULTS AND DISCUSSION

Performance of laying hen: The effects of dietary treatments on laying performance measured as egg production % (eggs/hen/day), egg weight (g), egg mass (g/hen/day), feed intake g/hen/day, feed conversion ratio (g feed/g egg) and Body weight change (g) are shown in Table 2.

Supplementation of probiotics and symbiotic caused higher egg production than control, but these differences were not significant statistically ($p>0.05$) while, organic acids supplementation (Galliacid) significantly ($p<0.05$) increased egg production from 88.5 to 97.3 (9.94%). Egg weight slightly improved ($p>0.05$) by dietary treatments. Supplementation of probiotics (Protexin and Clostat) improved egg weight by 8.2 and 6.11%, respectively. Organic acids Supplementation improved egg weight by 9.08%. Supplementation of probiotics, symbiotic and organic acids significantly ($p<0.01$) increased egg mass compared with control. The best egg mass were recorded with birds fed diet supplemented organic acids. The improving of egg mass were 14.18% with organic acids and about 6.61% with probiotics and symbiotic supplementation.

Feed intake was not affected by dietary treatments. Feed conversion ratio improved by dietary treatments being from 1.98 to 1.81, but these improvements were not significant ($p>0.05$).

Table 2: Effect of dietary treatments on performance of laying hens

Dietary treatments	Egg production, % eggs/hen/day	Egg weight (g)	Egg mass (g/hen/day)	Feed intake (g/hen/day)	Feed conversion ratio (kg feed/kg egg)	Body weight change+(g)
Control	88.50^b	57.30	52.90^c	105	1.98	172^{cd}
Probiotic (Protexin®)	91.90^b	62.00	56.20^b	107	1.90	312^a
Probiotic (Clostat®)	92.30^b	60.80	56.60^b	105	1.85	203^{bc}
Symbiotic (Dimound®)	91.80^b	59.60	56.40^b	103	1.83	117^d
Organic acids (Galliacid®)	97.30^a	62.50	60.40^a	109	1.81	239^b
Means	92.40	60.40	56.50	105	1.87	209
SE of means	±0.95	±0.85	±0.74	±1.13	±0.02	±18.72
Significances	*	ns	**	ns	ns	***

Means within each column with no common superscript are significantly different ($p<0.05$). Ns: Not significant *$p<0.05$ **$p<0.01$ ***$p<0.001$

The results showed that dietary treatments caused significant change ($p<0.05$) in body weight. Supplementation of probiotics and organic acids increased ($p<0.05$) body weight compared with control while, symbiotic supplementation was of no significant ($p>0.05$) effect on body weight.

Probiotics inclusion did not significantly influence egg weight. This result agreed with those reported by Mohan *et al.* (1995), Haddadin *et al.* (1996) and Chen and Chen (2004). Balevi *et al.* (2001) fed commercial multi strain probiotic to 40 week old layers and showed no significant differences in egg production and egg weight compared to the control. They stated that the difference between their results and previous works may be related to differences in the ages of the hens. Ramasamy *et al.* (2009) reported that inclusion of probiotic had no significant effect on egg production and egg mass. Also, other reports disagreed with the present results (Nahashon *et al.*, 1992; Tortuero and Fernandez, 1995) which might be related to the strain of bacteria, concentration and the form of bacteria used (viability, dryness or their products). Yoruk *et al.* (2004) and Panda *et al.* (2003) reported significant increase of produced egg mass in ISA-Brown and Leghorn laying hens fed diet included probiotic during the whole laying period. However, the results of Mutus *et al.* (2006), Ramasamy *et al.* (2009) and Zarei *et al.* (2011) showed that inclusion of probiotics had no significant effect on egg production and egg mass.

In the present study, the short period of the experiment may be a factor affects detecting clear effect of probiotics. Kurtoglu *et al.* (2004) showed that probiotics effect on egg production was not specific until day 60, but significant increase in egg production by probiotic supplementation were seen on days 60-90 of their experiment.

Adding symbiotic did not significantly affect egg production percentage which has been reported by Swiatkiewicz *et al.* (2010). Health of the gut is one of the major factors governing the performance of the birds (Paul *et al.*, 2007) and the profile of intestinal microflora plays an important role in gut health (Dhawale, 2005).

A positive effect of the prebiotics on some eggshell quality parameters had been reported by Swiatkiewicz *et al.* (2010). This is probably connected with the stimulation of mineral availability. On the other hand, the mechanism of the positive effect of prebiotics on mineral utilization can be attributed to the high solubility of minerals because of the increased production of short-chain fatty acids (Scholz-Ahrens *et al.*, 2007).

Dietary organic acids and their salts are able to inhibit microorganism growth in feed and consequently to preserve the microbial balance in the gastrointestinal tract, in addition by modifying intestinal pH. Organic acids also, improve the solubility of feed ingredients, digestion and absorption of nutrients (Vogt *et al.*, 1981; Patten and Waldroup, 1988; Owings *et al.*, 1990; Skinner *et al.*, 1991; Adams, 1999). The results of increasing egg production percentage due to adding organic acids is in agreement with those of Soltan (2008) who found that organic acid supplementation increased egg production by about 5.77% compared to untreated group. On the other hand, organic acids had no significant effect on egg weight. This finding agreed with those obtained by Yesilbag and Colpan (2006) and Soltan (2008).

Adding probiotics, symbiotic or organic acids significantly ($p<0.05$) increased egg mass. Soltan (2008) found that organic acid supplementation significantly improved egg mass by about 2.29 and 6.67% with 260 and 780 ppm of organic acid mixture, respectively. Hassanein and Soliman (2010) also found that adding live yeast (probiotics) to laying diets increased egg production percentage and egg mass paralleled to those of egg production. Moreover, Kizerwetter-Swida and Binek (2009) reported that probiotics have reduced the incidence and duration of poultry diseases. Apata (2008) and Kabir (2009) explained the mode of action of probiotics in poultry includes as maintaining normal intestinal microflora by competitive exclusion and antagonism, altering metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production, improving feed intake and digestion and stimulating the immune system.

Egg quality: Table 3 shows the effect of the different additives on egg quality measurements. Adding probiotic, symbiotic or organic acids did not significantly affect shape index, yolk index, yolk %, SWUSA, Haugh unit or specific gravity. However, such additives significantly ($p<0.05$) improved egg shell thickness. Shell thickness in mm recorded the lowest value being 0.32 for the control compared to a narrow range from 0.36 to 0.38 mm for the supplemented diets. These results are in agreement with those of Yalcin *et al.* (2008) who found that supplemental yeast culture had no effect on egg shape index, yolk index, yolk weight and Haugh units. The improvement in shell thickness may be a consequence of the increased mineral and protein absorption. This phenomenon of increased absorption reflected on increasing calcium and protein deposition of the shell and contributes to improve the quality which may results in reduced breaking of the shells. The same results were obtained by Soltan (2008) who reported that higher inclusion level of organic acids (780 ppm) improved egg shell quality. Also, Li *et al.* (2006) found that adding dried *Bacillus subtilis* culture increase egg shell thickness. On this regard, Panda *et al.* (2008) explained that egg shell quality improvement is under the influence of the intestinal Ca^{2+} absorption rate improvement, phenomenon facilitated by the presence within feed of some fodder additives like prebiotics. This beneficial effect on eggshell quality due to probiotic feeding may be attributed to a favorable environment in the intestinal tract by feeding of *L. sporogenes* which might have helped to assimilate more calcium which was evident by increased concentration of Ca in serum. Swiatkiewicz *et al.* (2010) found that selected feed additives which lower the pH of the diet and intestinal content can beneficially influence egg shell quality in older high producing laying hens.

The mode of action of organic acids was explained by (Park *et al.*, 2009) as low gastric pH accelerates the conversion of pepsinogen to pepsin which enhances the absorption rate of proteins and minerals. Additionally, organic acids are able to inhibit microorganism growth in the feed and as a result of this function they preserve the microbial balance in the gastrointestinal tract (Yesilbag and Colpan, 2006).

The obtained results showed also that adding probiotics, symbiotic or organic acids significantly ($p<0.05$) increased yolk color. These results are in agreement with Li *et al.* (2006) who found that adding dried *Bacillus subtilis* culture increase yolk color. In contrast, Wang *et al.* (2009) found that using phenyl acetic acid did not significantly affect yolk color.

Table 3: Effect of dietary treatments on egg quality of laying hens

Dietary treatments	Shape index	Shell thickness (mm)	Yolk color	Yolk index	Yolk (%)	SWUSA (mg cm^{-2})	Haugh unit	Specific gravity
Control	76.70	0.32^{c}	7.00^{b}	42.10	22.20	0.10	83.20	1.09
Probiotic (Protexin®)	78.00	0.38^{a}	7.40^{ab}	44.00	22.70	0.10	84.40	1.09
Probiotic (Clostat®)	78.40	0.37^{ab}	8.00^{a}	45.40	24.80	0.10	80.60	1.09
Symbiotic (Dimound®)	78.50	0.37^{ab}	7.80^{a}	44.00	22.80	0.11	90.80	1.09
Organic acids (Galliacid®)	77.80	0.36^{b}	7.80^{a}	43.30	22.90	0.10	83.60	1.10
Means	77.90	0.35	7.60	43.80	23.10	0.1o	84.60	1.09
SE of means	±0.55	±0.01	±0.12	±0.43	±0.34	±0.001	±1.32	±0.001
Significances	ns	*	*	ns	ns	ns	ns	ns

$^{a-d}$Means within each column with no common superscript are significantly different ($p<0.05$). Ns: Not significant *$p<0.05$

The results of the present study did not support the evidence suggests that symbiotics are more effective than either probiotics or prebiotics alone and that a mixture of probiotic strains may be more effective than the individual strains (Timmerman *et al.*, 2004).

The difference among the obtained results and previous works may be related to many factors. On the reported literature, responses to probiotics, prebiotics or organic acids supplementation are inconsistent. This led to abundant investigations on possible factors that could influence the responses to these additives. In general these additives have proved to be most effective under conditions of stress, possibly the presence of un-favorable organisms, extremes in ambient temperature, diseases, crowding and poor management. In commercial layers production one or more of these conditions are invariably present. Further possible causes of variations in response to probiotics, prebiotics and/or symbiotic supplementation in layers could be differences between strains, hybrids, age, plane of nutrition, nutrient composition of the diet and microbial population of gastrointestinal tract. Levels of inclusion of probiotics, prebiotics and/or synbiotic in the diet, duration of supplementation or other environmental conditions may be effective factors on variation response to such additives (Zarei *et al.*, 2011).

CONCLUSION

It could be concluded that adding probiotics, prebiotics, symbiotic or organic acids improved significantly egg production, egg mass and egg quality (egg shell thickness and yolk color). In the light of these findings, it is thought that these additives may be economically beneficial when used in laying hens. The superiority of organic acids (Galliacid®) in egg production and egg mass among the tested products had been proven. However, further investigations are needed for evaluating different sources and levels on performance and egg quality. Furthermore, since such supplementation did enhance egg production and egg quality traits especially shell thickness, studies on parents considering the effects upon hatchability are recommended.

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