

EFFECT OF DIETARY *LACTOBACILLUS* SUPPLEMENTATION ON PERFORMANCE OF LAYING HENS UNDER EGYPTIAN SUMMER CONDITIONS

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ABSTRACT

A 8-wk study was conducted to evaluate the effects of supplementing laying hen diets with commercially produced *Lactobacillus acidophilus* (Micro-Bac LA) on productive performance, egg quality and some blood constituents under Egyptian summer conditions. One hundred and thirty five Hy-Line® Brown pullets (48wk of age) were randomly allocated to one of five levels of *Lactobacillus acidophilus* (LA) supplementation (0, 100, 200, 300 and 400 mg/Kg diet). The mean cell count in the *Lactobacillus* preparation was 8×10^8 cfu/g. The group fed a basal diet without LA supplementation was considered as control group. Egg number, egg mass, feed intake and feed conversion ratio were significantly ($P < 0.05$) improved by about 7.3, 9.3, 10.9 and 11.0%; 9.9, 10.2, 11.9 and 13.2%; 3.6, 3.3, 3.6 and 2.2% and 5.5, 6.3, 7.7 and 9.7% in groups fed diets supplemented with 100, 200, 300, and 400 mg LA/Kg diet, respectively, compared to control group. Egg weight of hens fed supplemented diets was increased during the experimental period (48 to 56 wks of age) however, the significant effect was observed only in groups fed 100 and 400 mg LA/Kg diet. *Lactobacillus* supplementation significantly increased albumen quality, shell thickness and breaking strength at 52 and 56 wks of age. On the other hand, there were no significant differences among treated groups for albumin, yolk and shell percentages. Albumen height was significantly ($P < 0.05$) increased at 52 wks of age by LA supplementation.

Plasma Ca, total protein and globulin were significantly ($P < 0.05$) increased in groups fed treated diets compared with control group. *Lactobacillus* supplementation significantly ($p < 0.05$) decreased plasma phosphorus and A/G ratio. In contrast, plasma albumin was not significantly affected by feeding LA-supplemented diets. Both plasma and yolk cholesterol were significantly decreased with LA supplementation. Plasma cholesterol was reduced by about 9.9, 14.5, 17.8 and 21.7% and 18.3, 17.4, 24.4 and 29.9% and yolk cholesterol by about 16.5, 37.6, 40.9 and 43.2% and 26.2, 40.4, 36.9 and 41.5% for groups fed 100, 200, 300, and 400 mg LA/Kg diet at 52 and 56 wk of age, respectively.

In conclusion, these results suggested that *Lactobacillus* supplementation to laying hen diets had a beneficial effect on productive performance, egg quality under Egyptian summer conditions. In addition, *Lactobacillus* supplementation reduced yolk cholesterol and consequently, reducing the dietary intake of cholesterol by humans.

Keywords: *Lactobacillus*, ambient temperature, productive performance, egg quality, blood constituents, laying hen.

INTRODUCTION

Poultry are exposed to various factors of stress. Under Egyptian conditions, high ambient temperature is the major stress factor during summer season. High ambient temperatures have deleterious effects on growth and production of poultry. In laying hens, heat stress depresses egg production (Marsden *et al.*, 1987), egg weight (Peguri and Coon, 1991) and

shell quality (Balnave and Muheereza, 1997). This negative effect of heat stress is primarily due to reduced feed intake (Savory, 1986). Moreover, stress factors tend to create an imbalance in the intestinal microflora and lowering of body defense mechanisms (Jin *et al.*, 1997). Under this condition, antimicrobial feed additives such as antibiotics are often used to inhibit or eliminate harmful organisms in the intestine, and to improve growth and feed efficiency. Recently, there is international interest to use safety feed additives as substitute for antibiotics where, the continuous using of antibiotics in poultry feeds may result in the presence of antibiotic residues in poultry products and the development of drug-resistant bacteria in humans.

Probiotics have been introducing as an alternative to antibiotics. The term probiotic was intended to describe any microbial feed additive containing living *Lactobacillus acidophilus* (Parker, 1974). Now there are many types of probiotic preparations in the market. The modes of action of probiotics in poultry include: (1) maintaining a beneficial microbial population in the alimentary tract (Fuller, 1989); (2) improving feed intake and digestion (Nahashon *et al.*, 1993), and (3) altering bacterial metabolism (Cole *et al.*, 1987, Jin *et al.*, 1997).

Several studies have shown that the addition of probiotics to the diets of broilers and layers leads to improved performance (Jernigan *et al.*, 1985; Barrow, 1992; Jin *et al.*, 1997; Kalavathy *et al.*, 2003). Recently, considerable attention has also been paid to the potential of probiotics in altering lipid metabolism. This interest stems from the growing evidence of probiotics in reducing cholesterol concentrations in yolk (Mohan *et al.*, 1995; Abdulrahim *et al.*, 1996; Haddadin *et al.*, 1996) and serum in chicken (Mohan *et al.*, 1996; Jin *et al.*, 1998; Kalavathy *et al.*, 2003).

The objective of the present study was to investigate the effect of *Lactobacillus acidophilus* supplementation to laying hen diets on productive performance, egg quality and some blood constituents under Egyptian summer conditions.

MATERIALS AND METHODS

The present study was carried out at the Poultry Breeding Farm, Poultry Production Department, Faculty of Agriculture, Ain Shams University. This experiment was run during summer season. The averages ambient temperatures (Min. & Max.) recorded during the experiment period were 22.6 to 33.8 °C, respectively.

One hundred and thirty five 48 wks old of Hy-Line[®]Brown hens were maintained in layer batteries with three decks of five cages each until 56 wks of age. Birds were individually weighed and distributed randomly into five experimental groups of 27 hens with nine replicates (cages) of three hens each. A commercial product of *Lactobacillus acidophilus* (Micro-Bac LA[®]) produce by Pro-Byn, USA, was used in this experiment. The mean cell count of *L.acidophilus* in this product was 8×10^8 cfu /g. The first group was fed a basal diet containing 18% CP, 2775 Kcal ME/Kg without *L.acidophilus* supplementation (Table 1) and considered as a control group.

Table 1: Percentage composition of the basal diet

Ingredients	%
Ground yellow corn	61.80
Soybean meal	19.30
Corn gluten meal	2.90
Decorticated cottonseed meal	2.00
Corn gluten feed	4.00
Bone meal	1.80
Limestone	7.42
Salt	0.32
Vitamin and mineral premix*	0.40
DL-Methionine	0.04
L-Lysine	0.02
Total	100
Calculated Composition (% except energy)	
ME, Kcal /Kg	2775
Crude protein	8.00
Crude fat	2.90
Crude fiber	2.80
Calcium	3.75
Available phosphorus	0.40

*Each 2.5kg of vitamins and minerals premix contain: Vit. A, 12 mIU; vit. D₃, 4 mIU; vit. E, 15 g; vit. K, 2 g; vit. B₁, 1 g; vit. B₂, 8 g; vit. B₆, 2 g; vit. B₁₂, 10 mg; nicotinic acid, 30 g; Pantothenic acid, 10 g; Folic acid, 1 g; Biotin, 150 mg; Choline chloride, 40 mg; Copper, 5 g; Iron, 15 g; Manganese, 70 g; Zinc, 60 g and Selenium, 0.15 g.

L.acidophilus (LA) was mixed uniformly with control diet at levels of 100, 200, 300 and 400 mg/Kg diet for the 2nd, 3rd, 4th and 5th groups, respectively. Birds were subjected to 16L: 8D regime with free access to feed and water.

Body weight and feed intake was recorded every 28 days and the average feed intake per hen was then calculated. The Produced eggs per replicate cage were daily weighed and average egg mass per hen was then calculated at 52 and 56 wks of age. Feed conversion was calculated (g of feed intake/g of egg mass).

At 52 and 56 wks of age, two eggs from each replicate cage (18 from each treatment) were taken to determine the different measurements of both interior and exterior egg quality. The interior egg quality measurements were albumin and yolk percentages, albumin height (by using micrometer) and Haugh units{by the equation :Hu = 100 log(H+7.57-1.7W^{0.37})} while the exterior measurements were shell percentage, thickness(by using an electric caliper) and breaking strength (by using an apparatus of Fathi and El-Sahar, 1996).

Blood samples were withdrawn from the brachial vein of nine hens per group at 52 and 56 weeks of age by using heparinized syringe. The blood samples were immediately centrifuged at 3000 rpm for 10 minutes to separate plasma. Plasma samples were frozen at -20°C until assayed for cholesterol, total protein, albumin, Ca and P by a colorimetric method using

Lactobacillus supplementation had a favorable effect on feed conversion ratio during whole experimental period. Miles *et al.* (1981); Sellars (1991) and Haddadin *et al.* (1996) stated that presence of high numbers of *Lactobacilli* may increase motility of gut contents, and improve nutrient availability or absorption. In addition, improving feed conversion ratio associated with laying hens fed *Lactobacillus* supplemented diet could be attributed to the *Lactobacillus* stimulated appetite and increased fat, nitrogen, calcium, phosphorus, copper and manganese retention in layers as reported by Nahashon *et al.* (1994 & 1996). In addition, Fuller (1977) and Jin *et al.* (1997) reported that continuous feeding of probiotics to animals has been found to maintain the beneficial intestinal microflora in two ways: by competitive exclusion and by antagonistic activity towards pathogenic bacteria. In contrast, Goodling *et al.* (1987) reported that there were no increase in hen-day, egg production, feed efficiency and egg size in the case of layers fed *Lactobacillus* products during 48-week experimental period. The reasons for the ineffectiveness of *lactobacillus* could be attributed to the non-host-specific *Lactobacillus* used and the fact that the birds were being kept under relatively ideal conditions. Another reason may be due to the amount of *lactobacillus* used was too low to be effective.

Table (2): Body weight, egg number, egg mass, egg weight, feed intake, and feed conversion ratio of laying hens fed different levels of *Lactobacillus* from 48-56 wks of age (Means \pm SE).

Age (wk)	<i>Lactobacillus</i> levels				
	0	100	200	300	400
	Body weight, g				
48	1779 \pm 24.4	1789 \pm 27.9	1785 \pm 26.7	1788 \pm 25.1	1785 \pm 28.6
52	1814 \pm 35.2	1855 \pm 31.4	1818 \pm 29.8	1822 \pm 26.3	1846 \pm 33.7
56	1825 \pm 66.8	1888 \pm 67.1	1834 \pm 53.2	1842 \pm 59.4	1883 \pm 55.1
	Egg number/ hen				
48-52	21.41 ^b \pm 0.85	23.90 ^a \pm 0.63	24.00 ^a \pm 0.80	24.60 ^a \pm 0.66	23.99 ^a \pm 0.57
52-56	21.52 ^b \pm 0.96	22.15 ^b \pm 0.78	22.91 ^a \pm 0.68	23.00 ^a \pm 0.51	23.66 ^a \pm 0.73
48-56	42.93 ^b \pm 0.68	46.05 ^a \pm 0.55	46.91 ^a \pm 0.84	47.60 ^a \pm 0.75	47.65 ^a \pm 0.71
	Egg mass, g /hen				
48-52	1309 ^b \pm 27.1	1501 ^a \pm 40.1	1483 ^a \pm 28.7	1530 ^a \pm 38.7	1500 ^a \pm 20.7
52-56	1317 ^c \pm 42.7	1384 ^b \pm 21.9	1411 ^b \pm 39.4	1410 ^b \pm 17.9	1472 ^a \pm 24.1
48-56	2626 ^c \pm 27.4	2885 ^b \pm 33.4	2894 ^b \pm 47.2	2940 ^a \pm 28.5	2972 ^a \pm 39.4
	Egg weight, g				
48-52	61.14 ^c \pm 0.18	62.76 ^a \pm 0.34	61.80 ^b \pm 0.28	62.18 ^a \pm 0.25	62.50 ^a \pm 0.19
52-56	61.21 ^b \pm 0.29	62.47 ^a \pm 0.16	61.56 ^b \pm 0.37	61.30 ^b \pm 0.34	62.21 ^a \pm 0.28
48-56	61.17 ^c \pm 0.23	62.62 ^a \pm 0.27	61.68 ^b \pm 0.21	61.74 ^b \pm 0.17	62.46 ^a \pm 0.24
	Feed intake, g /hen				
48-52	3142 ^b \pm 37.2	3347 ^a \pm 34.1	3277 ^a \pm 55.4	3323 ^a \pm 34.1	3224 ^a \pm 39.2
52-56	3096 \pm 34.1	3114 \pm 43.5	3164 \pm 42.2	3146 \pm 53.9	3148 \pm 31.4
48-56	6237 ^b \pm 42.8	6462 ^a \pm 55.6	6441 ^a \pm 35.1	6460 ^a \pm 41.6	6372 ^a \pm 42.7
	Feed conversion ratio, g feed / g egg				
48-52	2.40 ^a \pm 0.08	2.23 ^b \pm 0.05	2.21 ^b \pm 0.03	2.17 ^b \pm 0.02	2.15 ^b \pm 0.04
52-56	2.35 ^a \pm 0.06	2.25 ^b \pm 0.02	2.24 ^b \pm 0.04	2.23 ^b \pm 0.04	2.14 ^b \pm 0.06
48-56	2.37 ^a \pm 0.05	2.24 ^b \pm 0.04	2.22 ^b \pm 0.06	2.20 ^b \pm 0.01	2.14 ^b \pm 0.03

^{a-c} Means within the same row with different letters are significantly differed

In conclusion, the *Lactobacillus* supplementation improved albumen quality. The result was in agreement with the findings of Tortuero and Fernandez (1995) who reported that *Lactobacillus* supplementation significantly improved albumen quality.

Egg shell quality

There was no significant difference among treated groups for shell percentage. Opposite trend was observed for shell thickness, where the dietary *lactobacillus* supplementation at all levels significantly increased shell thickness compared to control-fed group. With respect to breaking strength, it could be observed that the breaking strength of *Lactobacillus* fed-groups was significantly higher than control-fed group.

It could be concluded that the dietary *lactobacillus* supplementation significantly improved the eggshell quality. The last observation could be attributed to the *lactobacillus* supplementation improved the calcium retention, especially under unfavorable conditions e.g. high ambient temperatures (Nahashon *et al.*, 1994 & 1996). In addition, Mohan *et al.* (1995) reported that the probiotic supplementation improved shell thickness with fewer thin-shelled eggs. Robinson (1977) suggested that lactic acid secreted by lactobacilli might encourage better absorption of calcium and phosphorus from the digestive tract. Therefore, the eggshell quality improved. Likewise, Tortuero and Fernandez (1995) found that addition of *Lactobacillus* to the laying diet improved the shell thickness. This observation suggests that the usual decline in eggshell quality towards the end of the laying cycle was prevented by the presence of the ingested lactobacilli.

Blood parameters

Blood parameters of laying hens at 52 and 56 weeks of age as affected by *Lactobacillus* supplementation are listed in Table (4).

It could be observed that the dietary *lactobacillus* supplementation at all levels significantly increased plasma calcium compared to control-fed group. In contrast, the plasma phosphorus was significantly decreased when laying hens fed diet containing 100, 200, 300 and 400 mg LA/kg diet. Increasing plasma calcium may be explain the improving shell quality associated with laying hens fed *lactobacillus* supplemented diet. Heat stress caused a significant reduction in plasma calcium (Kalamah, 2001). The increase in plasma calcium in the result reported herein may be due to the beneficial effect of *Lactobacillus* addition on reducing the deleterious effect of heat stress by increasing the feed intake and consequently the amount of Ca consumed and/ or the increase of calcium retention as reported by Nahashon *et al.* (1994 & 1996).

Table 4: Some blood constituents and yolk cholesterol of laying hens fed different levels of *Lactobacillus* at 52 and 56 weeks of age. (Means \pm SE).

Age (wk)	<i>Lactobacillus</i> levels				
	0	100	200	300	400
	Plasma calcium (mg/dl)				
52	28.31 ^b \pm 1.09	29.19 ^a \pm 1.65	29.43 ^a \pm 2.01	28.84 ^a \pm 1.95	29.19 ^a \pm 2.10
56	27.53 ^c \pm 1.52	28.75 ^b \pm 0.98	29.92 ^a \pm 1.84	28.53 ^b \pm 2.41	29.01 ^b \pm 1.87
	Plasma phosphorus (mg/dl)				
52	5.60 ^a \pm 0.25	5.11 ^b \pm 0.56	4.90 ^b \pm 0.18	4.20 ^c \pm 0.37	4.82 ^b \pm 0.17
56	5.43 ^a \pm 0.54	4.57 ^b \pm 0.38	4.72 ^b \pm 0.24	3.95 ^c \pm 0.21	4.60 ^b \pm 0.13
	Total plasma protein (g/dl)				
52	6.17 ^b \pm 0.54	6.80 ^a \pm 0.49	6.72 ^a \pm 0.48	6.85 ^a \pm 0.57	6.82 ^a \pm 0.26
56	6.09 ^b \pm 0.48	6.72 ^a \pm 0.45	6.83 ^a \pm 0.43	6.79 ^a \pm 0.66	6.76 ^a \pm 0.47
	Albumen (g/dl)				
52	2.64 \pm 0.18	2.67 \pm 0.12	2.73 \pm 0.18	2.70 \pm 0.16	2.71 \pm 0.10
56	2.67 \pm 0.12	2.71 \pm 0.17	2.69 \pm 0.13	2.72 \pm 0.10	2.66 \pm 0.13
	Globulin (g/dl)				
52	3.53 ^c \pm 0.22	4.13 ^a \pm 0.21	3.99 ^b \pm 0.12	4.15 ^a \pm 0.10	4.11 ^a \pm 0.12
56	3.42 ^d \pm 0.17	4.01 ^c \pm 0.19	4.14 ^a \pm 0.15	4.07 ^b \pm 0.19	4.10 ^{ab} \pm 0.15
	A/G ratio				
52	0.73 ^a \pm 0.02	0.64 ^b \pm 0.07	0.68 ^b \pm 0.09	0.65 ^b \pm 0.07	0.66 ^b \pm 0.04
56	0.78 ^a \pm 0.05	0.68 ^b \pm 0.02	0.65 ^b \pm 0.08	0.67 ^b \pm 0.04	0.65 ^b \pm 0.09
	Plasma cholesterol (mg/dl)				
52	138.2 ^a \pm 9.4	124.5 ^b \pm 6.2	118.1 ^{bc} \pm 5.4	113.6 ^c \pm 8.2	108.2 ^c \pm 9.9
56	146.7 ^a \pm 12.9	119.9 ^b \pm 9.7	121.2 ^b \pm 12.7	110.9 ^{bc} \pm 4.5	102.7 ^c \pm 11.2
	Yolk cholesterol (mg/g)				
52	12.86 ^a \pm 0.58	10.74 ^b \pm 0.35	8.02 ^c \pm 0.60	7.60 ^c \pm 0.81	7.31 ^c \pm 0.45
56	12.71 ^a \pm 0.69	9.37 ^b \pm 0.62	7.57 ^c \pm 0.84	8.01 ^c \pm 0.75	7.43 ^c \pm 0.69

** Means within the same row with different letters are significantly differed (P \leq 0.05)

Lactobacillus could be important in the development of immune competence in animals, particularly when protection must be acquired against antigens likely to cause gut inflammatory reactions (Perdigon *et al.*, 1990). The previous results indicated that the total plasma protein was significantly increased when laying hens fed diet containing *Lactobacillus* at all levels. Opposite trend was noticed for plasma albumin, where there were no significant differences among treated groups for plasma albumin. In contrast, the *Lactobacillus* supplementation significantly increased plasma globulin compared to control-fed group. Concerning A/G ratio, it could be observed that *Lactobacillus* supplementation decreased the A/G ratio compared to control-fed group. Heat stress caused a significant reduction in plasma total proteins, albumin and globulins (Kalamah, 2001). Pollmann *et al.* (1980) stated that the *Lactobacillus* supplementation lead to elevated levels of total serum protein, globulin rather than albumen and increased white blood cell counts.

The current interest in reducing the dietary intake of cholesterol by humans, then the concept of producing eggs with reduced cholesterol

concentration might prove attractive as an aid to marketing. The previous results showed that the dietary *Lactobacillus* supplementation at all levels significantly decreased both plasma and yolk cholesterol. Plasma cholesterol was reduced by about 9.9, 14.5, 17.8 and 21.7% and 18.3, 17.4, 24.4 and 29.9% and yolk cholesterol by about 16.5, 37.6, 40.9 and 43.16% and 26.2, 40.4, 36.9 and 41.5% for groups fed 100, 200, 300, and 400 mg *Lactobacillus*/Kg diet at 52 and 56 wk of age, respectively. Gilliland *et al.* (1985) hypothesized that some *Lactobacillus* strains are able to incorporate cholesterol into the cellular membrane of the organism, thus, less cholesterol will be absorbed into blood. The hypocholesterolaemic effect may also be due to the ability of lactic acid bacteria to produce bile salt hydrolase, the enzyme responsible for bile salt deconjugation (Klaver and van der Meer, 1993). In addition, Abdulrahim *et al.* (1996) and Haddadin *et al.*, (1996) indicated that the *Lactobacillus acidophilus* reduced the cholesterol concentration in the eggs.

In conclusion, *Lactobacillus* supplementation to laying hen diets had a beneficial effect on productive performance, egg quality under Egyptian summer conditions. In addition, *Lactobacillus* supplementation reduced yolk cholesterol and consequently, reducing the dietary intake of cholesterol by humans.

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تأثير إضافة اللاكتوباسيلس إلى العلائق على أداء الدجاج البيض تحت ظروف الصيف

المصري

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أجريت هذه التجربة بغرض دراسة تأثير إضافة اللاكتوباسيلس إلى علائق الدجاج البيض على الأداء الإنتاجي وجودة البيض وبعض مقاييس السم تحت ظروف الصيف المصري. استخدم في هذه التجربة عدد 135 دجاجة بيضاء من سلالة الهاي لايسن البني عمر 48 أسبوع. تم توزيع الدجاجات على خمسة معاملات تجريبية وهي صفر، 100، 200، 300، 400 ملجم من مستحضر اللاكتوباسيلس التجاري الذي يحتوي كل جرام منه على 800 مليون خلية في المتوسط لكل كجم عليه. استمرت الفترة التجريبية لمدة 8 أسابيع (48-56 أسبوع). استخدمت الطيور المغذاة على العليقة القاعدية بدون إضافة اللاكتوباسيلس كمجموعة مقارنة. أدى إضافة اللاكتوباسيلس إلى تحسن معنوي في عدد البيض بمقدار 7.3، 9.3، 10.9 و 11% وكتلة البيض بمعدل 9.9، 10.2، 11.9 أو 13.2% و الغذاء المأكول بمعدل 3.6، 3.3، 3.6 و 2.2% ومعدل التحويل الغذائي بمقدار 5.5، 6.2، 7.7 و 9.7 في المجاميع المغذاة على 100، 200، 300 و 400 ملجم من مستحضر اللاكتوباسيلس/كجم عليه على التوالي مقارنة بالدجاجات المغذاة على عليقه كالتحكم. زاد وزن البيض خلال الفترة التجريبية من 48-56 أسبوع من العمر بإضافة اللاكتوباسيلس عند كل المستويات ولكن الفروق لم تكن معنوية إلا مع المجاميع المغذاة على 100 و 400 ملجم/كجم. أدى إضافة اللاكتوباسيلس إلى حدوث زيادة معنوية في جودة البيض وسمك القشرة بالإضافة إلى قوة الكسر عند عمر 52 و 56 أسبوع. لم يكن هناك فروق معنوية بين المجاميع المختلفة في نسبة كل من البيض والصفار والقشرة. زاد ارتفاع البيض بصورة معنوية عند عمر 52 أسبوع بإضافة اللاكتوباسيلس. زاد محتوى بلارما السم من الكالسيوم والبروتين الكلي والجلوبيولين بصورة معنوية مقارنة بمجموعة التحكم. أدى إضافة اللاكتوباسيلس إلى حدوث انخفاض معنوي لكل من فوسفور البلازما ونسبة الجلوبيولين إلى الجلوبيولين. إضافة اللاكتوباسيلس إلى علائق الدجاج البيضاء أدى إلى حدوث كوليسترول الصفار بمعدل 16.5، 37.6، 40.9 و 43.2% و 26.2، 40.4، 36.9 و 41.5% للمجاميع المغذاة على 100، 200، 300 و 400 ملجم من مستحضر اللاكتوباسيلس/كجم عليه عند عمر 52 و 56 أسبوع على التوالي مقارنة بالدجاجات المغذاة على عليقه كالتحكم.

ونستخلص من هذه التجربة أن استخدام اللاكتوباسيلس في علائق الدجاج البيض أثناء فصل الصيف له تأثيرات إيجابية معنوية على الأداء الإنتاجي وجودة البيض بالإضافة إلى تأثيره المخفض لكل من كوليسترول البلازما و صفار البيض.