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EFFECT OF USING OF CHLORELLA ALGAE (*CHLORELLA VULGARIS*) IN LAYER DIETS ON PRODUCTION PERFORMANCE AND *LACTOBACILLUS* COUNT IN THE INTESTINE OF HENS

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Abstract

This study was conducted at the Poultry Research Station in Abu Ghraib, Department of Agricultural Research, Ministry of Agriculture, the experimental field during three months (Three terms for a period of four weeks), from 10th of December 2019 to the 10th of January 2020. The study aimed to determine the effect of using different proportions of chlorella algae in layer hen ration and its effect on the hen's productive performance and numbers of lactobacilli bacteria in the intestine. The experiment included 400 laying hens (ISA Brown) of 54 week old which were fed according to the standard requirements mentioned in the guide for this breed (ISA Brown layer management guide). The hens raised using a 3 stage cages system with five hens in each cage. The birds were randomly distributed to five feeding treatments with four replications per treatment. The single replicate consists of 20 birds (80 birds per treatment). The treatments were as First treatment (T_1) without using chlorella algae (control) 'Second treatment (T_2) using 1% chlorella algae 'Third treatment (T_3) using 1.5% chlorella algae 'Fourth treatment (T_4) using 2% chlorella algae 'Fifth treatment (T_5) use of 2.5% chlorella algae The results indicated a significant improvement ($P<0.05$) in the egg production rate for T_2, T_3, T_4 treatments during the three trial periods (54-58, 58-62 and 62-66) a week, as well as the total period of the trial compared to the control treatment, and moral superiority ($P<0.05$) in the egg weight ratio for the T_5 treatment during periods (58-62 and 62-66) a week and the total period of the experiment compared to the control treatment. A significant increase ($P<0.05$) in the average egg mass for chlorella treatments (T_5, T_4, T_3, T_2) during the periods (58-62 and 62-66) a week and the total period of the experiment compared with the control treatment T_1 and the results showed a significant improvement ($P<0.05$) in the rate of feed conversion in favor of coefficients for chlorella algae (T_5, T_4, T_3, T_2) during the periods (58-62 and 62-66) a week and the total period of the trial compared with the control treatment T_1.

Key words : Chlorella algae, Layers, Production, Lactobacilli.

Introduction

Recent trends in research and studies in the field of poultry industry have increased to find the best fodder alternatives and nutritional additives to reduce costs and achieve the best fodder balance that meets the needs of domestic birds to achieve the best growth rates and high production speed (Leeson and Summers, 2005 and Blair *et al.,* 2018). Algae It is a large and diverse group of self-feeding living organisms that depend on themselves by the process of photosynthesis (Photoautotrophic), and algae grows in fresh and salt water and has attracted global attention in recent years because of its production of high-value chemical compounds as well as its benefit as energy producing cells (Greenwell *et al.,* 2010) Chlorella vulgaris is an important species of green algae, which lives in fresh water and is characterized by ease of cultivation, its high production and resistance to changing growth conditions and its high nutritional value that qualifies it to be a nutritional supplement for humans and animals, including poultry. It is characterized by its high content of protein, essential amino acids and fatty acids Saturated omega-3 and omega-6, antioxidants,

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carotenoids and vitamins (Ruggiero *et al*., 2015). Several experiments on the use of algae in poultry diets have shown that they have positive effects on live body weight and increase the rate and quality of egg production (Vijayavel *et al.,* 2007 and Shukla *et al*., 2011). Chlorella algae in diets of poultry can replace proportions from sources Protein (diets and protein concentrates) as well as the role of algae in improving productive and physiological performance and the health condition of birds by reducing the rate of mortality and increasing the body's resistance to diseases by increasing the activity of macrophages of white blood cells and thus increasing the immunity of birds and increasing rates of egg and meat production and lower concentration of triglycerides in Liver and improved egg quality produced by increasing egg shell thickness and a number of internal egg quality characteristics (Kotrbacek, 1994, Redel, 2001, Zheng *et al.,* 2012 and Englmaierová, 2013 and Gadiev *et al*., 2019). It can be used to produce table eggs, which is considered a functional feed with a high nutritional value in human nutrition (Matos *et al.,* 2017). In view of the global trend in the use of chlorella algae in diets of poultry birds and with the lack of studies on this topic, this study is considered the first in Iraq on the use of different levels of chlorella algae powder in diets of laying hens. The aim of this study is to conduct an experiment for laying hens to know the effect of using different levels (1%, 1.5%, 2%, 2.5%) of the chlorella moss powder in diets and their effect on the productive performance of laying hens and the numbers of Lactobacillus bacteria in the intestine.

Materials and Methods

This study was conducted at the Poultry Research Station in Abu Ghraib, Department of Agricultural Research, Ministry of Agriculture, the experimental field during three months (Three terms for a period of four weeks), from 10th of December 2019 to the 10th of January 2020. In the experiment, I used 400 ISA brown laying hens at the age of 54 weeks, and the breeding period was divided into 3 experimental periods (54-58) weeks, (58-62) weeks and (62-66) weeks old, and the fodder was presented according to the standard requirements mentioned in The breeding guide for this breed (ISA Brown layer managements guide) The chickens were raised using a 3-storey cage system with five chickens in one cage. The birds were randomly assigned to five feeding treatments at the rate of four replicates per treatment. The single repeater consists of 20 birds (80 birds). For one treatment) the T1 coefficients were the control treatment (without addition), T_5, T_4, T_3, T_2 using chlorella algae at rates of 2.5, 2, 1.5, 1 (%), respectively. A lighting program that includes 16 lighting hours and 8 hours of darkness / day was applied throughout the breeding period. The hall was equipped with air vacuums suitable for the size of the hall and the number of chickens, to ensure appropriate breeding conditions. Place the chicken under a healthy and preventive program that guarantees the health of the herd. Chlorella algae powder was prepared from China because it is not available in Iraq, and it is a product from Hunan Dye Natural Resource Inc. The material is a green powder and provides 50% total protein. This powder is safe for feed use, the material was kept in a dry and closed place. The characteristics of the egg production rate (H.D%), the egg weight rate, the egg

Table 1: Chemical composition and diet content of the experiment.

Feed composition	Treatments %				
	T_1	T_2	T_3	T_4	T_5
Corn	35	35	35	35	35
Wheat	35	35	35	35	35
Soybean Meal*	16	15	14.5	14	13.5
Protein concentration **	5	5	5	5	5
Chlorella powder	0	1	1.5	2	2.5
Fat	0.5	0.5	0.5	0.5	0.5
Di calcium phosphate	0.5	0.5	0.5	0.5	0.5
Salt	0.3	0.3	0.3	0.3	0.3
Limestone	7.7	7.7	7.7	7.7	7.7
Total	100	100	100	100	100
Chemical composition* **					
Kcal/kg	2806.15	2826.7	2837	2847.3	2857.6
Crude protein %	16.68	16.79	16.85	16.9	16.96
Fat %	3.12	3.24	3.3	3.36	3.43
Crude fiber%	2.55	2.63	2.67	2.71	2.75
Lysine%	0.86	0.87	0.87	0.88	0.88
Methionine + cystine%	0.65	0.65	0.65	0.65	0.65
Calcium %	3.36	3.36	3.36	3.36	3.36
Available phosphorus%	1.75	1.75	1.75	1.75	1.75

* The soybean meal used from an Argentine source has a crude protein content of 48% and 2440 kcal / kg energy represented.** Protein concentrate used animal produced from a Dutch company (imported) WAFI contains 40% crude protein, 2157 kilocalories / kg representative energy protein, 5% raw fat, 3.20% raw fiber, 3.04% calcium, 2.65% phosphorous, 3.85% Lysine, 3.70% methionine, 4.13% methionine + cysteine, 0.43% tryptophan, 1.80% threonine. And it contains a mixture of vitamins and minerals that secure the need of birds from these elements. *** Chemical composition for the diet content was according to NRC (1994).

mass rate and the feed conversion coefficient were measured and the numbers of lactobacilli bacteria in the intestine were calculated. An experiment was used to study a single factor effect (5 × 4) applied with a complete random design (CRD) to study the effect of treatment and period on different traits and the mean differences between the averages were compared to the Duncan (1955) polynomial test. And use the program S.P.S.S. (2018) in statistical analysis of data.

Results and Discussion

Egg production ratio (% H.D)

Table 2 shows the effect of using different proportions of chlorella algae in laying hens diets in the egg production rate (HD%) and the results indicate a significant difference ($P < 0.05$) in all addition treatments and for all trial weeks in the egg production ratio, as the two treatments excelled T_4 and T_3 with additives percentages of 1.5% and 2% chlorella, respectively, in the percentage of egg production compared to the control treatment T_1 (without addition) during the period (58-54) weeks, whereas the treatments T_5 and T_2 did not differ with the proportions of adding 1% and 2.5 % Chlorella, respectively, was significant with all trial treatments, and during the period (58-62) weeks, significantly ($P < 0.05$), T_4, T_3, T_2 treatments were significantly superior in the egg production rate over the control T1 treatment, whereas the T_5 (2.5%) chlorella did not differ Morally with all experiments of the experiment, and the period (62-66) weeks, in which the production rate did not differ from the previous period, and the transaction T_5 (2.5%) did not record significant differences with all the experiments of the experiment, while the total period of the experiment was a confirmation of moral superiority ($P < 0.05$) for T_4, T_3, T_2 coefficients for T_1 control treatment. There were no significant differences for T_5 treatment with trial coefficients in egg production rate. H.D%.

Weight of eggs (g)

Table 3 shows the effect of using different proportions of chlorella algae in laying hens diets in the average egg weight (g), and the results indicate that there were no significant differences in the average egg weight during the period (54-58) weeks between the coefficients of the experiment. As for the period (58-62) weeks, we notice a significant increase ($P < 0.05$) in favor of the T_5 treatment (by adding 2.5% chlorella), whereas the T_4, T_3 and T_2 coefficients (with ratios of adding 2,1.5,1%), respectively, were not recorded. Significant differences between it and treatment T_1 on the one hand, and between it and treatment T_5 on the other hand. As the age of the chickens reached to a period of (62-66) weeks, the T_5 treatment recorded the highest mean egg weight (significantly $<P < 0.05$) over the rest of the experiment, and the treatments T_4 and T_3 were significantly superior ($P < 0.05$) over the control T_1 treatment and was not recorded Treatment T_2 significant differences with T_4, T_3, T_1 treatments during the period (62-66) weeks, and the total period of the experiment indicates a significant increase ($P < 0.05$) in egg weight in favor of treatment T_5 on T_2 and T_1 treatments, whereas T_4 and T_3 treatments did not They indicate significant differences with the rest of the coefficients of the experiment.

Egg mass (g/bird/day)

Using of different proportions of chlorella algae in laying hens diets and its effect on the average of egg mass shown in table 4, as the first period (54-58) weeks did not record any significant differences between the treatments. As the age of the chickens progressed to a period of (58-62) weeks, a significant moral superiority emerged in favor of the co-use treatments of chlorella compared to the control treatment, as the T_5, T_4, T_3, T_2 coefficients recorded significant superiority ($P < 0.05$) compared to the control treatment T_1, while the period (62-66) A week, T_5 and T_4 treatments were recorded with rates (2% and 2.5%) respectively, chlorella significantly ($P < 0.05$) over treatments T_1 and T_2 in the average of egg mass, T_3 and T_2 treatment achieved significant superiority at the expense of control treatment T_1. Treatment T_3 did not indicate significant differences with the T_5, T_4, T_2 coefficients during the same period, and the results of the total period of the experiment indicated a significant moral superiority of the coefficients for using chlorella algae (T_5, T_4, T_3, T_2) over the T_1 control treatment in the average of egg mass.

Feed Intake

One of the important indicators to determine the efficiency of the feed provided to the birds and their feed content, and is the main determinant of production efficiency. Table 5 shows the effect of using different proportions of chlorella algae in laying hens' diets on the feed Intake (g / bird / day), as the results indicated the lack of There were significant differences between trial treatments when using chlorella algae at rates of 2.5, 2, 1.5, 1% during trial periods from 54 weeks to 66 weeks and the total period.

Feed Conversion coefficient

Table 6 shows the effect of using different proportions of chlorella algae in laying hens diets in the characteristic of feed conversion coefficient, and the results during the period (54-58) weeks indicate that there were significant differences ($P < 0.05$) for the benefit of using chlorella,

Table 2: Effect of using different percentages of chlorella algae in laying hens diets in egg production rate (H.D%) (mean ± standard error).

Treatments	Periods / weeks			Total Period
	54-58	58-62	62-66	(54-66)
T_1	^{B}0.25±79.58	^{B}0.28±81.17	^{B}1.17±81.13	^{B}0.55±80.63
T_2	^{AB}1.94±84.84	^{A}0.54±86.58	^{A}0.51±85.33	^{A}0.68±85.58
T_3	^{A}2.25±86.17	^{A}0.69±85.25	^{A}0.73±86.71	^{A}0.91±86.04
T_4	^{A}1.12±86.54	^{A}0.83±85.38	^{A}0.41±85.38	^{A}0.61±85.77
T_5	^{AB}2.65±82.71	^{AB}2.28±83.08	^{AB}2.26±83.21	^{AB}2.28±83.00
Significance	*	*	*	*

ABC Means with different superscripts within the same column differ significantly (*p < 0.05), N.S: p > 0.05.

Table 3: Effect of using different percentages of chlorella algae in laying hens diets in egg weight (g) (mean ± standard error).

Treatments	Periods / weeks			Total Period
	54-58	58-62	62-66	(54-66)
T_1	1.51±65.35	^{B}0.47±65.77	^{C}0.45±64.99	^{B}0.25±65.54
T_2	0.73±65.54	^{AB}0.50±66.29	^{BC}0.29±65.48	^{B}0.43±65.77
T_3	0.46±65.40	^{AB}0.42±67.22	^{B}0.74±66.12	^{AB}0.48±66.25
T_4	0.46±65.86	^{AB}1.00±66.64	^{B}1.09±66.33	^{AB}1.17±66.10
T_5	0.18±67.06	^{A}0.50±68.08	^{A}0.31±68.87	^{A}0.24±68.01
Significance	N.S	*	*	*

ABC Means with different superscripts within the same column differ significantly (*p < 0.05), N.S: p > 0.05.

Table 4: Effect of using different percentages of chlorella algae in laying hens diets in the average of egg mass (g/bird/day) (mean ± standard error).

Treatments	Periods / weeks			Total Period
	54-58	58-62	62-66	(54-66)
T_1	0.14±52.41	^{B}1.14±53.37	^{C}1.02±52.74	^{B}0.74±52.84
T_2	1.40±55.60	^{A}0.74±56.89	^{B}0.45±55.88	^{A}0.40±56.01
T_3	1.68±56.36	^{A}0.46±58.28	^{AB}0.92±56.62	^{A}0.88±57.33
T_4	0.97±56.52	^{A}0.36±56.56	^{A}0.83±57.31	^{A}0.82±56.67
T_5	1.73±55.46	^{A}1.61±56.65	^{A}1.67±57.34	^{A}1.61±56.47
Significance	N.S	*	*	*

ABC Means with different superscripts within the same column differ significantly (*p < 0.05), N.S: p > 0.05.

Table 5: Effect of using different percentages of chlorella algae in laying hens diets on feed consumption rate (g / bird / day) (mean ± standard error).

Treatments	Periods / weeks			Total Period
	54-58	58-62	62-66	(54-66)
T_1	0.77±104.66	0.98±101.62	1.35±101.05	1.02±102.53
T_2	0.37±103.26	0.56±102.78	1.08±101.31	0.55±102.44
T_3	0.73±103.37	0.66±103.00	1.19±101.99	0.65±102.92
T_4	0.14±103.37	0.44±103.39	0.65±102.41	0.34±102.85
T_5	0.95±102.91	0.69±103.59	0.91±102.80	0.77±103.10
Significance	N.S	N.S	N.S	N.S

ABC Means with different superscripts within the same column differ significantly (*p < 0.05), N.S: p > 0.05.

as two treatments T_4 were recorded, T_3, dietary conversion coefficient decreased compared to T_1 control treatment. T_5 and T_2 treatments did not show significant differences with trial treatments during the first period. As for the second period (58-62) weeks, significant superiority (P <0.05) for T_5, T_4, T_3, T_2 coefficients continued with rates of use of 1%, 1.5%, and 2% chlorella, respectively, compared with the control treatment T_1 (without addition). The last period of the experiment (62-66) weeks of chicken age witnessed the best feed conversion coefficient during the experiment, as all coefficients of using chlorella T_5, T_4, T_3, T_2 excelled at rates 2.5,2,1.5,1 (%) significantly (P <0.05).) On the treatment of control T1, as the overall period of the experiment showed the moral superiority of the co-use treatments of chlorella (T_5, T_4, T_3, T_2) and with different ratios on the control treatment T_1 in the coefficient of feed conversion.

Determination of Lactobacilli count in Jejunum

The numbers of lactobacilli bacteria were converted from large numbers to simplified logarithmic numbers for ease of statistical analysis. From the Fig.1, the effect of using different proportions of chlorella algae in diets of laying hens is shown in the logarithmic count of Lactobacilli bacteria in the intestine person, as we note the increase in significance (P <0.01) in the numbers of Lactobacilli bacteria in favor of most of the coefficients of using chlorella algae compared to the control coefficient and the numbers increased with increasing the percentage of addition, as we note that the T_5 and T_4 coefficients with 2% utilization rates 2.5% chlorella have significantly outperformed (P <0.01) over the rest of using coefficients Chlorella algae recorded the highest number of bacteria, while treatment T_3 recorded a highly significant (P <0.01) superiority over the T_2 and T_1 treatments in the intestinal Lactobacilli numbers, whereas the T_1 and T_2 treatments did not differ from each other significantly when calculating count of Lactobacilli in the intestine.

This improvement in most productive traits represented by the rate of egg production, the

Table 6: Effect of using different percentages of chlorella algae in laying hens diets in feed conversion coefficient (feed g / g egg mass) (mean ± standard error).

Treatments	Periods / weeks			Total Period
	54-58	58-62	62-66	(54-66)
T_1	^{A}0.01±2.00	^{A}0.03±1.91	^{A}0.04±1.92	^{A}0.03±1.94
T_2	^{AB}0.04±1.86	^{BC}0.01±1.82	^{B}0.03±1.81	^{B}0.01±1.83
T_3	^{B}0.05±1.84	^{BC}0.01±1.77	^{B}0.01±1.78	^{B}0.02±1.80
T_4	^{B}0.03±1.83	^{BC}0.02±1.81	^{B}0.02±1.81	^{B}0.02±1.82
T_5	^{AB}0.05±1.85	^{B}0.04±1.83	^{B}0.05±1.79	^{B}0.04±1.83
Significance	*	*	*	*

ABC Means with different superscripts within the same column differ significantly (*p < 0.05), N.S: p > 0.05.

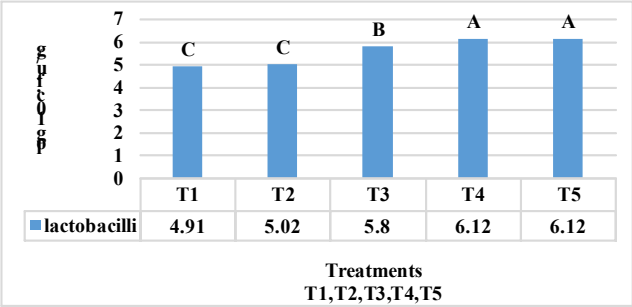

Fig. 1: Effect of using different percentages of chlorella algae in laying hens diets in the logarithmic numbers of Lactobacilli bacteria (CUF / g) in Jejunum (mean ± standard error).

Letters on the columns indicate significant differences (P <0.01).

weight of the eggs produced, the egg mass and the efficiency of nutritional conversion when adding chlorella algae in different proportions to the laying of diets of chickens compared with the control treatment (without addition) may be attributed to the high nutritional value of the components of chlorella algae, foremost of which is the protein of value High biosynthesis, which competes with plant protein sources such as soybeans and animal protein concentrates that are used in poultry feeds, as the protein ratio reaches more than 50% (Andrade *et al.,* 2018). The synthesis of essential amino acids in chlorella algae is similar to the composition of animal protein (Becker, 2004) Chlorella is also an antioxidant (Sheih *et al.,* 2009), a donor of methyl groups that inhibits free radicals and lipid peroxide and stimulates natural antioxidants in the body as well as enzymatic antioxidants (Halliwell and Gutteridge, 1989) as antioxidants preserve and protect essential substances Necessary for the formation and growth of ovarian follicles, which mainly consist of fatty substances, and the role of antioxidants in diets. Poultry that works to protect the lipoprotein and other fatty compounds, which is a key part of the egg yolk component, protecting it from oxidation and providing these substances in the necessary proportions, and then having maturation of ovarian follicles with less time in favor of chlorella moss treatments compared to the control treatment (Sturkie, 1986). The role of antioxidants It works to regulate the process of representation of fats in the body, as well as sedimentation of raw materials for the growth and maturation of ovarian follicles and thus an increase in the rate of egg production in favor of chlorella moss transactions, and between Kotrbáèek *et al*., (2015) that there are positive roles of chlorella moss in improving the internal environment of the digestive system and increasing benefit From feed and the high numbers of lactobacillus bacteria in the intestine, which is one of the indicators of improving the health status of the bird in general and the digestive system and benefiting from feed in particular, and this certainly is reflected in the absorption of nutrients as much and consequently the improvement of egg production rates and the weight of eggs produced, and this is shown by the results of our study The apparent improvement in the number of lactobacilli bacteria and the increase in the percentage of addition (Fig. 1). The reason may be The other is in improving the egg production rate because chlorella algae contains many vitamins such as vitamin E and carotenoids (Andrade *et al.,* 2018) which have positive effects in increasing egg production, through their effect in increasing the secretion of sex hormones as vitamin E stimulates the release of the hormone released Luteinizing hormone (LHRH) from the hypothalamus and the role of vitamin A by its effect on the pituitary gland thus enhancing the secretion of FSH, LH, progesterone and estrogen, which leads to enhanced egg production because there is a positive correlation between the concentration of these hormones in the blood and egg production (Al-Daraji *et al.*, 2008). Likewise, the presence of a high concentration of carotenoids in the yolk with a higher utilization rate in the bush and therefore an increase in the weight of the egg yolk, which is the first determinant of the size of the rest of the other egg components, which is the egg and egg shell, since increasing the weight of the yolk leads to an increase in the precipitation of egg white by twice the weight of the yolk (Saleh *et al.,* 2017 and Fouad *et al.,* 2018) The egg weight recipe is an important economic trait that is a correct indication of its size, which contributes to determining the price as it is no less important than the characteristic of egg production, which are an important target for laying hens, and the reason may be due to increased Weight of the egg with increasing the percentage of added chlorella to the increase in the

percentage of long-chain polyunsaturated fatty acids in the chlorella moss, which contributes to the increase in the percentage of egg yolks (Borowitzka, 1988, Stadelman and Cotterill, 1995). Free radicals have the ability to break down cell membranes by oxidizing unsaturated fatty acids and having a negative effect on the hepatocytes, which affects the rate of formation and manufacture of yolk components by the liver (Anton, 2007a) and the role of antioxidants in protecting fats and polyunsaturated fatty acids (PUFA) from oxidation This is reflected in the increased sedimentation of unsaturated fatty acids in the yolk leading to an actual increase in the yolk first and this is reflected in the average egg weight in general. Egg mass is a mathematical trait that measures its number based on the number of eggs produced first and the weight of eggs produced second (North, 1984, Stadelman and Cotterill, 1995). Studies show that the number of eggs produced and their weight increases linearly as the chicken ages, which positively affects the mass of eggs produced. Therefore, the treatments that achieved the highest rates of egg production and the weight of eggs achieved the highest rates of egg mass produced and that the increase in egg production or the weight of eggs and other qualities of other eggs is only the fruit of the efficiency of converting inputs to outputs of economic value. the results of our experiment clearly indicate an improvement in the nutritional conversion coefficient for the use of chlorella algae in all periods of the experiment and between Englmaierová (2013). Chlorella algae will fill and provide the body's requirements for different nutrients, specifically essential amino acids and trans fatty acids and not increase the feed consumed Compared to the improvement in the rate of egg production and egg weight. The results of the experiment showed an increase in the number of Lactobacilli bacteria in the intestine with a high percentage of chlorella algae in the diet. This may be due to the containment of chlorella algae on some active substances, including Chlorellin, which showed their roles as an anti-harmful bacteria without affecting the beneficial bacteria in the gut (Jensen, 2001). Also, the presence of other active substances in the chlorella algae represented by tocopherol and multiple sugars and linolenic fatty acid which have vital roles in the activity of microorganisms as this group of active substances affects the permeability of cell membranes and thus influence the activities and activity of microorganism in the intestine, thus the important role of moss Chlorella through active substances in the balance of microorganisms and the environment entering the intestine by increasing the numbers of beneficial bacteria Lactobacilli in the intestine (Malanga *et al.,* 1997; Safafar *et al.,* 2016.(We conclude from this study that the use of chlorella algae powder in laying hens diets has a positive effect on improving the productive performance and health status of birds through its role in the process of balancing the internal environment of the intestine by increasing the numbers of beneficial bacteria.

References

Al.Daraji, H.J., W.K. Al-Hayani and A.S. Al-Hassani (2008). Hematology of birds blood, Ministry of Higher Education and Scientific Research, University of Baghdad - College of Agriculture.

Andrade, L.M., C.J. Andrade, M. Dias, C.A.O. Nascimento, and M.A. Mendes (2018). Chlorella and Spirulina Microalgae as Sources of Functional Foods. *Nutraceuticals, Food Suppl*., 45–58.

Andreeva, A.V., O.N. Nikolaeva, E.R. Ismagilova, V.R. Tuktarov, R.G. Fazlaev, A.I. Ivanov, O.M. Altynbekov, G.M. Sultangazin, I.M. Urmanov and A.Z. Khakimova (2018). Effect of probiotic preparations on the intestinal microbiome. *J. Eng. Appl. Sci*., **13:** 6467–6472.

Anton, M. (2007). Composition and structure of hen egg yolk.Pages 1–6 in Bioactive egg compounds. Springer.

Becker, W. (2004). The nutritional value of microalgae for aquaculture. In: Richmond A (ed) Handbook of microalgal mass cultures. CRC Press Inc. Boca Raton, Florida, 380–391.

Blair, R. (2018). Nutrition and Feeding of Organic Poultry, 2nd Edition. CABI.

Borowitzka, M. A. (1988). Vitamins and fine chemicals from micro-algae.in Cambridge University Press.

Englmaierová, M., M. Skøivan and I. Bubancová (2013). A comparison of lutein, spray-dried Chlorella, and synthetic carotenoids effects on yolk colour, oxidative stability, and reproductive performance of laying hens. *Czech J. Anim. Sci.*, **58:** 412–419. Available at http://www.agriculturejournals.cz/web/cjas.htm?volume=58 & firstPage=412&type=published Article (verified 7 March 2019).

Fouad, A.M., W. Chen, D. Ruan, S. Wang, W. Xia and C. Zheng (2018). Effects of dietary lysine supplementation on performance, egg quality, and development of reproductive system in egg-laying ducks. *J. Appl. Anim. Res*., **46:** 386–391.

Gadiev, R.R., D.D. Khaziev, C.R. Galina, A.R. Farrakhov, K.D. Farhutdinov, I.Y. Dolmatova, M.A. Kazanina and G.F. Latypova (2019). The use of chlorella in goose breeding. *AIMS Agric. Food*, **4:** 349–361.

Greenwell, H.C., L.M.L. Laurens, R.J. Shields, R.W. Lovitt and K.J. Flynn (2010). Placing microalgae on the biofuels priority list: A review of the technological challenges. *J.R. Soc. Interface*, **7:** 703–726.

Halliwell, B. and J.M.C. Gutteridge (1989). Free radicals in biology and medicine. Clarendon.

Jensen, G.S. (2001). Blue-green algae as an immuno-enhancer and biomodulator. *J. Am. Nutraceutical Assoc*., **3:** 24–30.

Kotrbáèek, V., J. Doubek and J. Doucha (2015). The chlorococcalean alga Chlorella in animal nutrition: a review. *J. Appl. Phycol*., **27:** 2173–2180 Available at http://link.springer.com/10.1007/s10811-014-0516-y (verified 7 March 2019).

Kotrbacek, V., R. Halouzka, V. Jurajda, Z. Knotkova and J. Filka (1994). Increased immune response in broilers after administration of natural food supplements. *Vet. Med. (Praha).*, **39:** 321–328.

Leeson, S. and J.D. Summers (2005). Commercial Poultry Nutrition Third Edition. 3RD ed., University Books P.O. Box 1326 Guelph, Ontario, Canada N1H 6N8 Nottingham University Press Manor Farm, Church Lane, Thrumpton, Nottingham, NG11 0AX, England.

Malanga, G., G. Calmanovici and S. Puntarulo (1997). Oxidative damage to chloroplasts from Chlorella vulgaris exposed to ultraviolet B radiation. *Physiol. Plant.*, **101:** 455–462.

Matos, J., C. Cardoso, N.M. Bandarra and C. Afonso (2017). Microalgae as healthy ingredients for functional food: a review. *Food Funct*., **8:** 2672–2685.

North, O.M. (1984). Commercial Chicken Production Manual. 3rd ed. AVI. Publishing Company. Inc. Westport, Connecticut.

Redel, H. and B.U. (2001). Effect of algae biomass on growth of Peking ducks. Jahresbericht Landesanstalt Landwirtschaft Brand, **5:** 112–113.

Ruggiero, M.V., D. Sarno, L. Barra, W.H.C.F. Kooistra, M. Montresor and A. Zingone (2015). Diversity and temporal pattern of Pseudo-nitzschia species (Bacillariophyceae) through the molecular lens. *Harmful Algae*, **42:** 15–24.

Safafar, H., P. Uldall Nørregaard, A. Ljubic, P. Møller, S. Løvstad Holdt and C. Jacobsen (2016). Enhancement of protein and pigment content in two Chlorella species cultivated on industrial process water. *J. Mar. Sci. Eng*., **4:** 84.

Saleh, A.A., B. Gálik, H. Arpášová, M. Capcarová, A. Kalafová, M. Šimko, M. Juráèek, M. Rolinec, D. Bíro and A.M. Abudabos (2017). Synergistic effect of feeding Aspergillus awamori and lactic acid bacteria on performance, egg traits, egg yolk cholesterol and fatty acid profile in laying hens. *Ital. J. Anim. Sci*., **16:** 132–139.

Sheih, I.C., T.K. Wu and T.J. Fang (2009). Antioxidant properties of a new antioxidative peptide from algae protein waste hydrolysate in different oxidation systems. *Bioresour. Technol.*, **100:** 3419–3425. Available at http://www.sciencedirect.com/science/article/pii/S0960852409001151.

Shukla, S.P., J. Kvíderová and J. Elster (2011). Nutrient requirements of polar Chlorella-like species. *Czech Polar Reports*, **1:** 1–10.

SPSS® 27.0. Computer Software, 2018. SPSS Inc. Headquarters, 233 p., Wacker Drive, Chicago, Illinois. 60606, USA.

Stadelman, W.J. and O.J. Cotterill (1995). Egg Science and Technology. An Imprint of the Haworth Press. INC. 4th ed. Food Products Press, New York. London, UK.

Sturkie, P.D. (1986). Avian Physiology. 4th ed. Springer Verlary. New York.in Springer.

Vijayavel, K., C. Anbuselvam and M.P. Balasubramanian (2007). Antioxidant effect of the marine algae Chlorella vulgaris against naphthalene-induced oxidative stress in the albino rats. *Mol. Cell. Biochem*., **303:** 39–44.

Zheng, L., S.T. Oh, J.Y. Jeon, B.H. Moon, H.S. Kwon, S.U. Lim, B.K. An and C.W. Kang (2012). The dietary effects of fermented Chlorella vulgaris CBT®on production performance, iver lipids and intestinal microflora in laying hens. *Asian-Australasian J. Anim. Sci*., **25:** 261–266.