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Effect of Adding Different Levels of *Portulaca oleracea* L. Seeds and Leaves Powder to The Diet on Productive and Physiological Performance of Broiler Chickens (Ross 308)

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Abstract: This study was conducted in the Poultry Field of the College of Agriculture, Basrah University for the period from 20/9/2019 to 24/10/2019 to investigate the effect of adding different levels of Portulaca oleracea L. seeds and leaves powder on some physiological characteristics of broilers. A total 216 one day old unsexed Ross-308 broiler chicks were used in this study. The chicks were randomly distributed into six treatments replicated three times (12 chicks each replicate). The experiment was designed as a completely randomized design (CRD). The first treatment was control (basal diet), P. oleracea seed powder was added as 5 & 10 g.kg⁻¹ for the 2nd and 3rd treatments. Leaves powder was added as 5 and 10 g.kg⁻¹ to the basal diet in treatment 4th and 5th. In the 6th treatment 5 gm of seed and 5 gm of leaves powder was added to the basal diet. The results showed that there was a significant (P <0.05) decrease in serum glucose, cholesterol and triglyceride concentrations in all additive treatments compared to control. There was also a significant decrease (P <0.05) in Escherichia coli bacteria count, as well as a significant improvement (P < 0.05) in the number of Lactobacilli bacteria in all addition treatments compared to the control group. The addition of *P. oleracea* seeds and leaves powder had no significant effect on some haematological indices and on the total protein concentration in the serum. The study confirms the importance of adding powder of seeds or leaves in the broiler diet because it has a positive role in improving some of the physiological and microbial blood characteristics of the broiler.

Keywords: Portulaca oleracea, Broiler chicks, Physiological, Microbial characteristics.

Introduction

The poultry industry is witnessing an increasing growth in production, either meat or eggs, which requires providing balanced diets that meet the increase in the number of birds on the one hand and the rapid broiler growth on the other hand, with the necessity of the diet linked to the quality of the product and the extent of acceptance by the consumer (Oz *et al.*, 2017). As a result of the harm caused by the antibiotics used in feeding poultry to human and animal health through the resistance of pathogens and their residues in poultry meat (Soltan *et al.*, 2008; Dalkili & Guler, 2009). So, the researchers tended to use medicinal plants and

herbs as safe feed additives in poultry diets, as they have a positive role in stimulating growth, improving the immune system and physiological traits of birds (Jugl-Chizzola et al., 2006; Özer et al., 2007; Al-Salhie & Al-Waeli, 2019; Hussein & Jassim, 2019; Sultan et al, 2019; Al-Salhie & Makki, 2020). In addition to stimulating beneficial microorganisms within the intestines of birds, which contributes to the digestion of many nutrients and make them available to birds (Tekeli et al., 2006). Therefore, it has become a modern practice to include poultry diets natural antioxidants to increase bird productivity and resistance to many common diseases (Ognik et al., 2016).

Portulaca oleracea plant is one of the natural additives because it contains many biologically active compounds such as sugars, proteins and fats (Aberoumand, 2009), its high content of minerals such as calcium, potassium, magnesium and iron (Uddin et al., 2012), in addition beta-carotene, glutathione, to melatonin (Naeem & Khan, 2013). This plant is rich in essential amino acids and linoleic acid (omega 3) in addition to tocopherol and ascorbic acid, these compounds improve meat quality, flavour, and total acceptance (Uddin et al., 2014). The addition of P. oleracea seed powder to the quail and broiler diet improved the production and enhanced the immune system (Konca et al., 2015; Kartikasari et al., 2018). When leaves powder was used, birds showed better performance and physiological parameters (Ghorbani et al., 2014; Habibian et al., 2017).

This study was conducted to find out the impact of *P. oleracea* seeds and leaves powder on some physiological traits of broiler and its effect on the intestine's microorganisms.

Materials & Methods

This study was conducted in the Poultry Fields of the Consulting Office of the College of Agriculture, University of Basrah for the period from 20/9/2019 to 24/10/2019 for 35 days. A total of 216 unsexed one day old Ross 308 chicks with an average weight of 38 g were purchased from a private Al-Taji hatchery in Baghdad province. The chicks were randomly distributed into six treatments, at the rate of three replicates per treatment and 12 birds per replicate in. The chicks were raised in 18 cages (70 × 70cm), with a height of 30 cm from the floor.

All necessary managements for rearing practices from providing heat and ventilation within the ideal limits for the growth of chicks. The chicks were *ad-libitum* fed on a starter diet for 1–21 days and a final ration for the period 22-35 days (Table 1). Chemical analysis of P. oleracea seeds and leaves powder were carried out according to AOAC (2016) (Table 2). The included experiment six experimental treatments, the first treatment was control (the basal diet), and *P. oleracea* seeds powder was added to the basal diet at levels of 5 or 10 (g.kg⁻ ¹) in the second and third treatment respectively, and leaves powder at levels of 5 or 10 $(g.kg^{-1})$ in the fourth and fifth treatments respectively, and mixing of seeds and leaves powder at level 5 + 5 (g.kg⁻¹) in the sixth treatment throughout the trial period.

Blood samples (5 ml.bird⁻¹) were collected from the birds during their slaughtering at the age of 35 days (3 birds per treatment). Samples distributed to two types of tubes the first type was free of coagulation material was placed after taking blood samples in a centrifuge (3000 rpm) to separate the blood components. Serum kept at a degree (-20) $^{\circ}$ C to estimate total

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protein, glucose, cholesterol and triglycerides following the instructions in the manual attached with the kits for each estimate.

Feed ingredients	Starter diet (%) 1-21 days	Finisher diet (%) 22-35 days				
Corn	58.00	60.00				
Wheat	4.00	4.00				
Soy bean meal (48%)	31.00	29.00				
Plant oil	1.00	1.00				
Protein concentrate $(40\%)^*$	5.00	5.00				
Lime stone (CaCO3)	0.70	0.70				
Vitamins & minerals premix	0.15	0.15				
Table salt (NaCl)	0.15	0.15				
Total	100	100				
Calculated chemical analysis**						
Metabolizable energy (kcal.	3010	3035				
_Kg ⁻¹)						
Crude protein (%)	22.37	21.58				
Raw fat (%)	2.76	2.82				
Crude fibre (%)	3.72	3.62				
Calcium (%)	0.63	0.61				
Available phosphorous (%)	0.30	0.33				
Lysine (%)	1.19	1.14				
Methionine + Cysteine (%)	0.83	0.81				
Energy: protein	134.55	140.64				

Table (1).: Ingredients and chemical composition of basal diet.

* Protein concentrate (Brocorn-5 special) produced by Allblasserdam-Holland Wafi B. V. contain 40% protein, 2107 Kcal.kg⁻¹ metabolizable energy, 5% fat, 2.20% crude fiber, 7.10% moisture, 28.30% crude ash, 4.2% calcium, 2.65% total phosphorus, 4.65% available phosphorus, 3.85% lysine, 3.70% methionine, 4.12% methionine+ cysteine, 0.42% tryptophan, 1.70% sodium, 4.20% chloride, 200 mg.kg⁻¹ Cu, 1.600 mg.kg⁻¹ Mn, 2000 mg.kg⁻¹ Zn, 2000 mg.kg⁻¹ Fe, 20.00 mg.kg⁻¹ Iodine, 5.00 mg.kg⁻¹ Se.

** Chemical analysis according to NRC (1994).

Table (2) : Chemical analysis of *P. oleracea* seeds and leaves powder based on dry weight.

Chemical composition (%)							
Sample	Dry matter	Raw fat	Crude protein	Total ash	Dissolved carbohydrates	Crude fibre	Metabolizab le energy kcal. Kg ⁻¹
Watercress seeds powder	91.4	11.03	11	10.7	54.27	13	2939.183
Watercress leaves powder	98.27	5.16	24	13.33	48.85	8.66	2753.757

The complete quantity of lipid peroxidation products in serum was tested using the thiobarbituric acid (TBA) technique, measuring reactive spectrophotometric malondialdehyde (MDA) materials at 532 nm. The second type of tube contains an EDTA anticoagulant to estimate some of the blood characteristics, which included the number of red and white blood cells. PCV and haemoglobin concentration (Al-Daraji et al., 2008). Also, the bursa of fabricius gland was isolated from the carcasses of birds at the age of 35 days and was weighed to determine the relative weight concerning the body weight (Al-Fayyad et al., 2011).

The intestinal microbial characteristics were studied by taking a sample of 1g of intestinal contents from the jejunum under aseptic conditions and transferred by sterile bottles to the laboratory within a period not exceeding 40 minutes and after making decimal dilutions with peptone water, the total, *E. coli* and Lactobacilli bacteria count were performed as described by Harrigan & McCance (1976).

A completely randomized design (CRD) was used for statistical analysis. Mean differences were tested by the revised least significant differences (RLSD) with the SPSS program (SPSS, 2012, version 21).

Results & Discussion

Blood characteristics

The effect of adding different levels of P. oleracea seeds and leaves powder on some haematological parameters was shown in table (3). As it is clear that there was no significant effect on the numbers of red and white blood cells, haemoglobin concentration and the PCV among all experimental treatments. These results were not in agreement with those of Sadeghi et al. (2016) who found significant differences in blood indices (RBC, WBC, PCV and Hb) of broiler chicks fed a ration with P. oleracea seed and leaves powder (1.00, 0.75, 0.50, 0.25,0)% compared to control. Also, it did not agree with Habibian et al. (2017) that there were significant differences P <0.05 in the blood indices (RBC, WBC, PCV and Hb) of broiler chicks fed a ration with P. oleracea seed and leaves powder (0, 1.5, 3.5 and 4.5) g. kg^{-1} , where 4.5 g. kg⁻¹ gave the best results compared to other treatments at 49 days of age. The reason is due to the different age of birds, as well as the difference in the levels of addition of seed powder.

 Table (3). The effect of adding P. oleracea seeds and leaves powder on some blood indices of broiler chicks at the age of 35 days (Mean± SD)

Treatments	RBC	WBC	Hb	PCV %
	$(x10^6 \text{ cell.ml}^{-1})$	$(x10^3 \text{ cell.ml}^{-1})$	(gm.100 ⁻¹ ml)	
T1	2.38±0.14a	21.86±0.58a	7.14±0.05a	27.00±0.58a
T2	2.73±0.00a	21.86±0.02a	7.27±0.00a	27.67±0.33a
T3	2.54±0.19a	21.86±0.02a	7.22±0.06a	28.33±0.33a
T4	2.61±0.07a	21.84±0.02a	7.27±0.01a	27.33±0.88a
T5	2.54±0.19a	21.87±0.00a	7.26±0.02a	27.00±0.58a
T6	2.48±0.14a	21.87±0.00a	7.25±0.01a	27.68±0.33a

T1= control, T2=adding 5 g.kg⁻¹ *P. oleracea* seed powder, T3=10g.kg⁻¹ *P. oleracea* seeds powder, T4=5 g.kg⁻¹ *P. oleracea* leaves powder, T5=10 g.kg⁻¹ *P. oleracea* leaves powder, and T6=5+5 g.kg⁻¹ *P. oleracea* seed+ leaves powder. The same letters vertically are not significant at (P<0.05).

Biochemical parameters

Table (4) shows the effect of different levels of P. oleracea seeds and leaves powder on serum biochemical parameters of 35 days old broiler chicks. The absence of significant effect among all levels of addition and control treatment in total protein concentration is evident. However, there was a significant decrease (P < 0.05) in the glucose concentration in T2-T6 serum compared to the control treatment in which the concentration of glucose was 288.37 (mg.100 ml⁻¹), and the T4 treatment recorded the lowest concentration of glucose (236.25 mg.100 ml⁻¹). This decrease is attributed to the presence of active compounds in the seeds and leaves P. oleracea, as it has some flavonoids that act similar to insulin action and increase glucose transporters and thus are able to reduce and lower the level of glucose in the blood (Kim *et al.*, 2005).

It was also noted from table (4), a significant decrease in $(P \le 0.05)$ in the cholesterol concentration was achieved with seed and leaf of P. oleracea treatments (T2, T3, T4, T5 and T6) compared to the control treatment. The low cholesterol level in the serum could be due to the presence of a high percentages of oleic acid in the seeds and leaves of the P. oleracea, which improves the health and immune status of the bird and thus lowers the level of cholesterol in the blood (Konca et al., 2015). Likewise, the role of fatty acids, especially (omega 3), works to reduce cholesterol by transporting it to the intestine and oxidized by bile secretions (Uddin et al., 2014).

 Table (4). Effect of P. oleracea seed and leaves powder on some biochemical parameters of broiler chicks (Mean± SD)

Treatments	Total protein	Glucose	Cholesterol	Triglycerides
	$(g.100 \text{ ml}^{-1})$	$(mg.100ml^{-1})$	(mg.100ml ⁻¹)	$(mg.100 ml^{-1})$
T1	4.50±0.24a	288.37±4.90a	162.00±2.31a	125.18±1.74a
T2	4.88±0.40a	270.48±5.51a	105.54±2.33c	110.29±1.22b
T3	4.55±0.21a	277.77±4.22a	124.08±3.47b	105.76±1.14c
T4	4.44±0.00a	236.25±6.96c	122.14±2.80b	114.96±1.47b
T5	4.15±0.01a	240.29±3.20c	125.84±4.67b	105.53±1.50c
T6	4.57±0.05a	263.20±5.31b	120.73±3.00b	94.61±1.79d

Different letters vertically are significant at (P<0.05), T1=control, T2=5g.kg⁻¹ *P. oleracea* seed powder, T3= 10 g.kg⁻¹ *P. oleracea* seed powder, T4= 5g.kg⁻¹ *P. oleracea* leaves powder, T5= 10 g.kg⁻¹ *P. oleracea* leaves powder, T6= $(5g+5g).kg^{-1}$ *P. oleracea* seed and leaves powder.

Table (4) has shown the effect of the additional levels on the level of triglycerides. It was noticed that a significant decrease (P <0.05) in the concentration of triglycerides in the blood serum concerning the seeds and leaves powder of *P. oleracea* compared to the T1 control (125.18 mg.100ml⁻¹), the most

significant decrease in T6 (94.61mg.100ml⁻¹). The decrease in the concentration of triglycerides in the blood serum as a result of the adding seeds and leaves powder of *P*. *oleracea* is due to the high level of linolenic acid that prevents the synthesis of fatty acids and triglycerides in the liver (Gill *et al.*, 1997).

These results agree with those of Ghorbani *et* (2016), and Habibian *et al.* (2017), who proved that *P. oleracea* seed and leaves powder caused a reduction in cholesterol and triglycerides of broiler chicks.

Immunity characters

The effect of adding different levels of Portulaca oleracea seeds and leaves powder on the immune characteristics of broilers at the age of 35 days (Table 5). The results indicated that there was no significant effect on fabricius gland and gland index. However, there was a significant (P<0.05) on some oxidant indices as thiobarbituric acid (TBA) level of chicks' serum fed diets contain P. oleracea seeds or leave powder, as TBA levels of treatments 2, 3, 4, 5 and 6 decreased (P<0.05) in comparison with the control group. Where the occurrence of oxidative rancidity is considered a criterion for increasing the oxidative indicators in the blood serum and as a result of this oxidation, aldehyde compounds are formed that interact these compounds with acid TBA (Zeb, 2015).

al. (2013), Konca et al. (2015), Sadaghi et al. Malondydehyde (MDA) It is considered a measure of the oxidation of unsaturated fatty acids and its knowledge of peroxidation of fats and this peroxide that works to decompose unsaturated fatty acids and thus get rid of the fats accumulated in the membranes and prevent the formation of aldehydes. The common way to measure MDA is the interaction of Thiobarbituric acid (TBARS) with Thiobarbituric acid materials (TBA) at 532 nm reduction due to the addition of P. oleracea seeds or leaves powder may be attributed to the presence of lipid peroxide that degrades unsaturated fatty acids in cellular membranes by self-eliminating free radicals to form (hydroperoxides) fat and thus preventing the formation of aldehyde compounds because increasing malondydehyde means to increase the effectiveness of free radicals (Zeb & Ullah, 2016).

Treatments	Fabricius gland (%)	Fabricius gland	TBA(µ mol.ml ⁻¹)
		index	
T1	0.177±0.009a	1.000±0.000a	0.2020±0.0289a
T2	0.179±0.013a	1.043±0.058a	0.167±0.0018b
T3	0.175±0.014a	1.053±0.022a	0.1567±0.0027c
T4	0.180±0.014a	1.040±0.010a	0.1433±0.0026d
T5	0.169±0.012a	1.063±0.012a	0.0970±0.000f
T6	0.171±0.014a	1.037±0.013a	0.1217±0.0024e

Table (5). The effect of P. oleracea seed or leaves powder on fabricia gland, gland index and TBAof broiler chicks at the age of 35 days (Mean± SD).

Different letters vertically are significant at (P<0.05), T1=control, T2=5g.kg⁻¹ *P. oleracea* seeds powder, T3= 10 g. kg⁻¹ *P. oleracea* seeds powder, T4= 5 g.kg⁻¹ *P. oleracea* leaves powder, T5= 10 g. kg⁻¹ *P. oleracea* leaves powder, T6= (5g+5g). kg⁻¹ *P. oleracea* seed and leaves powder.

Jejunum total number of bacteria, *E. coli* and Lactobacilli

Table (6) shows the total bacteria count, E. coli and Lactobacilli in the jejunum of broiler chicks at the age of 35 days. A significant difference was observed in the total bacteria count, E. coli bacteria and Lactobacilli among all levels of addition and control treatment. The results of the statistical analysis indicated that there was a significant decrease (P < 0.05) in the number of total bacteria and E. coli in all levels of addition compared with the control treatment, which recorded the highest number of total bacteria and colon bacteria (76.00 and 361.67 respectively). The lowest levels of this bacterium were recorded in the treatments of the P. oleracea leaves, followed by the treatments of the P. oleracea seed. Whereas, the numbers of Lactobacilli bacteria increased significantly (P<0.05) in all addition treatments compared to the control, and the highest increase in their numbers was recorded in the P. oleracea leaves, followed by the seed's treatments. Perhaps microbial changes in the intestinal environment of the jejunum part of the small intestine are due to the effect of the P. oleracea on many pathogenic microbes as a

result of the presence of inhibitory substances and other killers' substances against many harmful bacteria and non-important ones (Ghorbani et al., 2013). Seeds and leaves of P. oleracea contain many antioxidant and antibacterial and fungi substances (Smith et al., 2003), and anti-toxic materials (Simopoulos et al., 1992). These results are similar with those of Zhao et al. (2013). There was a significant decrease (P<0.05) in the number of coliform bacteria (E. coli), while there was a significant increase in the number of Lactobacilli bacteria in the intestine of broilers fed on feeds to which watercress leaves were added at levels (0, 0.2)and 0.4% compared with the control at the age of 42 days. Sadeghi et al. (2016) found was a significant decrease (P < 0.05) in the number of coliform bacteria (E. coli), while there was a significant increase in the number of Lactobacilli bacteria in the intestine of broiler meat fed on feeds to which watercress seeds were added at levels (0, 0.25, 0.50, 0.75 and 1.00,) % compared to control at 43 days old. The reason is due to the difference in the proportions of the addition of seed powder and watercress leaves in the bush, as well as the difference in the age of the broiler chicks.

Table (6): Effect of P. oleracea seeds and leaves powder on the number of microbes in the
jejunum of broiler at the age of 35 days (means \pm SD).

Treatments -	Number of bacteria (log ₁₀ CFU.g ⁻¹)			
Treatments	Total bacteria	Lactobacilli	E. coli	
T1	$361.67^{e} \pm 1.45$	$143.00^{d} \pm 1.15$	$76.00^{d} \pm 1.15$	
T2	$266.33^{a} \pm 1.45$	$164.00^{\rm b} \pm 2.31$	$55.00^{b} \pm 2.52$	
T3	$280.33^{b} \pm 1.45$	$159.00^{bc} \pm 2.89$	$52.67^{b} \pm 1.20$	
T4	$292.33^{\circ} \pm 1.76$	$177.33^{a} \pm 2.19$	$43.33^{a} \pm 1.45$	
T5	$289.00^{\circ} \pm 1.53$	$155.67^{c} \pm 2.91$	$61.67^{c} \pm 3.28$	
T6	$305.00^{d} \pm 1.53$	$162.33^{bc} \pm 1.45$	$54.33^{b} \pm 2.40$	

Different letters vertically are significant at P<0.05, T1=control, T2=5g.kg⁻¹ *P. oleracea* seed powder, T3= 10 g. kg⁻¹ *P. oleracea* seed powder, T4= 5 g.kg⁻¹ *P. oleracea* leaves powder, T5= 10 g. kg⁻¹ *P. oleracea* leaves powder, T6= (5g+5g). kg⁻¹ *P. oleracea* seeds and leaves powder.

Conclusion

Addition of either 5 or 10 g. Kg⁻¹ from *P*. *oleracea* seeds or leaves powder has no significant impact on blood RBC, WBC, Hb, and PCV. However, serum triglycerides, glucose and cholesterol as well as TBA were significantly reduced. There was a significant reduction in all types of bacteria (Total bacteria, Lactobacilli and *E. coli*) due to the addition of *P. oleracea* seeds or leaves powder.

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Conflict to interest There is no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

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تأثير اضافة مستويات مختلفة من مسحوق بذور وإوراق البقلة .Portulaca oleracea L الى العليقة في بعض الصفات الفسلجية والميكروبية لفروج اللحم

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المستخلص: اجريت هذه الدراسة في حقل الدواجن التابع المكتب الاستشاري في كلية الزراعة، جامعة البصرة للمدة من *Portulaca* التي المكتب الاستشاري في كلية الزراعة، جامعة البصرة للمدة من *Portulaca* (Ros-2019/20 لدراسة تأثير اضافة مستويات مختلفة من مسحوق بذور وأوراق البقلة *Portulaca* (Ros-308) *objected* في بعض الصفات الفسلجية لفروج اللحم ، استخدم بالتجربة 216 فرخاً من فروج اللحم (Ros-308) *objected* في بعض الصفات الفسلجية لفروج اللحم ، استخدم بالتجربة 216 فرخاً من فروج اللحم (Ros-308) بعمر يوم واحد غير مجنسة وزعت عشوائياً على ست معاملات تجريبية وبواقع ثلاثة مكررات للمعاملة الواحدة، 12 فرخاً لكل مكرر وفق التصميم العشوائي الكامل (CRD). كانت المعاملة الاولى السيطرة (العليقة الاساسية) وأضيف مسحوق بذور البقلة الى العليقة الاساسية) وأضيف مسحوق بذور البقلة الى العليقة الاساسية) وأضيف مسحوق بذور البقلة (غم.كذم⁻¹¹) في معاملة الاولى السيطرة (العليقة الاساسية) وأضيف مسحوق بذور البقلة (غم.كذم⁻¹¹) في معاملة الرابعة والثالثة ومسحوق أوراق البقلة بالمستويين 5 و 10 (غم.كفم⁻¹) بالمعاملة الثانية والثالثة ومسحوق أوراق البقلة بالمستوين 5 + 5 (غم.كفم⁻¹) في المعاملة السادسة على التوالي .اظهرت النتائج حصول انخفاض (20.0<P) في تركيز كلوكوز في مصل الدم في جميع معاملات الاضافة مقارنة (بلاسير ووراق البقلة مالمرت النتائية والثالثة مصحوق أوراق البقلة مسحوق بذور والوراق البقلة مالمستوي 5 + 5 (غم.كفم⁻¹) في المعاملة السادسة على والوراق البقلة والمستوي 5 + 5 (غم.كفم⁻¹) في معاملة السادسة على والوراق البقلة بالمستوي 5 + 5 (غم.كفم⁻¹) في معاملة السادسة على والوراق البقلة بالمستوي 5 + 5 (غم.كفم⁻¹) في معاملة السادسة على والوراق البقلة والزلاية في مصل الدم في جميع معاملات الاضافة مالوران التوالي .اظهرت معنوي في تركيز الكولستيرول والكليسيريدات الثلاثية في مصل الدم في جميع معاملات الاضافة مالوران بالميون وي والوراق البقلة معادي وي والوراق البقلة مالامي في مصل الدم عند اضافة مسحوق بذور واوراق البقلة مقارنة بيقية المعاملات. كما حصول انخفاض معنوي (20.0>P) في اعداد بكتريا المضافة ماحوي الورارق البقلة مالان وي الكولي في معاملة الامي في تركيز الكولي في تركيز الكولي في معامل وي والدواية معامل الذم على وي الكولي في مركوا وفي تركولي وفي تركي والوران

الكلمات المفتاحية: البقلة، فروج اللحم ،الصفات الفسيولوجية ،الميكروبية.

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