Effects of the Manufactured Bacterial Preparation on Microbial Characteristics of Broilers

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Abstract:

This experiment was conducted in the poultry field of the department of Animal Production at the College of Agriculture at Basrah University for the period from 25/3/2021 to 28/4/2021, in which 450 broiler chicks, Ross 308 strain, one day old and an average weight of 43 g/chick were used, Chicks were raised in a three-storey battery system, and each floor contains a cage of 1.5 x 1 m^2 dimension. Chicks were randomly distributed to 10 experimental treatments, with 45 chicks for each treatment. One treatment included three replicates (15 chicks/duplicate), including the transactions, T1: Negative control coefficient (a basal diet without supplement). T2: Positive control treatment, adding dried skim milk at a level of (1)g/L drinking water. T3, T4, T5: adding the Manufactured Bacterial Preparation at a level (0.5, 1,2) g/kg feed, respectively. T6, T7, T8: adding the Manufactured Bacterial Preparation at a level (0.5, 1,2) g/L drinking water, respectively. T9: adding the Manufactured Bacterial Preparation at a level of (0.5) g/kg feed, and (0.5) g/L drinking water. T10: adding the Manufactured Bacterial Preparation at a level (1)g/kg feed, and (1)g/L drinking water. The results indicated that there is a significant decrease ($P \leq 0.05$) in the logarithmic indicators of coliform bacteria with a significant increase ($P \leq 0.05$) in the numbers of lactic acid bacteria in favor of the processed bacterial culture treatments compared to the two control treatments.

Keywords: Lactic Acid Bacteria, *Lactobacillus*, Microbial Count, chicken intestines.

Introduction:

It has become necessary to spread the concept of biosecurity within the poultry fields; Because of its great role in preventive medicine and its economic impact. The meaning of the term "Biosecurity" is comprehensive, and it is difficult to limit it to a specific definition. Still, it is developing according to its use. It means that in poultry farming projects, harmful microorganisms are isolated as much as possible except for those obtained by birds through water or feed, which are frequently different from the microorganisms that grow in poultry intestines (Hwang and Singer, 2020).

Because of the difficulty of controlling microorganisms, specifically pathogenic ones, many of those interested in poultry farming projects have contributed to the use of antibiotics to reduce or

reduce pathogenic bacteria, to enhance biosecurity, but this was negatively reflected by the emergence of bacterial species resistant to antibiotic treatments such as *Salmonella* and Coliform bacteria. Which are endemic to most poultry farms (Alnajjar and Alemadi, 2017); Because it has resistance against to some types of antibiotics, with made the World Health Organization forbids the use of some types of these antibiotics in poultry farming for fear of passing them on to the consumer (Agboola *et al.*, 2015).

To enhance the concept of bio-security, which contributes to achieving microbial balance and supporting the digestive system with beneficial bacteria, which is ultimately reflected in improving the health status of poultry birds, as indicated by Mahmmod *et al.* (2014), contributed to a significant decrease in the number of coliform bacteria in the jejunum region of the small intestine compared to the control. Ahmed and Manati (2015) noted that the use of probiotics contributes to the establishment of microbial balance by reducing the numbers of total bacteria and coliform bacteria and increasing the numbers of lactic acid bacteria compared to the control treatment.

It was noted in an experiment conducted on broilers that the probiotic was used at a weight of 80 g per kg of feed, as it was pointed out that there were no significant differences in the numbers of lactic acid bacteria and coliform bacteria in the fasting area. However, there were arithmetic differences in favour of the probiotic treatment compared to the control treatment (Adli and Sjofjan, 2020). The latest studies published within the current period, which were carried out by Zhang *et al.* (2021), when adding 1% of the probiotic consisting of *Lactobacillus acidophilus* bacteria at several 5×10^9 (cfu/g), added to 10 ml of distilled water and presented to broilers, where the study confirmed that the probiotic contributes to increasing the numbers of lactic acid bacteria and reduces the numbers of coliform bacteria, in favour of the probiotic treatment compared to the control treatment.

In continuation of the previous studies, the current research intends intending to study the effect of using the manufactured bacterial preparation, one gram of which contains no less than16 $\times10^9$ (cfu /g), with seven different bacterial types of lactic acid bacteria (Al-Salhi, 2022) In to the microbial characteristics of broilers.

Materials and Methods:

Experience design:

This experiment was conducted in the poultry field of the department of Animal Production at the College of Agriculture at Basrah University, from 25/3/2021 to 28/4/2021, for 35 days. 450 experimental broiler chicks, Ross 308 strain, were used in this experiment. With an age of one day and an average weight of 43 g/chick, the chicks were raised in a three-storey battery system. Each floor contains a cage with dimensions of 1.5 x 1 m^2. The sexed chicks were randomly distributed over ten experimental treatments, with 45 chicks. For each treatment, and one treatment included three replicates (15 chicks/duplicate), the experimental treatments were organized as follows:

T1: Negative Control Treatment, basal diet (without any addition).

T2: Positive Control Treatment, adding powdered skim milk at a level of (1) g / L of drinking water.

T3: Adding manufactured bacterial preparation at a level of (0.5) g/kg of feed.

T4: Adding manufactured bacterial preparation at a level of (1)g/kg of feed.

T5: Adding manufactured bacterial preparation at a level of (2)g / kg of feed.

T6: Adding manufactured bacterial preparation at a level (0.5) g / L of drinking water.

T7: Adding manufactured bacterial preparation at a level of (1)g/L of drinking water.

T8: Adding manufactured bacterial preparation at a level of (2)g / L of drinking water.

T9: Adding manufactured bacterial preparation at a level of (0.5) g/ kg of feed and (0.5) g / L of drinking water.

T10: Adding manufactured bacterial preparation at a level of (1)g / kg of feed and (1)g / L of drinking water.

Chick management:

The room temperature was regulated according to the mercury thermometer from the age of one day until the age of 35 days using the electric heating system and air intakes, according to the broiler breeding guide, and the 24-hour continuous lighting system was adopted throughout the breeding period while providing all the conditions for raising broilers. The water to which the different doses of the manufactured bacterial preparation were added was prepared (according to the symbols of the treatments mentioned above), as presented in inverted 6-litre plastic buckets, to facilitate the process of drinking water freely. Plastic with a diameter of 38 cm. The birds were fed two types of rations: the starter ration from (1-21) days of age, which contained 23.20% crude protein and 2913 kcal/kg of energy as a representative feed, and a final ration from the age of (22-35) days. It also contained 19.72% crude protein and 3164 kcal/kg of feed as representative energy, and table (1) shows the chemical composition of the feed used in the experiment.

Table(1): Chemical composition of basal diet used in the experiment and its chemical analysis

Ingredients	Starter (1-21) day	Finisher (22-35) Day
Yellow corn	42	50
Wheat	17.2	15
Soybean meal (48%)	32	24
Protein (40%)	5	5
Primex	1	1
Plant oil	0.5	3.2
Limestone	2	1.5
Salt	0.3	0.3
Total	100	100
Calculated chemical composition		
Crude protein %	23.2	19.72
Metabolizable energy	2913	3164
Energy-to-protein ratio	125.55	160
Crude fibre %	3.9	3.45
Calcium %	1.07	0.88
Available phosphorus %	0.48	0.34
Methionine %	0.5	0.45
Lysine%	1.2	1.02
Methionine + Cysteine %	0.87	0.68

•Protein concentrate: Produced by the Dutch Brocon Company, it contains 40% crude protein and 2107 (kcal/kg) represented energy, 5% fat, 4.20% calcium, 2.65% phosphorous, available phosphorous 4.68%, 3.70% methionine, 0.66% Cysteine, 3.85% lysine, 20.2 crude fiber, 12.4% methionine and cysteine.

•The chemical composition of the materials included in the diets was calculated according to the recommendations of the NRC(1994) .

The cultural media used in the study:

The culture media used in the study was prepared according to the manufacturer's instructions and then sterilized by an Autoclave at a temperature of 121°C, and at a pressure of 15 pounds/inch ^{2}, for 15 minutes, then left until its temperature drops to 45° C, to be poured in dishes, under sterile conditions inside (Hood), according to the study scheme, where the study included the use of a variety of culture media, including the following (Da Silva *et al*,. 2019).

Nutrient Agar:

It was prepared according to the instructions of the British company (Oxoid), the incubation at 35 °C for 24-48 hours, after which the developing colonies in the dishes were counted, numbered between 30-300 territories, and it was used in the total plate count.

MacConkey Agar:

It was prepared according to the instructions of the Indian company (HIMEDIA), the incubation at 37° C for 24-48 hours, after which the developing colonies of red or pink color were counted, and it was used to estimate the numbers of coliform bacteria.

M.R.S Agar:

It was prepared according to the instructions of the Indian company (HIMEDIA), the incubation at 37°C for 48-72 hours under anaerobic conditions and used for counting and microbial isolation of lactic acid bacteria.

Peptone Water 0.1%:

Prepared by dissolving 1 g of peptone in one litre of distilled water, distributed to laboratory tubes, sterilized tubes at 121°C for 15 minutes and used in decimal dilutions for bacterial counting.

Estimation of bacterial colonies in the jejunum region:

Total Bacteria:

Microbial counting of total bacteria was carried out by pouring plate method (Da Silva *et al.*, 2019) by taking 1 gm of intestinal contents and transferring them to a test tube containing 9 ml of 0.1% peptone water, and shaking it well to obtain a homogeneous bacterial culture, several decimal dilutions of this diluent were made, then 1 ml of these series of dilutions was transplanted to Petri dishes, then 15-20 ml of medium was added after nutrient agar, stirring and homogenizing the agar was, left to solidify. The plates were incubated under air conditions at 35°C for 24 hours. The developing colonies were counted employing a colony counter and the number of bacteria (cfu/g) calculated was by multiplying the average number of colonies for two plates × the inverted dilution.

Lactic Acid Bacteria:

The same method (Da Silva *et al*,. 2019), was adopted to estimate the numbers of lactic acid bacteria using an MRS Agar culture medium. The dishes were incubated in anaerobic conditions inside the incubator at 37°C for (48-72) hours.

Coliform Bacteria:

The casting method mentioned by (Da Silva *et al*,. 2019), was adopted to estimate the numbers of coliform bacteria using a MacConkey Agar culture medium, and they were incubated in aerobic conditions inside the incubator at a temperature of 37°C for 24-48 hours, during which the red or pink colonies were counted.

Statistical analysis:

The complete random design (CRD) was used to study the effect of different treatments on the studied traits. The significant differences between the means were compared by the Duncan Test polynomial under the significance level of 0.05 (Duncan,1955). The program SPSS (2018) was used in the statistical analysis.

Results and Discussion:

The effects of a Manufactured Bacterial Preparation on the logarithmic numbers of bacteria

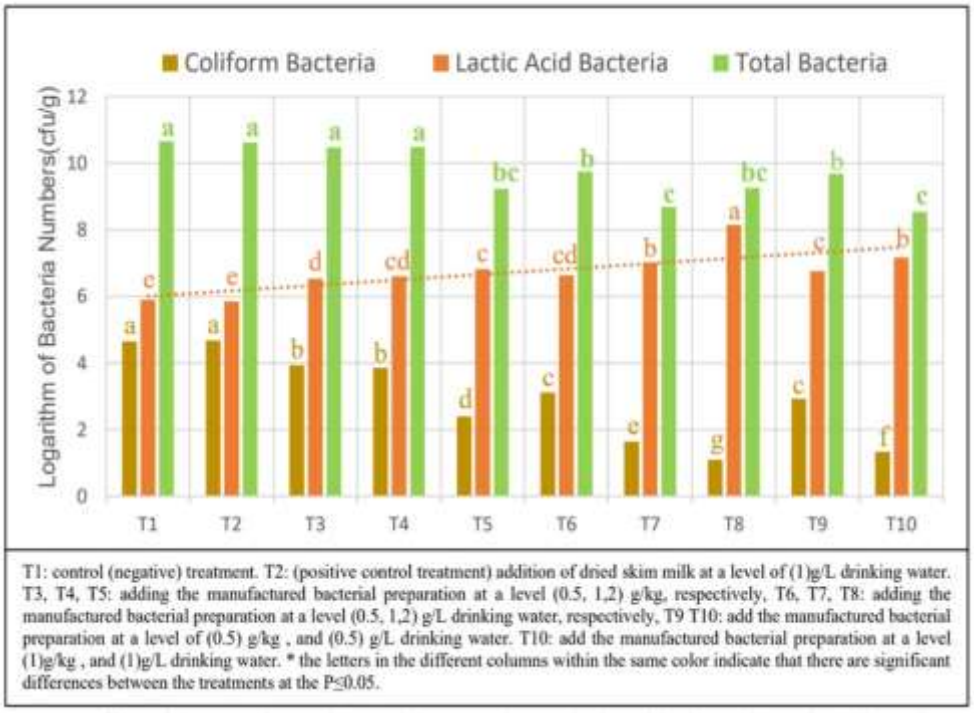
Fig. (1) shows the effect of the Manufactured Bacterial Preparation on the logarithmic numbers of total bacteria, lactic acid bacteria and coliform bacteria in the small intestine (the jejunum) of broilers at the age of 35 days. As we notice in the total bacteria index that, there are no significant differences between the treatments (T1, T2, T3 and T4). The results also did not record significant differences between treatments T5 and T8 on the one hand, between (T6 and T9), and between T10 and T7) on the other hand.

The results showed that there were significant differences ($P \leq 0.05$) in favour of treatments T1, T2, T3, and T4) compared to the remaining group of treatments (T5, T6, T7, T8, T9 and T10). In the indicator of lactic acid bacteria, it was noticed that a significant ($P \leq 0.05$) superiority was obtained in the logarithmic numbers of lactic acid bacteria in favour of treatment (T8), which included adding (2 g) of the manufactured product to a litre of drinking water over the rest of the experimental treatments. While the two control treatments did not record significant differences between them (T1 and T2), it was noted that no significant differences occurred between some treatments of the manufactured preparation (T3, T4, T5, T6 and T9), and the results did not show any significant differences between the two treatments (T7 and T10), although there were arithmetic differences between the coefficients of the manufactured preparation, which were associated with the level of addiction. With regard to the indicators of coliform bacteria a significant ($P \leq 0.05$) decrease was gradually observed in the logarithmic numbers of coliform bacteria, in favour of the treatments of the manufactured product. On the contrary, as shown in the indicators of lactic acid bacteria, (by increasing the concentration of lactic acid bacteria, the indicators of lactic acid bacteria decreased coliform bacteria in the same treatments), while the two control treatments did not record any significant differences between them.

The increase in the numbers of lactic acid bacteria in the treatments of the manufactured bacterial preparations was due to the effects of the preparations on seven different types of lactic acid bacteria, which work synergistically in confronting coliform bacteria and other pathogenic types under study, which contributed to reducing their numbers in the treatments of the manufactured bacterial preparations. The change of the environment of the small intestine to an acidic environment, as well as the result of its secretion of a wide range of metabolic products, which are considered inappropriate for the activity of pathogenic bacteria, is what achieves the principle of microbial balance and improves the functions of the digestive system, which is ultimately reflected in the promotion of the general health of broilers and increased production.

This result agreed with what was observed by Mahmmod *et al.* (2014). Furthermore, adding the Iraqi probiotic (1kg/100kg) in to the diet contributed to a significant decrease in the numbers of coliform bacteria in the jejunum region of the small intestine compared to the control. And to the findings of Ahmed and Manati (2015) that the use of probiotics contributes to the establishment of microbial balance by reducing the numbers of total and coliform bacteria and increasing the numbers of lactic acid bacteria compared to the control treatment.

It did not agree with the findings of both Adli and Sjofjan (2020) in not obtaining significant differences in the numbers of lactic acid bacteria and coliform bacteria in the jejunum region, despite the presence of arithmetic differences in favour of the treatment of the probiotic compared to the control treatment, in their study conducted on broilers, in which the probiotic was used at a weight of 80 grams per kg of feed, while it agreed with the findings of Zhang *et al.* (2021) when adding 1% of the probiotic to 10 ml of distilled water, provided to broilers, which confirmed. The study found that the probiotic contributes to increasing the numbers of lactic acid bacteria and reduces the numbers of coliform bacteria, favouring the probiotic treatment compared to the control treatment.



T1: control (negative) treatment. T2: (positive control treatment) addition of dried skim milk at a level of (1)g/L drinking water. T3, T4, T5: adding the manufactured bacterial preparation at a level (0.5, 1,2) g/kg, respectively, T6, T7, T8: adding the manufactured bacterial preparation at a level (0.5, 1,2) g/L drinking water, respectively, T9 T10: add the manufactured bacterial preparation at a level of (0.5) g/kg , and (0.5) g/L drinking water. T10: add the manufactured bacterial preparation at a level (1)g/kg , and (1)g/L drinking water. * the letters in the different columns within the same color indicate that there are significant differences between the treatments at the P≤0.05.

Fig. (1) Effect of the Manufactured Bacterial Preparation on the logarithmic numbers of total bacteria, lactic acid bacteria and coliform bacteria in the small intestine (jejunum) of broilers at the

Conclusion:

Enhancing the normal intestinal flora of broilers with different levels of the manufactured bacterial preparation has effectively contributed to creating a microbial balance; as a result of competitive exclusion in obtaining attachment sites for cell receptors of the intestinal wall, which contributes to the expulsion of large numbers of harmful bacteria from the gut, thus improving microbial characteristics and ultimately improving production performance.

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تأثير المُستخضر البكتيري المُصنع في الصفات الميكروبية لفروج اللحم

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الملخص:

أُجريت هذه التجربة في حقل الدواجن التابع لقسم الإنتاج الحيواني في كلية الزراعة بجامعة البصرة للمدة من 2021/3/25 ولغاية 2021/4/28 ، استخدمَ فيها 450 وحدة تجريبية من أفراخ فروج اللحم ، سلالة Ross 308 بعمر يوم وأحد وبمعدل وزن43 غم/ فرخ ، رُبيت الأفراخ في نظام البطاريات ذاتِ ثلاثة طوابق ، كل طابق يحتوي على قفص بأبعاد 1.5× 1 م^2 ، وزعت الأفراخ عشوائياً على عشر معاملات تجريبية بواقع 45 فرخاً لكل معاملة ، وتضمنت المعاملة الواحدة ثلاثة مكررات (15 فرخ/ مكرر) ، حيث شَملت المُعاملات ، T1: (معاملة السيطرة السالبة) ، عليقة قياسية من دون إضافة. T2: (معاملة السيطرة الموجبة) إضافة الحليب الفرز المجفف بمستوى (1) غم / لتر ماء الشرب. T3، T4، T5: إضافة المستخضر البكتيري المُصنع بمستوى (0.5، 1، 2) غم / كغم علف، على التوالي ، T6، T7، T8: إضافة المستخضر البكتيري المُصنع بمستوى (0.5، 1، 2) غم / لتر ماء الشرب،على التوالي. T9: إضافة المستخضر البكتيري المُصنع بمستوى (0.5) غم / كغم علف، و(0.5) غم / لتر ماء الشرب. T10: إضافة المُستخضر البكتيري المُصنع بمستوى (1) غم / كغم علف ، و (1) غم / لتر ماء الشرب. أشارت النتائج الى وجود انخفاض معنوي ($P\leq0.05$) في المؤشرات اللوغارتيمية لبكتيريا القولون مع حصول زيادة معنوية ($P\leq0.05$) في أعداد بكتيريا حامض اللاكتيك لصالح معاملات المستخضر البكتيري المُصنع بالمقارنة مع معاملتي السيطرة .

الكلمات المفتاحية : بكتيريا حامض اللاكتيك ، العد الميكروبي ، أمعاء الدواجن .