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Producing a Biologically Metabolized Substance and Scientifically Proving Its Efficiency in Reducing Ammonia Gas Associated with Poultry Farming

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Abstract

The dispersion of ammonia gas NH_3 in poultry farming operations is considered a significant challenge that poses a threat to production. Given its direct impact on the general welfare of poultry, this will negatively impact the quality of the final product. The medication underwent a metabolic transformation in the laboratory, which involved a sequence of three successive events. Lactic acid bacteria were isolated and identified from the ileum area of the small intestine of Poultry during the early phase. During the following stage, the bacteria that were separated were stimulated on a sterile skim milk medium for three consecutive occasions. The third stage entailed the generation of physiologically metabolized molecules referred to as "postbiotics" or biologically active compounds.

During the 35-day raising period, three experimental treatments (T_0, T_1, and T_2) had this chemical given to the broilers' meals at different levels (0, 1, and 2 grams/kg feed, respectively. To determine the material's efficacy in lowering NH_3 ammonia gas emissions and their effect on manufacturing.

The experiment involved developing a novel technique to accurately quantify ammonia gas (NH_3) in poultry farms. This approach had not been previously employed in Iraq, the Arab world, or maybe even globally. The study conducted on this topic was substantial.

According to the statistical analysis, the postbiotic T_1 and T_2 treatments significantly reduced the rates of ammonia gas NH_3 compared to the control treatment T_0 *($P \leq 0.05$)*.

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Keywords
Biologically Metabolized, Reducing Ammonia, Poultry Farming, Lactic Acid Bacteria.

Introduction

Over the past few years, there has been a significant surge in poultry breeding initiatives. Due to the rising demand for its meat and egg products, the company has implemented intensive breeding systems and expanded its projects to accommodate the growing population density. However, these measures have resulted in the release of significant amounts of waste, specifically bird droppings, which are considered major environmental pollutants. It is crucial to adopt appropriate recommendations for treating this waste to mitigate its negative impact [1].

Ammonia gas, NH3, is the most hazardous pollutant. It is produced through the anaerobic fermentation of uric acid (60-65) %, ammonia salts 10%, and urea (2-3) %. Additionally, nitrogen and undigested proteins found in waste contribute to its presence [2].

The risk associated with the dispersion of ammonia gas in poultry farming projects resides in its detrimental effects on human health, particularly for individuals employed in these projects. Consequently, it will hurt public health indicators. Additionally, ammonia gas directly affects various physiological systems in poultry birds, including the respiratory and immune systems, as well as causing a loss of appetite. When the concentration of ammonia gas in the feed surpasses 20 parts per million (PPM), it can be detected by its odor and the irritation it causes to human eyes. This high level of ammonia can also be identified by observing a color change from yellow to brown in a turmeric leaf. The presence of ammonia in the feed has a direct impact on the overall performance and behavior of the project. [3].

Given the significant risks posed by NH_3 ammonia gas emissions from poultry farming projects to public health, both for humans and animals, as well as the overall air quality, the concept of developing a biologically metabolized substance has been proposed. The objective is to demonstrate its effectiveness in laboratory and field settings in reducing the levels of ammonia gas associated with poultry farming. To mitigate environmental pollution caused by noxious gases and promote the intestinal microbiota of poultry birds with metabolites from beneficial bacteria, which enhance overall bird health and productivity, as well as foster the adoption of natural or

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organic products that have a positive impact on human health, antibiotics should be avoided. A manufacturer with detrimental effects on avian species and consumers.

Previous efforts were made to mitigate the release of ammonia gas NH_3 associated with poultry farming. These efforts focused on treating poultry bedding, which is a significant contributor to gas production. The methods employed included the addition of ferrous sulphate and phosphoric acid [4] and zeolite [5]. While some individuals utilised alum in their approach [6], these prior endeavours were solely dependent on one aspect, namely the mattress, and overlooked the primary factor, which is the digestive system. The digestive system is regarded as the principal source responsible for the processes of food digestion, decomposition, and gas production.

In addition, there is a progressive decline in the use of brushes and other rudimentary methods. Due to advancements in poultry breeding initiatives, over 70% of global egg production and 65% of meat production now occur in cage systems that do not utilize bedding. This is significant since bedding is known to facilitate reactions that lead to the formation of hazardous ammonia gas. The efforts persisted beyond this stage and shifted towards specifically targeting the digestive tract. To achieve a state of microbial balance and reduce the population of harmful bacteria, it is necessary to simulate the intestinal flora. This is crucial for supporting the health of the digestive tract. Although antibiotics have been widely used for this purpose, their negative impact on consumers has led to their ban by the World Health Organization in some countries [7].

Subsequently, attention shifted towards improving the composition of gut bacteria using probiotics. These probiotics have demonstrated their effectiveness in comparison to antibiotics and their significant contribution to promoting public health and boosting production is widely acknowledged by researchers. For instance, Xie *et al*. [8] observed that exposing birds to a probiotic containing 10^{8} colony forming units per gramme (cfu/g) of lactic acid bacteria *Lactobacillus reuteri* enhances villi height and improves the intestinal lining significantly when compared to the control treatment. Zhang *et al.* [9] reported that the inclusion of 1% of the probiotic containing *Lactobacillus* had a significant impact. The inclusion of 5×10^{9} *Lactobacillus acidophilus* (cfu/g) per liter of water for broilers with *Lactobacillus reuteri* bacteria resulted in a notable enhancement in average body weight, weight increase, and feed conversion factor when compared to the control treatment.

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As a result, prominent corporations began manufacturing and selling probiotics commercially, leading to their widespread dominance in the local market. Hence, it was imperative to discover a more efficient and cost-effective substitute that faithfully replicates the gut microbiota. To maximize cost-effectiveness, the substance is sourced from the digestive system of local birds, which is separated for optimal benefits.

Methods

1. Design the experiment

The experiment was designed according to Figure (1) after the average weight of the chicks reached 43 g. These chicks were raised in cages inside an experimental hall designated for research work. They were provided with rations (Table 1), and all appropriate conditions were prepared for the chicks, according to the recommendations of the Aviagen Company. Global [10].

2. Prepare the metabolized substance (Post biotic)

The metabolized material was prepared according to three successive stages and according to Figure.(2)

Cultivation media used in the experiment

After the culture media for the experiment were prepared as directed by the manufacturer, they were autoclaved at 121°C and 15 pounds/inch^2 of pressure for 15 minutes to kill any bacteria or viruses. After cooling to 45°C, they were ready to be poured. Within a sterile environment (the hood), in dishes, since the experiment made use of several culture mediums, such as the following.

1. M.R.S Agar

Prepared as directed by the manufacturer (Himedia, India), used for isolating lactic acid bacteria (LAB).

2. M.R.S Broth

Prepared as directed by the manufacture (Himedia, India), used for activating LAB.

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3. Skim Milk

It came from the Basrah city market. To activate and grow bacterial cultures, it was autoclave sterilized at 121°C for 5 minutes

Gram stain

Gram stain kit prepared by Titan Biotech. LTD, India, was used to stain glass slides; to reveal their microscopic and morphological characteristics

Lactic acid bacteria

Estimating the populations of lactic acid bacteria using MRS Agar culture media was done following the approach stated by Da Silva *et al.* [11]. Using an oxygen-free environment within the incubator, the plates were heated to 37 °C for a duration of 48 to 72 hours. We utilized it to check the quantity, purity, and viability of the bacteria we isolated.

Isolation of lactic acid bacteria

Samples were collected from the ileum region using sterile cotton swabs after the birds were slaughtered and portions of their intestines were removed. Cotton swabs were used to inoculate the Petri dishes containing the MRS Agar medium. Following this, the Petri dishes were put in an incubator under anaerobic conditions at a temperature of 37°C for 48 hours.

Activation of bacterial isolates

To activate the isolates before each test, we used cotton swabs to disseminate the developing colonies over the solid medium MRS Agar. Then, we moved them to an anaerobic incubator and kept them at 37°C for 48 hours.

Diagnosis of bacterial isolates

The growth colonies were sub-cultured three times to obtain pure colonies the growing isolates were identified using phenotypic, microscopic, and biochemical analyses [12] in the following ways: -

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Appearance examinations

We examined the morphological features of the bacterial colonies after they had grown on MRS Agar culture media. These included colour, size, height, colony edge, overall look, and the emission of the typical lactic acid odour upon opening the dishes.

Microscopic examinations

The chosen colonies were placed on sterile Agar MRS medium plates and left to incubate at 37°C for 48 hours. Following this, all bacterial cultures were Gram stained in preparation for microscopic examination. To determine the shape and movement of the bacteria, the hanging drop method was used for movement analysis. [13].

Biochemical tests

Johnson &Case [14] listed a battery of confirmatory biochemical tests for bacterial colonies, including the following: catalase, oxidase, peroxidase, growth at various temperatures, bile salt resistance, low pH resistance, litmus milk medium growth, ammonia production from indole, and arginine degradation:

Measurements related to the production of ammonia gas (NH_3) in manure

Electric oven drying at 60°C for 24 hours followed by electric grinder grinding of waste samples before ammonia gas NH_3 measurements. To reduce to a fine powder; to be ready to do the next calculations:

1. Measure pH values

5 g of the waste sample was mixed well with 10 ml of distilled water, and left for 10 minutes, then the pH was measured with a pH meter. [15, 16].

2. Measurement of uric acid concentration

The concentration of uric acid was measured according to what was indicated by Kim and Patterson [17], which consists of placing 0.5 g of the dried waste sample in a 100 ml beaker containing 50 ml of borate buffer solution and shaking for 30 minutes at room temperature, then placing samples were centrifuged at 5000°C for 10 minutes.

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The concentration of uric acid was then measured using the kit supplied by the French company BIOLABO according to the steps indicated by the supplied company and using a spectrophotometer to read the samples, at a wavelength of (520 *nm*), and the concentrations were calculated according to the following equation [18]:

$$\textit{Uric acid concentration } (mg/100ml) = \frac{\textit{Sample absorbance}}{\textit{Absorbance of standard solution}} \times \textit{Concentration of standard solution}$$

3. Measure the amount of nitrogen

The amount of total nitrogen in waste was measured using a semi-micro Kjeldahl device [19].

4. Measure the concentration of ammonia NH_3

Kim & Patterson [17] provided the method for measuring ammonia NH3 concentration; following centrifugation and sample preparation, the French business BIOLABO (2003) provided the procedures for measuring concentration.

5. Measurement of ammonia gas production, NH_3

The test was done by collecting 3 g of waste for each replication of every experimental treatment, resulting in a total of nine samples. The bird droppings were stored in 200 ml plastic bottles that were firmly sealed with a rubber ring. The bottles were equipped with electrical sensors. Electrodes, operating under the Arduino system, were securely installed to measure ammonia gas. Figures 4, 5, and 6 provide a visual representation of this installation. Additionally, 6 ml of distilled water was added to the electrodes. Subsequently, the samples were placed in a poultry-raising facility for 48 hours to simulate rearing conditions and allow ample time for the fermentation process to conclude. The measuring procedure involved individually attaching the electrodes of each sample to the computer-programmed Arduino board and systematically recording the data in sequence.

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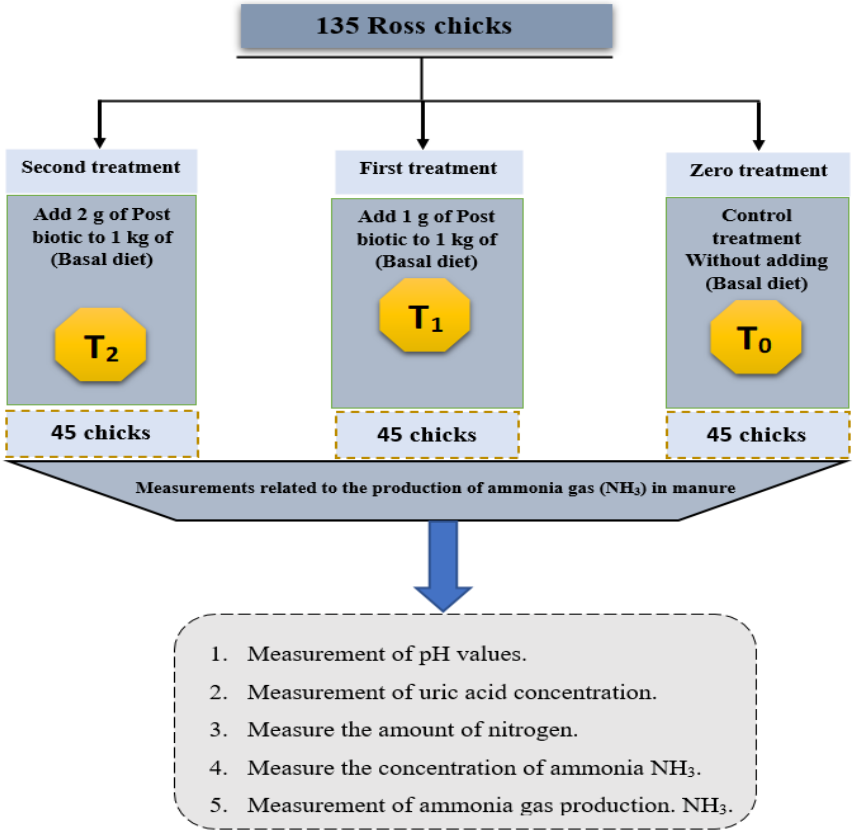

Fig. 1 - Chick's experiment design diagram

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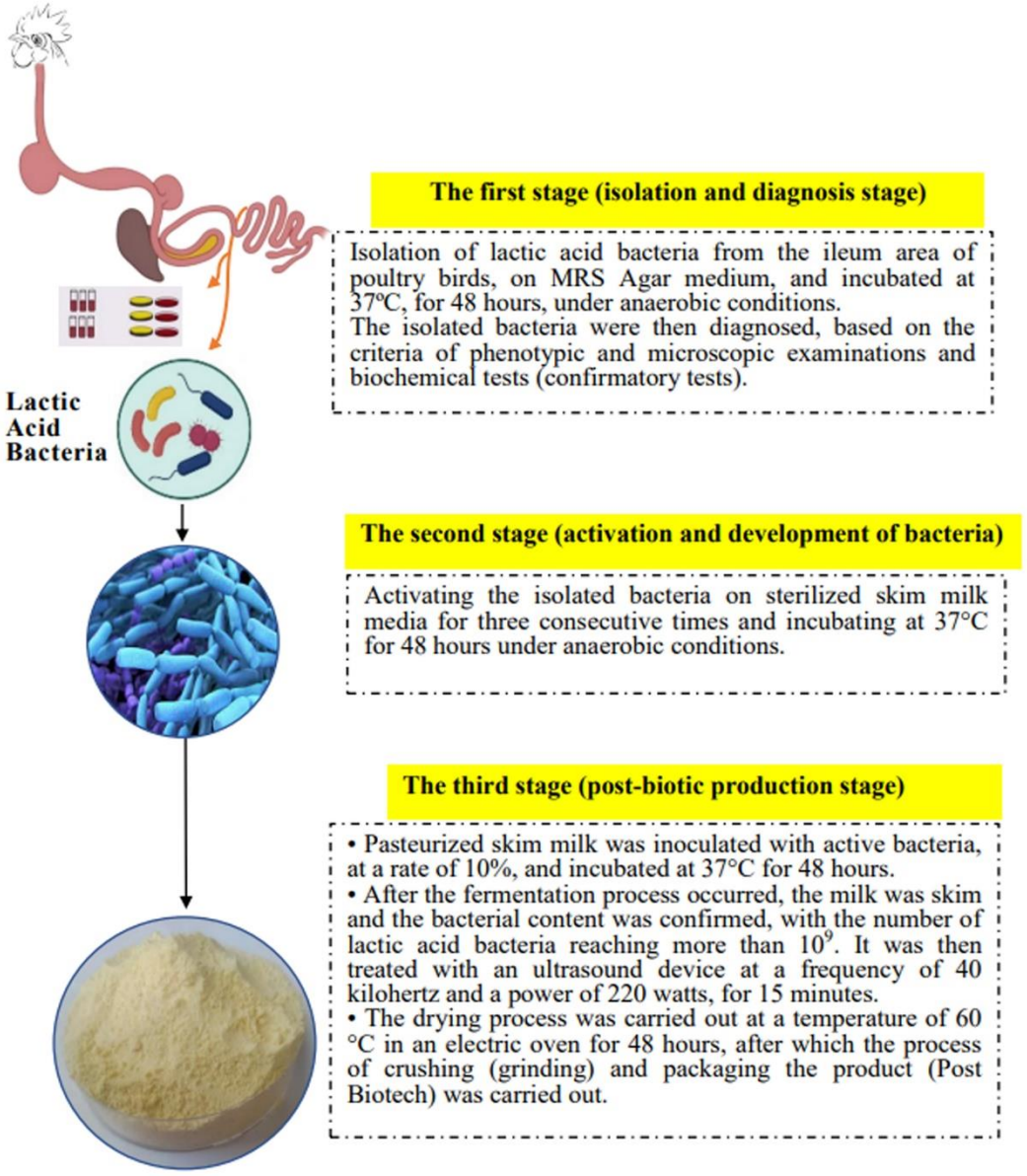

Fig. 2 - Stages of preparing postbiotics

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Table 1 - Diets and their chemical composition

Ingredients	Starter diet(1-21) days	Final diet (22-35) days
Yellow Corn	42	50
Wheat	17.2	15
Soybean Meal (48%)	32	24
Protein Concentrate (40%)	5	5
Mixture Of Vitamins and Minerals	1	1
Vegetable Oil (Sunflower)	0.5	3.2
Limestone	2	1.5
Table Salt	0.3	0.3
The Total	100	100
Calculated Chemical Composition		
Crude Protein %	23.43	20.03
Energy Represented as Kkg/Kg Feed	2956.76	3204.46
Energy to Protein Ratio	126.16	159.98
Crude Fiber %	4.11	3.58
Calcium %	1.32	1.1
Available Phosphorus %	0.47	0.46
Methionine %	0.43	0.42
Lysine%	1.24	1.03
Methionine + Cysteine %	0.9	0.81

- Protein concentrate: produced by the Dutch company Brocon, contains 40% crude protein, 2107 (kcal/kg) represented energy, 4.20% calcium, 2.65% phosphorus, 4.68% available phosphorus, 3.70% methionine, 0.66% cysteine, 3.85% Lysine, 2.20 crude fibres, 12.4% methionine and cysteine.
- The chemical composition of the materials included in the composition of the diet was calculated according to the recommendations of the NRC [20].

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Statistical analysis

Using SPSS [21] for statistical analysis, we compared means and found statistically significant differences using the Duncan [22] multinomial test, with a significance level of 0.05.

The effect of Post biotics on the pH values of waste

Table (2) clearly shows the impact of postbiotics on waste pH levels. There are no notable variations ($P \leq 0.05$) among the experimental treatments throughout the initial week. The second treatment, T2, which added 2 g Post biotic/kg feed, had the most noticeable impact on lowering average pH values in the second week. From the third to the fifth week, the metabolized substance (postbiotics) started to show its effectiveness in the digestive tract environment, and its effectiveness doubled. The fact that the experimental treatments significantly *($P \leq 0.05$)* reduced average pH values more effectively than the control treatment proved this.

Table 2 - **Effect of postbiotics on the pH values of waste**

Treatments	First week	Second week	Third week	Fourth wee	Fifth week
T_0	0.02±7.17	[a] 0.02±7.22	[a] 0.03±7.31	[a] 0.03±7.52	[a] 0.05±7.73
T_1	0.03±7.14	[a] 0.01±7.13	[b] 0.02±7.06	[b] 0.08±6.84	[b] 0.01±6.56
T_2	0.03±7.07	[b] 0.03±6.9	[c] 0.01±6.71	[c] 0.04±6.47	[c] 0.03±6.18
Sec.	**N. S**	*	*	*	*

T_0: control treatment (without addition). T_1 and T_2 add (1 and 2) g Post biotic/kg feed, respectively.
*Different letters indicate significant differences at the 0.05 probability level.
*The symbol N.S indicates that there are no significant differences between the averages of the coefficients.

Effect of postbiotics on the uric acid concentration of waste

Tabulated in Table (3) is the impact of Post Biotech on the waste's uric acid content. There is no discernible difference between the experimental therapies in the first two weeks. But there was a marked decline from week three through week five when the trial came to a close. There was a statistically significant difference *($P \leq 0.05$)* in the amount of uric acid in the excrement (mg/100 ml) between the experimental treatments and the control group, concerning both the amount of

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addition and treatment type.

Table 3 - **Effect of postbiotics on the uric acid concentration of waste (mg/100 ml)**

Treatments	First week	Second week	Third week	Fourth wee	Fifth week
T_0	0.02±3.24	0.05±3.44	[a] 0.02±3.52	[a] 0.03±3.60	[a] 0.02±3.65
T_1	0.03±3.24	0.04±3.38	[b] 0.05±3.30	[b] 0.03±3.23	[b] 0.01±3.18
T_2	0.04±3.20	0.10±3.33	[b] 0.08±3.24	[b] 0.06±3.12	[b] 0.07±3.04
Sec.	**N. S**	**N. S**	*	*	*

T_0: control treatment (without addition). T_1 and T_2 add (1 and 2) g Post biotic/kg feed, respectively.
*Different letters indicate significant differences at the 0.05 probability level.
*The symbol N.S indicates that there are no significant differences between the averages of the coefficients.

Effect of postbiotics on the amount of nitrogen in waste

Table (4) displays the impact of Post Biotech on the nitrogen content of garbage. There were no notable variations *(P≤0.05)* among the experimental treatments throughout the initial week. However, during the second week, it became clear that the second therapy, T_2, was much more effective in lowering the nitrogen level. Although there are no notable distinctions between treatments T_0 and T_1, experimental treatments T_1 and T_2 showed a significant advantage *(P≤0.05)* in nitrogen content in waste compared to the control treatment T_0 from the third week to the fifth week.

Table 4 - **Effect of postbiotics on the Amount of Nitrogen in Waste (mg/100 g)**

Treatments	First week	Second week	Third week	Fourth wee	Fifth week
T_0	0.02±1.06	[a] 0.02±1.18	[a] 0.05±1.33	[a] 0.02±1.57	[a] 0.03±1.81
T_1	0.01±1.05	[a] 0.02±1.07	[b] 0.02±1.02	[b] 0.01±0.81	[b] 0.04±0.64
T_2	0.00±1.01	[b] 0.03±0.87	[c] 0.06±0.70	[c] 0.05±0.52	[c] 0.03±0.38
Sec.	**N. S**	*	*	*	*

T_0: control treatment (without addition). T_1 and T_2 add (1 and 2) g Post biotic/kg feed, respectively.
*Different letters indicate significant differences at the 0.05 probability level.
*The symbol N.S indicates that there are no significant differences between the averages of the coefficients.

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Effect of Post-Biotech on the ammonia concentration of waste

A look at Table (5) reveals how Post Biotech affected the waste ammonia content. There is no discernible difference between the experimental therapies throughout the first two weeks. Throughout the experiment, from week three to week five, the ammonia concentration in the waste decreased significantly *(P≤0.05)* when compared to the control treatment and different levels of addition. The lowest concentration was 30.72 ppm in the second treatment, 38.23 ppm in the first, and 55.28 ppm in the control treatment. A pattern emerged according to the levels of addiction.

Table 5 - Effect of postbiotics on the ammonia concentration of waste (ppm)

Treatments	First week	Second week	Third week	Fourth wee	Fifth week
T_0	31.94±1.78	1.64±39.63	[a] 1.51±49.07	[a] 1.15±52.93	[a] 1.60±55.28
T_1	3.56±31.35	38.57±1.43	[b] 1.64±43.52	[b] 1.58±41.83	[b] 1.16±38.23
T_2	31.09±1.51	1.81±36.82	[b] 1.53±40.38	[c] 1.21±35.78	[c] 1.63±30.72
Sec.	**N. S**	**N. S**	*	*	*

T_0: control treatment (without addition). T_1 and T_2 add (1 and 2) g Post biotic/kg feed, respectively.
*Different letters indicate significant differences at the 0.05 probability level.
*The symbol N.S indicates that there are no significant differences between the averages of the coefficients.

Indicators of NH_3 ammonia gas emission within five weeks

You can see the indications of the five-week emission of NH_3 ammonia gas in Figure (3). All of the experimental treatments show no discernible changes after one week. There was a clear disparity between the control treatment and the first two Post Biotech treatments (T_1 and T_2) in terms of the reduction in ammonia gas emission indicators (NH_3) during the second week, indicating that the Post Biotech treatments were effective in lowering the rates of ammonia gas. With an index of 0.18 ppm, the second treatment (T_2) reduced ammonia gas (NH_3) at the fastest rate; the first treatment (T_2) reduced it to 10.32 ppm, and the control treatment (T_0) reduced it to 2.01 ppm. In other words, the second treatment was significantly better than the other two treatments *(P≤0.05)*, while the first treatment was better than the control treatment in lowering NH_3 ammonia gas levels.

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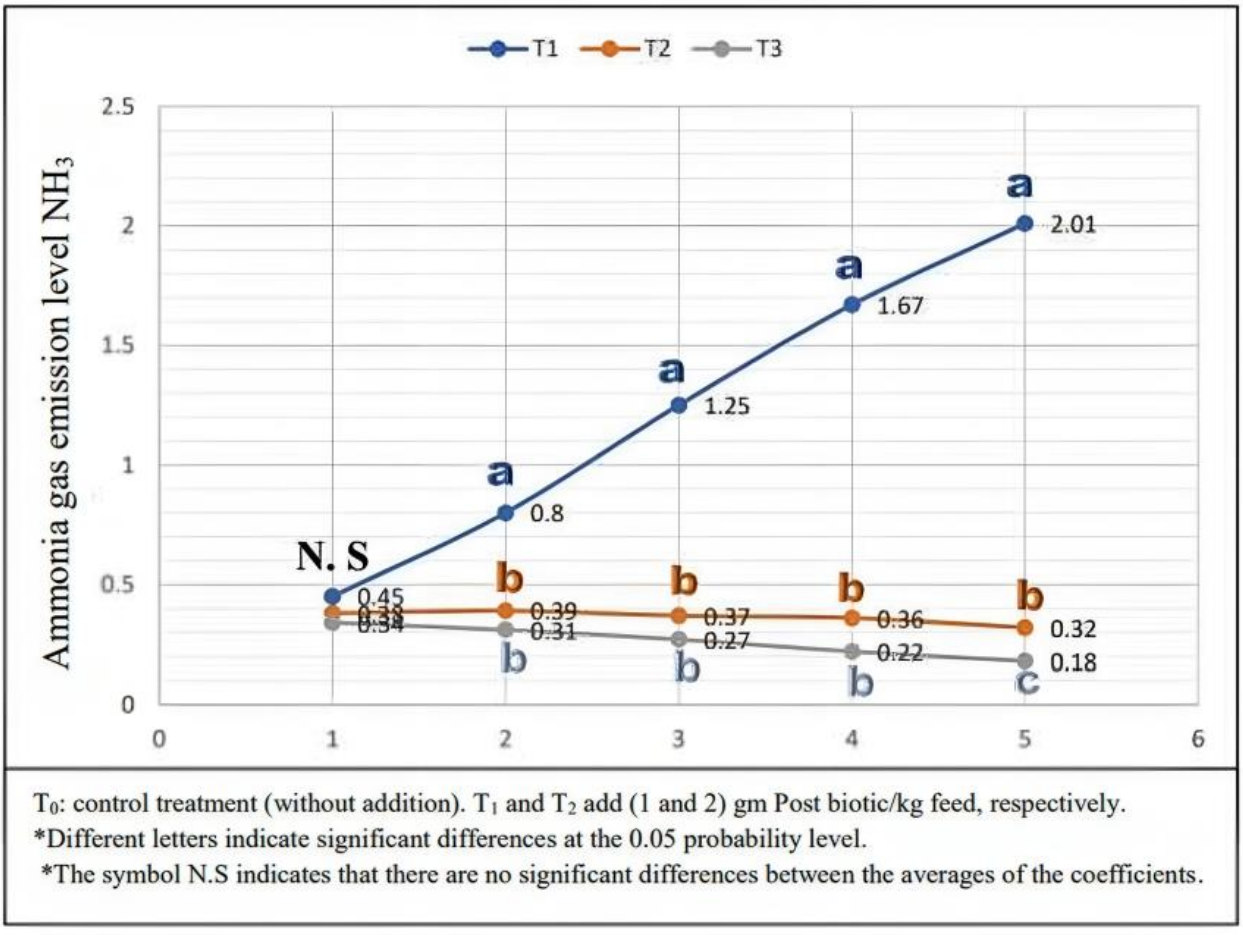

T_0: control treatment (without addition). T_1 and T_2 add (1 and 2) gm Post biotic/kg feed, respectively.
*Different letters indicate significant differences at the 0.05 probability level.
*The symbol N.S indicates that there are no significant differences between the averages of the coefficients.

Fig. 3- NH_3 ammonia gas emission indicators per 3 g within 48 hours for 5 weeks

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Discussion

The decrease in the average pH values in the waste, for the treatments that contained Post biotics, occurred due to the creation of microbial balance as a result of taking multiple doses of Post biotics throughout the experiment, which led to lowering the pH and modifying the environment of the digestive canal. Thus the environment would be unsuitable for the growth of bacteria. Harmful substances, always compete with the host's body (chicks) in feeding on protein, so removing them from the digestive tract will reduce the risk of protein turning into harmful ammonia [23].

These results were consistent with what was observed by Mi *et al.* [24], who indicated that the pH of feces (bird droppings) is very important in the release of ammonia gas (NH_3) and that its decrease in the droppings will lead to a reduction in gas production.

The decrease in the concentration of uric acid in waste is attributed to an increase in the process of synthesis or metabolism. Because it is closely linked to body weight gain in normal cases, and this has become clear through the rise in body weight and weight gain indicators, Figures (4 and 5), when studying the effect of biologically metabolized matter (Post biotic) on these characteristics. 69

This result agreed with Qorbanpour *et al.* [25] in that enhancing intestinal flora with lactic acid bacteria or their products contributes to reducing the rate of uric acid concentration in broilers, as a result of increased building processes and decreased catabolism.

Reducing the secretion of nitrogen, which is excreted in the form of uric acid and undigested protein in fecal waste, plays an essential role in reducing the emission of ammonia gas (NH_3) released from the interaction of waste contents, and one of the most important and safest ways to reduce it is to increase its benefit by improving the environment of the digestive tract of poultry birds Nahm, [26], which occurred as a result of adding the metabolic products of lactic acid bacteria to diets. It is no secret to specialists that the biological role that these products play when enhancing the intestinal flora with beneficial bacteria and their products in general, thus increasing absorption and benefiting from it in building various body tissues, and the results of this experiment confirmed This is when calculating the economic feasibility in studies of change in body weight.

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One way to help chickens achieve a healthy microbiome is to supplement their diet with lactic acid bacteria or metabolites. The elimination of many harmful bacteria from the digestive tract and the increased colonization of the intestinal wall by lactic acid bacteria, which improved the digestion coefficient and increased the benefit from food mass, as shown by lowering the pace of waste excretion enhances microbiological properties, which in turn improves production performance by lowering the rate of emission of toxic gases linked with the waste [1].

Similar to the decrease in the indicators above (pH, uric acid, and the amount of nitrogen) in the waste, the concentration rate of ammonia and ammonia gas decreased, because these indicators are closely related to a positive, direct relationship between them, meaning that the Postbiotic treatments contributed effectively to creating a physiological effect on the intestines. Minutes of broiler chickens, according to the above explanation, contributed to creating environmentally friendly conditions and thus will be reflected in the quality of production.

These results were supported by other studies, in that strengthening the digestive tract with a probiotic that contains lactic acid bacteria can reduce the concentration of ammonia and ammonia gas in the waste of broilers [27, 28].

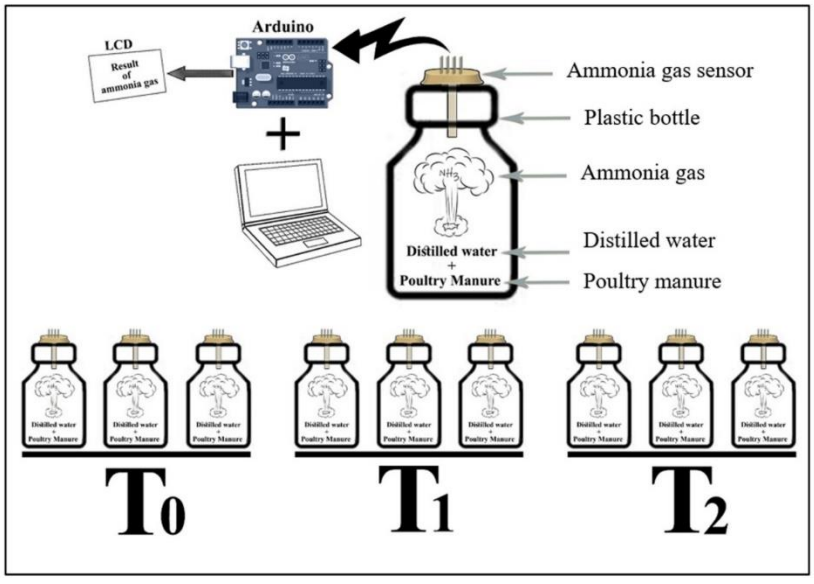

Fig. 4 - Schematic of connecting samples to the Arduino device

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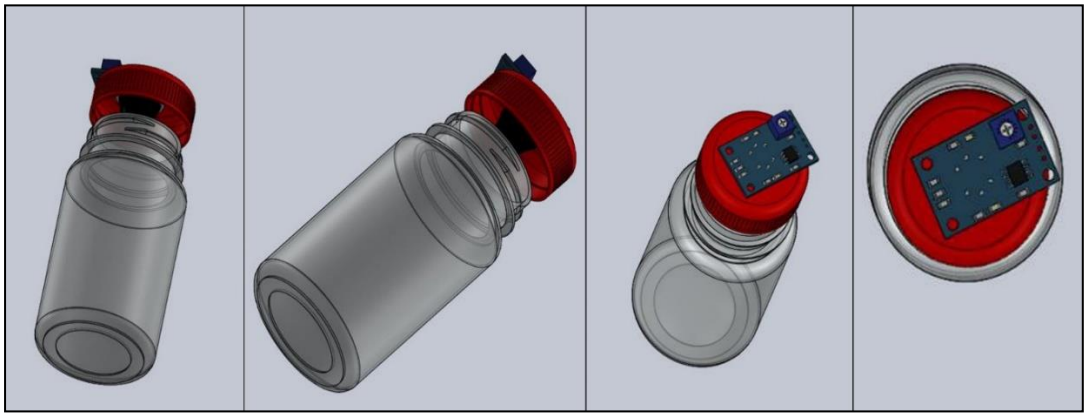

Fig. 5 - A tripartite design for sample collection containers equipped with a sensor to measure ammonia gas, NH_3

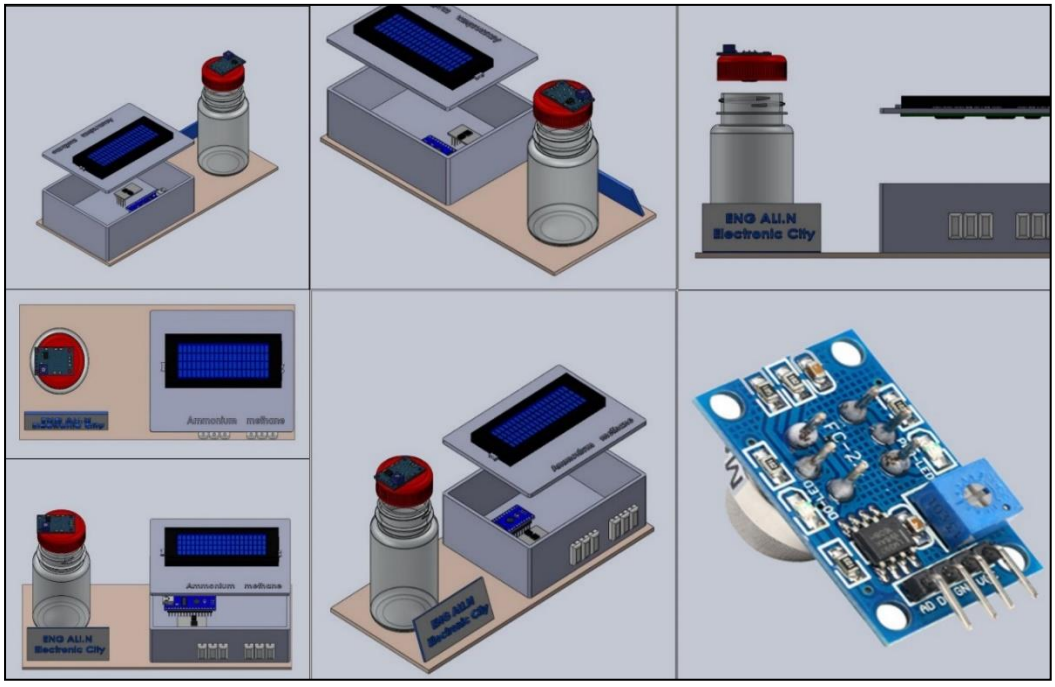

Fig. 6 - The three-dimensional design of the innovative method for measuring

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ammonia gas, NH_3

CONCLUSIONS

The decrease in the rates of the studied indicators: pH, uric acid, and the amount of nitrogen, in the waste, contributed to reducing the rate of concentration of ammonia and ammonia gas, because these indicators are closely related to a positive direct relationship between them, that is, the Post biotic T_1 and T_2 parameters contributed effectively to causing Physiological effect on the small intestine of broilers, which contributed to creating environmentally friendly conditions, by reducing NH_3 ammonia gas levels, in favor of the Post biotic T_1 and T_2 treatments compared to the T_0 control treatment.

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CONFLICT OF INTEREST

The authors stated that there are no conflicts of interest with the publication of this work.

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