Special Issue:

Veterinary Medicine between Sustainable Development and Public Health to Confront Global Changes

Effect of Biocid® as a Feed Additive on Growth, Blood, Immunity and Bacteriological Indices of Chicken Broilers

Dalia Hamid Mansour[1], Mohamed Elshabrawy Ghanem[2], Marwa Hassan[3], Yousry Ibrahim[4], Ibrahim M. Hegab[5]*

[1]Department of Avian and Rabbit Diseases, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, 41522, Egypt; [2]Department of Theriogenology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, 41522, Egypt; [3]Department of Animal Hygiene, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, 41522, Egypt; [4]El-Hoda Company for Mining and Agricultural Development, Cairo, Egypt; [5]Department of Animal Behavior and Management, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, 41522, Egypt.

Abstract | The aim of this study is to examine the effect of Biocid® as a feed additive on broilers final, carcass and organs weight, serum chemistry including albumin, total protein, alanine transaminase, aspartate aminotransferase, alkaline Phosphatase, gamma-glutamyl transferase, urea and creatinine as well as examining the histological alterations in internal and lymphoid organs and bacteriological analysis of cecal bacterial count. One hundred and five chicks were divided into 3 groups, Group A, in which chicks (n=35) was vaccinated and taken Biocid® for the entire experimental period (31 days) in both feed and drinking water. Group B, in which chicks (n=35) was vaccinated with no Biocid® supplementation. Group C, in which chicks (n=35) was not vaccinated but taken Biocid® only in feed. Birds were weekly weighted. Ten birds per group were slaughtered then blood samples were collected for serum analysis. After slaughter, organs were collected and weighted. Intestinal swabs were collected for bacteriological investigations immediately. Final body and carcass weights were significantly (P=0.007 and P= 0.009, respectively) improved in groups (AandC) received Biocid® (13.5% and 8.7 %, respectively) as well as an elevated levels of albumin (P=0.03) in group A (8.8%) compared to groups B and C. while the other biochemical parameters including total protein, liver enzymes, creatinine Alkaline Phosphatase and urea in serum did not show any significant alterations between treatment groups. Spleen, thymus and bursa were remarkably (P<0.0001, P<0.0001 and P=0.0007, respectively) increased in weight in Group A (2.33±0.74, 4.05±1.28 and 7.88±2.49, respectively) compared to other treatment groups. Total coliform and lactobacillus counts were significantly (P=0.02 and P=0.001, respectively)) higher in group A (8.75±0.56 and 8.27±0.47, respectively) compared to other groups. In conclusion, the current study is a preliminary investigation of the potential of Biocid® usage as feed additive to broilers ration formula which might enhance body weight, shorten the rearing period and improve the immune status of the chicken.

Keywords: Biocid®, Silica, Organic acid, Broilers, Amnio acids

Received | June 24, 2024; **Accepted** | August 18, 2024; **Published** | October 17, 2024
***Correspondence** | Ibrahim M. Hegab, Department of Animal Behavior and Management, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, 41522, Egypt; **Email:** ibrahim_hegab@vet.suez.edu.eg
Citation | Mansour DM, Ghanem ME-S, Hassan M, Ibrahim Y, Hegab IM (2024). Effect of Biocid® as a feed additive on growth, blood, immunity and bacteriological indices of chicken broilers. Adv. Anim. Vet. Sci. 12(s1): 220-231.
DOI | https://dx.doi.org/10.17582/journal.aavs/2024/12.s1.220.231
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

Poultry farming is a major income source for small raisers in developing countries. It participates in providing the daily protein needs of humans through meat and eggs utilization (Abdou *et al.*, 2023). A predictable need for animal protein has exceedingly increased in recent years. Therefore, during the last decade, the poultry industry has drastically expanded to be able to produce large quantities of meat to cover the high protein requirements of the increasing population worldwide (Choi *et al.*, 2023). Rearing of broilers for commercial purposes was frequently being expanded and their meat was presented as superior economical alternative sources of animal protein (Ayalew *et al.*, 2022; Eissawy *et al.*, 2023). The high levels of broiler production and competent feed conversion are essential of the modern poultry industry, which to a certain extent could be achieved using specific feed additives.

One of the most common feed additives that is widely used in poultry farming is antibiotic additives which has been supplemented as growth promoters to poultry rations to stabilize the intestinal microbial flora, enhance the overall performances and preclude some particular intestinal pathology (Rafiq et al., 2022). This ultimately leads to an increase in growth and final body weight as addition of antibiotics to broilers' diet could increase body weight gain by 5.8 % (Rahman *et al.*, 2012). This enhancement might be explained by improved appetite and feed conversion efficiency, stimulate the immune system, and increased vitality and regulation of the intestinal microflora (Peric *et al.*, 2009). However, the growing concern for development of antimicrobial resistance besides the consumer preference for antibiotic-free products has driven many countries to prohibit or restrict the utilization of antibiotics as feed additives (Fonseca *et al.*, 2024).

Following the ban of antibiotics, probiotics are feed additives that have become more popular in poultry farming which are more universal and can be simply combined with other feed additives (Krysiak *et al.*, 2021). The introduction of probiotic increases immunity of the gastrointestinal tract, more effective of microbial control, improved feeding efficiency, more antioxidant capacity, energy digestibility and are not as harmful to the environment as antibiotics which positively affect animal health as well as production performance (Al-Fatah, 2020). Yet, although fewer compared to antibiotics, probiotics usage might encompass several concerns such as their risks, handling procedures, and adaptability to the environment. For example, some probiotic strains will participate in the spread of antibiotic resistance (enterococcus) and produce toxin substances (Jha *et al.*, 2020). Moreover, the conditions of using probiotics must be thoroughly observed as supplementation of probiotics during high temperature induced changes in the structure of fatty acids (FA) and secondary bacterial structures of the probiotics (Dianawati *et al.*, 2013). In addition, the overdose of probiotics may cause a deterioration in the productive capacity in poultry (Haines *et al.*, 2015).

Some dietary products such as prebiotics, yeast culture, essential oils and spices are therefore being evaluated to replace antibiotics and probiotics in broilers diets. Moreover, organic acids were also reported to improve productivity of pigs and poultry (Khan *et al.*, 2022; Mahmoud *et al.*, 2022). They have been previously utilized in feed preservation, shielding feed from bacterial and fungal obliteration (Karpiński and Ożarowski, 2024). They occur naturally mostly in fruits and are also manufactured in commercial quantity using hydrocarbons. The potential of organic acids to reinforce growth of monogastric animals has been proven formerly due to their antibacterial power and encouragement of villus growth (Nhara *et al.*, 2024). They can be added to poultry rations at a concentration of 500g./ton for mold prevention and 2.5–3.0 kg/ton to decrease food pH and control *Salmonella* (Banupriya *et al.*, 2016). Broilers fed on diets supplied with organic acids had significantly higher body weights and feed conversion ratio (Adil *et al.*, 2010). Organic acids alone or their combinations are regarded safe, and execute equivalent functions like antibiotics (Khan *et al.*, 2022).

Biocid® is a newly developed product recently manufactured by Al-Ahram Mining Company. The product comprises various ingredients including organic acids, amino acids and silica (Supplementary Certificates 1 and 2). Diet supplementation with amino acids had been previously reported to increase broilers production (Johnson *et al.*, 2020) through upregulation of protein translation, increasing feed conversion ratio, improve intestinal integrity and immune function. Similarly, organic acids help restoration of intestinal microbiota and inflammation reduction which improve digestion of protein and increase growth and feed conversion ratio (Khan and Iqbal, 2015). Finally, silica enhances enzymatic activities, besides the protein and mineral digestion in broilers which improve growth and productive performance (Burton *et al.*, 2020). Biocid® can be administered either in drinking water and/or diet. We hypothesize that dietary supplementation of Biocid®, as a source of organic acids, amino acid and silica, will improve the performance, intestinal histomorphology and bacteriological profile, and serum biochemistry of vaccinated and un-vaccinated broiler chicken during the rearing period.

MATERIALS AND METHODS

BIRDS AND MANAGEMENT

This experiment was performed at the animal educational and research farm, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt. All experimental procedures were approved, prior to the commencement of work, by the animal ethics committee of the Faculty of Veterinary Medicine, Suez Canal University Approval No. (SCU-VET-REC- 2024034). This experiment was conducted on a total 105 one-day-old broilers chicks purchased from a commercial hatchery. Welfare concerns to minimize stress were followed according to Nielsen *et al.* (2021) where chicks were kept in floor pens supplied with dry sawdust which was periodically monitored for dryness and dampened sawdust was regularly removed with dry one. Ventilation hoods and ceiling fans were installed to ensure fresh air supply and removal of ammonia and foul air during the experiment. Optimum stocking density (7 birds/m^2) was followed according to Amer *et al.* (2023). Temperature was pointed at 34 °C during the 1 week and regularly lowered till it reached 25 °C at the end of the experiment. The rearing environment was illuminated at 20 lux minimum, with functional areas of resting (e.g. dark brooders) offering intensities down to 0.5 lux. Chicks were provided with 23 hrs. of light up to day 3 of life with a gradual decrease of the photoperiod to 16–17 hrs. at day 7 till the end of the experiment. Chicks have ad libitum easy access to all feeders and drinkers throughout the experiment. Birds were daily checked for signs of animal-based measures (ABMs) of welfare which included distress calls, feather and body dirtiness, huddling, panting, lethargy, total mortality, injurious pecking, hock burn, footpad dermatitis and body weight loss Nielsen *et al.* (2021). No apparent signs of distress were observed during the experiment.

Experimental design

Upon arrival, chicks were visually examined for any deformities and signs of health and unhealthy chicks were excluded from the experiment. Chicks were split randomly to 3 treatment groups having five replicates of seven birds each. The first group (Group A) received the Biocid® compound in both drinking water and feed and birds were vaccinated according to the regular vaccination protocol. Biocid® was mixed in diet in a concentration of 1 g/Kg diet, while in drinking water it was added according to the manufacturer protocol as from Day 1-7 in a concentration of 0.5 g/liter and from the 9th day till the end of the experiment (31 days old) as 1 g/liter. Group (B) did not receive the Biocid® compound neither in diet nor the drinking water but received the regular vaccination protocol. The final group (Group C) received the Biocid® compound only in feed (not in drinking water) as 1gm/Kg diet but did not get the regular vaccination protocol throughout the experiment. Upon arrival to the poultry house, chicks were weighed individually, and their initial body weight was recorded. The final body weight of chicks in each group was recorded at 31 days old at the end of the experiment prior to slaughter using an electronic digital balance (SF-400D; ATOM, China).

Sampling

At 31 days, ten birds from each group were slaughtered, and the blood was collected into 2 tubes; the first ones were allowed to clot and then centrifuged for 15 min at 3,000 x *g*. The obtained sera were transferred to Eppendorf tubes and stored at -20°C until needed for further biochemical analysis. The second tube was ethylenediaminetetraacetic acid (EDTA) tube for hematological analysis. After blood collection, birds were eviscerated and tissue samples including cecum, lymphoid organs (Thymus, Bursa and Spleen) and liver and kidney were collected from the slaughtered birds and fixed in 10% neutralized formalin for histological examination. One gram of cecal contents of five slaughtered birds in each group were collected after slaughter and transferred to a sterile test tube with 9 ml sterile Tryptic Soy broth.

Histological examination

The fixed tissue samples were transported to the pathological laboratory of the faculty of veterinary medicine, Suez Canal University, for further processing. The tissues were dehydrated with increasing concentrations of ethyl alcohol (70%, 90%, 96%, and 100%), cleared in xylene, and embedded in paraffin. The paraffin blocks were then cut using a microtome into four 5-μm-thick discontinuous paraffin-embedded sections per sample, which were then stained with hematoxylin and eosin and examined under a light microscope (Olympus C X40). The representative fields were photographed. The digital images of cecal tissues were captured and analyzed for morphometric measurements including villi length and width (μm). A computer morphometric program (Quick Photo Micro 3.2, PROMICRA, s.r.o.) was used for the histomorphometry measurements of the cecal samples.

Bacteriological examination

The mixture of the cecal contents and broth were diluted tenfold serially using a vortex (WiseMix, VM-10, Korea) and drop plate procedure described by Herigstad *et al.* (2001). Thirty μl of the diluted sample was plated onto various media: standard plate count agar (Plate Count Agar, 1.46365, Millipore, Germany) for total bacterial count (TBC); MacConkey Agar (100205 Millipore, Germany) and EMB agar (Lab M, LAB061, UK) for total coliform count; and lactobacillus agar (MRS AGAR), (TM medium, India) for lactobacilli count. Total aerobic bacteria (TBC) and total coliforms were grown at 37°C for 24-48 hours, Lactobacilli were incubated in a 3% CO_2 atmosphere at 37°C for 48–72 h. The results were expressed as means ± standard error (in $\log_{10}$ cfu/g).

Carcass traits and meat quality

After slaughter, the birds bled and then weighed to obtain the weight after slaughter. Birds were de-feathered, eviscerated, and weighed again to determine the carcass weight. Weight of different organs including liver, spleen, bursa and thymus was recorded (0.01 g). The thigh and breast muscles were dissected and weighed using electronic balance (0.01 g).

Statistical analysis

SPSS 22.0 software (Armonk, NY: IBM Corp. Spss, Inc, Chicago, IL, USA) was used to analyze all data. One-way ANOVA model was used after checking the assumption of normality of distribution and homogeneity of variances of every single dependent variable using Shapiro-Wilk and levene's test, respectively, to investigate the effect of Biocid® treatment on different parameters with a single independent variable was investigated (The effect of Biocid® treatment) while dependent variables include the growth performance and carcass traits, hematological and biochemical parameters, histo-morphometric analysis of intestine and total bacterial count of caecal contents. Duncan's post hoc multiple comparisons test where appropriate. Dependent variables (Final body weight, Spleen, thymus and breast weights and ALT and AST serum levels) that did not follow the assumptions of the parametric One-way ANOVA were analyzed using the non-parametric Kruskal–Wallis test. The level of significance at which the null hypothesis was rejected was $\alpha = 0.05$. The results were expressed as the mean ± SE.

RESULTS AND DISCUSSION

Growth performance and carcass traits

The overall influence of Biocid® addition on growth and carcass traits was listed in Figures 1 and 2. No significant mortalities were observed between treatment groups. Initial body weight was approximately similar with no significant difference amongst the treatment groups. Broilers in groups A and C received Biocid® treatment showed significantly higher final body weight (P=0.007, 2041.50±64.92 g and 1955.50±37.16 g, respectively), weight after slaughter (P=0.0095, 1934.00±60.84 g and 1866.50±35.79 g, respectively) and carcass weight (P= 0.009, 1565.50±57.84 g and 1441.50±23.44 g, respectively) by the end of the rearing period. Nevertheless, both thigh (P=0.36, Group (A): 589.50±18.23 g, (B): 557.50±20.87 g, (C): 590.50±14.60 g) and breast (P=0.51, (Group (A): 642.50±46.35 g, (B): 614.50±40.09 g, (C): 651.00±13.80g) muscles weights did not significantly differ between the treatment groups (Figure 1). Similarly, the weight of the internal organs varied between the treatment groups that the spleen (2.33±0.18 g), bursa (4.05±0.33 g) and thymus (7.88±0.61 g) in Group A were significantly heavier (P<0.0001, P<0.0001 and P=0.0007, respectively) than groups B (Spleen: 1.31±0.09g, Bursa: 2.42±0.22g, Thymus: 5.35±0.43g) and C, while the liver weight (Spleen: 1.39±0.43g, Bursa: 2.09±0.77g, Thymus: 5.24±1.16g) did not show any significant variations (P=0.91) between the experimental groups (Figure 2).

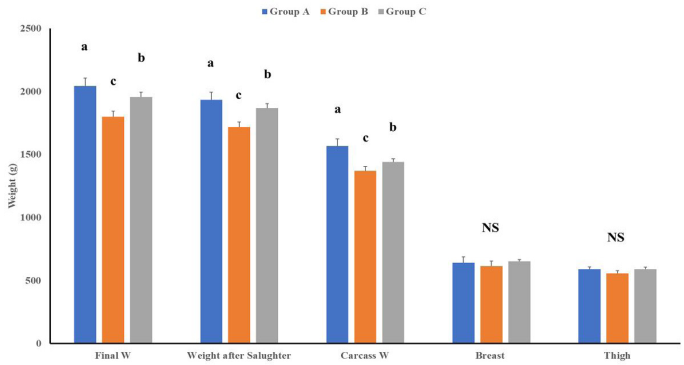

Figure 1: Growth performance and carcass traits (g) in different groups (Mean±SE). Columns with different superscripts are significant at p≤0.05. NS: non-signifcant.

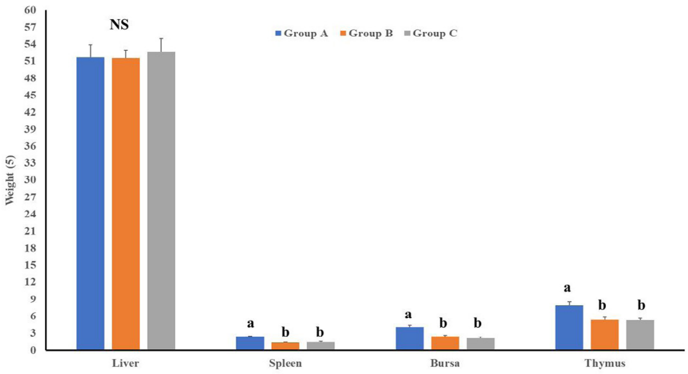

Figure 2: Weight of different internal organs (g) in different groups (Mean±SE). Columns with different superscripts are significant at p≤0.05. NS: non-signifcant.

Haematological and biochemical analyses

There were non-significant variations in the erythrocytic counts between treatment groups, but the total leukocytic count was significantly (P = 0.05) lower in group (C) compared to the other groups (Table 1). Biochemical analysis of serum samples revealed that broilers in Group (A) had significant higher albumin levels (P = 0.03) compared to groups B and C while Biocid® treatment did not alter the other biochemical parameters in the treatment groups (Table 1).

Histopathological results

Histo-morphometric analysis of intestine

Groups A and C which received Biocid® showed elongated villi and increased numbers of goblet cells than group B which received a regular diet without Biocid®. Histo-morphology of intestinal villi was assessed by measuring the villus height and crypt depth. The villi length showed

Table 1: Haematological and Biochemical parameters (Mean±SE) among the different treatment groups.

Parameter	Group A	Group B	Group C	P value
RBCs ($\times 10^6/\mu l$)	2.98±0.25	3.67±0.50	2.37±0.31	0.10
Total leuckocytic count (/µl)	27333 ± 4372^a	28667 ± 1764^a	17333 ± 1453^b	0.05
Albumin (g/dl)	1.175 ± 0.02^a	1.08 ± 0.05^b	1.03 ± 0.02^b	0.03
Total protein (g/dl)	2.58±0.08	2.40±0.12	2.33±0.13	0.35
ALT (g/dl)	2.50±0.22	3.60±0.55	2.50±0.22	0.13
AST (g/dl)	468.00±29.30	561.80±114.10	409.80±11.56	0.37
Alkaline Phosphatase	5485.00±1228.00	7228.00±1905.00	8005.00±963.90	0.52
GGT	26.50±2.73	26.20±1.87	25.00±2.61	0.90
Urea	3.50±0.39	4.60±0.33	3.50±0.39	0.07
Creatinine (mg/dl)	0.24±0.01	0.26±0.01	0.26±0.004	0.10

Means with different superscript within the same row are significantly different (P<0.05).

significant increase (*P*<0.05) in villi length in groups A (25.78%) and group C (13.63%) compared to group B and so, these groups showed a better absorptive surface of intestinal epithelium resulting in a better nutrient utilization. The crypt depth showed significant differences (*P*<0.05) between the treatment groups with increased depth in groups C(48.30%) and A (39.46%) compared to group B (Figure 3). The Goblet cell hyperplasia was abundant and numerous in groups A and C.

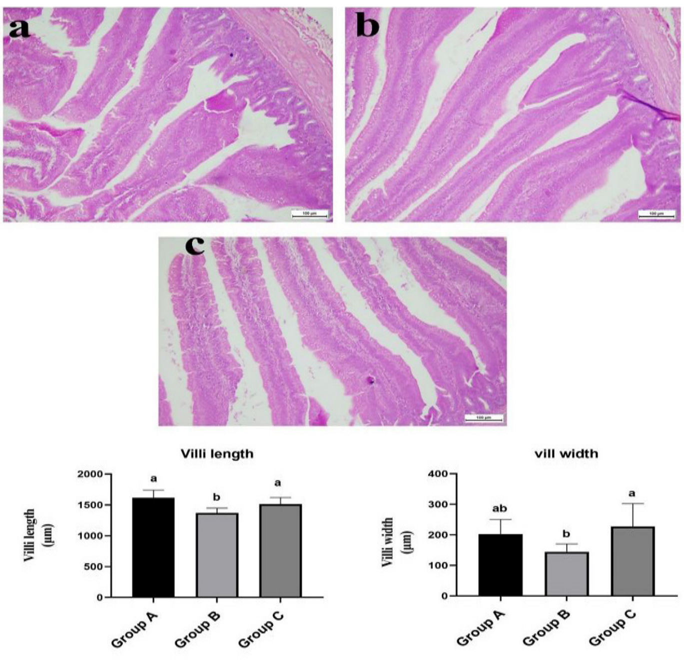

Figure 3: Photomicrograph of intestine histomorphometry. stained by H and E stain Scale Bar: 100 µm. A (received Biocid® and Newcastle vaccine), b (vaccine only) and basal diet, c (Biocid® only). Mean (±SE) of Villi length and width (µm) of the section. Columns with different superscripts are significant at p≤0.05.

Lymphoid organs

The bursa of group A showed normal structure. A typical fold is covered with epithelium and contains numerous lymphoid follicles separated by thin bands of fibrous connective tissue. Group B showed mild alterations such as mild depletion in medulla with expansion of interfollicular spaces by edema. Also, group C showed normal structure without any histopathological alternations. The thymus of group A showed normal structure. Moreover, group B showed numerous Hassall 's corpuscle which consisted of aggregate of large epithelial cells. Within the corpuscle are vacuoles containing necrotic granulocytic leukocytes. Group C also showed normal structure. Spleen of group A showed hyperplasia of lymphoid follicle. The arterioles are surrounded by a diffuse population of T-lymphocytes. that form the periarteriolar lymphoid sheath. There was also a lymphoid follicle composed of B-lymphocytes. The spleen of groups B and C showed normal structure (Figure 4).

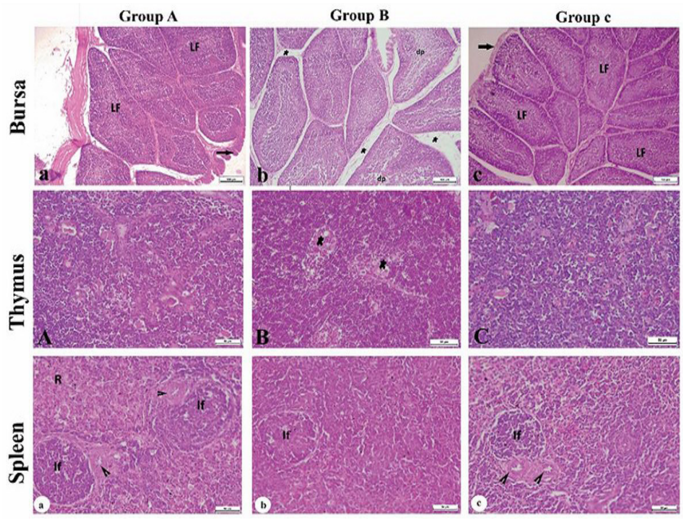

Figure 4: Photomicrograph of bursa of the three group (a, b, c), stained by H and E stain Scale Bar: 100 µm. Lf: lymphoid follicle, star shape: edema and arrow: epithelium. Photomicrograph of thymus of three group (A, B, C), stained by HandE stain Scale Bar: 50 µm. star shape: Hassall 's corpuscle. Photomicrograph of spleen of the three group (a, b, c), stained by HandE stain Scale Bar: 50 µm. Lf: lymphoid follicle and head arrow: arteriole.

Table 2: The bacterial count (Mean±SE) of caecal content among the different treatments.

Bacterial count (log_{10} CFU/g)	Group A	Group B	Group C
Total bacterial count (TBC)	9.3±0.56	8.23±0.53	7.73±0.50
Total coliform count (TCC)	8.75±0.56[a]	7.69±0.35[ab]	7.02±0.22[b]
Total lactobacillus count (TLC)	8.27±0.47[a]	8.16±0.30[a]	6.65±0.16[b]
Correlation coefficient with TBC	0.855**	0.632*	0.862**

-Means with different superscript within the same row are significantly different (P<0.01). *Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

Liver and kidney

The hepatic tissue of group A showed some mild focal leukocytic infiltration around central vein, mild disarrangement and vacuolation of hepatocytes. Group B showed moderate vacuolation of hepatocytes and desquamation epithelium. In addition, Group C showed normal hepatic plates with mild disarrangement of hepatocyte. The renal tissue of group A showed mild histopathological alterations such as infiltration of intratubular spaces by inflammatory cells and vacuolation of renal tubules. Group B also showed shrinkage of glomeruli with expansions of bowman's capsule and massive infiltration of inflammatory cells and increase spaces between renal tubules. Finally, group C showed normal kidney structure (Figure 5).

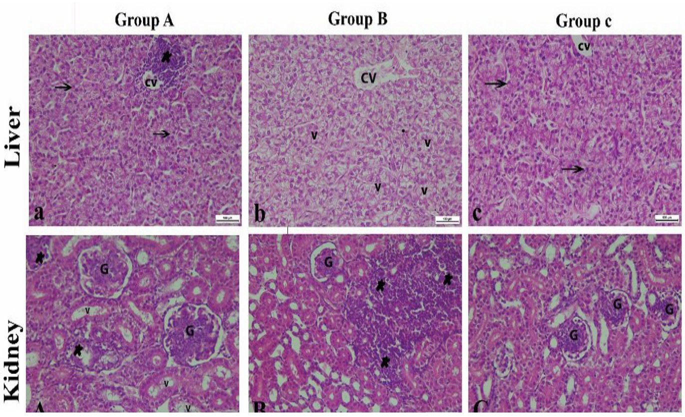

Figure 5: Photomicrograph of liver of three group (a, b, c), stained by HandE stain Scale Bar: 100 µm. v: vacuolation and arrow: hepatocytes. Photomicrograph of kidney of the three group (A, B, C), stained by HandE stain Scale Bar: 50 µm. star shape: leucocytic infiltration, G: gormouli.

The total bacterial count of caecal contents

The total bacterial count of caecal contents in the different treatment groups were presented in Table 2. The current findings showed that there was no significant difference in the total bacterial count of the caecal content (log_{10} CFU/g) between the three groups. Total coliform count (TCC) (log_{10} CFU/g) displayed a significant (P=0.02) increase in groups A (24.64%) and B (9.54%) when compared to group C,. In terms of Total lactobacillus count (log_{10} CFU/g), both groups A and B demonstrated a significant ($p=0.001$) increase (24.36% and 22.71 %, respectively) when compared to group C. Total bacterial count (TBC) was substantially correlated ($P < 0.01$) with both Total coliform count (TCC) (log_{10} CFU/g) and Total lactobacillus count (log_{10} CFU/g) in group A, but in groups B and C, the TBC was significantly ($P < 0.05$ and $P < 0.01$) associated with TCC, respectively.

Data illustrated in Table 3 revealed no significant change between the three treatment groups in the caecal content of Coliform/ Lactobacillus, Lactobacillus/ Coliform, Coliform/ TBC, and Lactobacillus/ TBC ratios.

Table 3: Ratio between different bacterial counts among the different treatments.

Ratio	Group A	Group B	Group C
Coliform/Lactobacillus	1.06±0.05	0.95±0.04	1.05±0.02
Lactobacillus/ Coliform	0.96±0.04	1.08±0.05	0.95±0.02
Coliform/TBC	0.94±0.03	0.95±0.05	0.93±0.04
Lactobacillus/TBC	0.89±0.3	1.02±0.07	0.88±0.04

The previous approach of using antibiotics as feed additives has been banned due to various concerns. Recent trends of using alternative approaches such as organic acids as feed additives in gain more popularity among poultry farmers as useful replacements for antibiotics (Chattopadhyay, 2014). The presented findings examined the effect of Biocid®, a novel product containing organic and amino acids and silica, as a feed additive in poultry diet.

The current findings clearly showed that the growth performance of birds received Biocid® both in group A (diet and drinking water) and group C (diet only) was significantly higher than birds in the control group (Group B) that did not receive the Biocid® treatment. This increase in body and carcass weights could be explained via various mechanisms. First, the organic acid components in the Biocid® may have a growth-promoting effect result in better feed intake, the drop in pathogenic microbial load, and the provision of a suitable environment for the growth-enhancing bacteria (Khan *et al.*, 2016). Chowdhury *et al.*, (2009) reported that addition of 0.5% citric acid enhance feed intake, growth, carcass yield in broiler. Live body weight increased significantly in chicks fed a diet supplied with acetic acid, citric acid, and lactic acid (Khan *et al.*, 2022). Different

Links Researchers

modes of action of organic acid supplementations can be explained such as they restrain the growth pathogenic bacteria and promotes pH reduction in the gastrointestinal tract of birds, improve pepsin activity, eventually enhance nutrients digestibility. Moreover, it helps counteracting the effect s of toxic substances secreted by certain bacteria and its colonization, which can reduce inflammation and competition with pathogenic microbes (Ma *et al.*, 2021). Organic acids can work on pathogenic bacteria by infiltrating the bacterial membrane, suppressing ATP synthesis and/or interrupting the bacterial membrane and denatures their DNA. All these actions ultimately establish eubiotic intestinal environment which improves gut health and nutrient digestibility and absorption (Pirgozliev *et al.*, 2019).

In addition, amino acids constituents of the Biocid® may increase growth, feed utilization efficiency, and carcass yields (Attia *et al.*, 2020) which was clearly observed in groups A and C. Increasing amino acid density in broilers diet had been observed to increase the final body weight and production performance, whereas decreasing their density reduced growth and live weight (Nasr and Kheiri, 2011). Another advantage of the essential amino acid constituents of the commercial Biocid®, including arginine, threonine, tryptophan and valine, made it possible to further reduce the crude protein levels in broiler diets (Attia *et al.*, 2022) which might be an economical prospective for poultry farmers. Also, they decrease the nitrogen loss during protein metabolism that leads to low ammonia excretion in the environment and improves growth performance of birds. Moreover, they chelated trace minerals decreasing the circulatory and intestinal levels of heat shock protein 70 (HSP70) and pro-inflammatory cytokine gene expression, optimizing broilers response to stress (Baxter *et al.*, 2020). Feeding diets with supplementary amino acid contents can alleviate certain intestinal diseases in broilers such as necrotic enteritis augmenting the digestion and absorption. Amino acids supplementation enhance cecal butyric and total short-chain fatty acids production which increasing productivity and improve immunity which have the significant as potential immunomodulating agents to improve the immune function of broiler chickens (Xu *et al.*, 2018). They also participate in protein synthesis, methylation reaction of DNA, elimination of reactive oxygen species (ROS) and acts as glutathione (GSH) precursor– a tripeptide, which decreases ROS and thus protects cells from oxidative stress (Alagawany *et al.*, 2020). Silica is another compound of Biocid® which had been previously reported to have positive effects on growth performance (Burton *et al.*, 2020) similar to the results found in groups (A and C). Silica was used as a unique feed additive owning sizeable surface area, high porosity, strong cation exchange activities, and more active sites, carrying the potential for detoxification and decontamination of feed with mycotoxins (Pappas et al., 2010). Moreover, they can execute several functions including toxin binding, improvement of the enzymatic activity in the small intestine, and control of ammonia emission into the environment. Finally, it could change GIT enzymatic secretion by raising the GIT fluids' pH which could, in turn, reduce intestinal inflammation and improve nutrient digestibility and absorption leading to higher productive performance in broilers (Ghazalah *et al.*, 2021).

Serum biochemical profile did not reveal any significant differences between treatment groups, except for albumin which was significantly higher in group A compared to the other treatment groups. This increase in serum concentrations of albumin could be interpreted by boosting dietary protein utilization via stimulation of digestive enzymes as protease (Tarek *et al.*, 2022) besides improvement of liver function to synthesize albumin. These results are in partial accordance with Natsir *et al.* (2017) who noted that dietary organic acids mixed with broilers diet increased serum total protein and albumin. Also, total leucocytic count was significantly lower in the non-vaccinated group C which received Biocid® through feed than the other treatment groups. The increased total leucocytic count in vaccinated groups may be due to the marked lymphocytosis in response to vaccination due to the vaccine's immunostimulatory effect as a response to antigenic stimulation caused by vaccine administration (Saleh *et al.*, 2011). This result can be fortified by our findings that birds in group (A) showed increased weights of immune organs (Spleen, bursa and thymus) which reflects better immune status (Martínez *et al.*, 2021) than the group (B) which received the vaccination but did not receive Biocid®. Another noticeable finding is the responses of broiler that received Biocid® (groups A and C) might not be similar. Biocid® mixing with diet and drinking water in group (A) may optimize growth, immune and productive performance in broilers rather than diet only as body and carcass weights and weights of immune organs in group A were higher than group B which may indicate better utilization of the product by increasing concentration when added to both diet and the drinking water.

In the current study, Biocid® treatment exerted beneficial effects on the gut morphology of assessed birds in both A and C groups. Birds treated with Biocid® had significantly increased villi length and width and goblet cell numbers than the control birds (group B). Also, increased villus height and width is indicative of increased epithelial cell turnover and a well-differentiated intestinal mucosa, more active cell growth and regeneration, which can promote enzyme secretion, improve digestion, and expand the surface area available for absorption (Wang *et al.*, 2021) suggesting improved digestive and absorptive capacity which was manifested by the high productive performance of both groups in terms of body and carcass weights (Al-Garadi *et al*., 2022). The villus crypt is thought as the villus factory and deeper crypts reveal fast tissue turnover to allow regeneration of the villus as required in response to normal sloughing or inflammation from pathogens or their toxins and high requirements for tissue (Awad *et al*., 2009). Furthermore, in this study, both A and C groups showed significant increase in the number of goblet cells. Goblet cells are the main secretory cells responsible for mucin production which may contribute to several functions including lubricating intestinal surfaces, trapping and neutralizing bacteria, detoxifying heavy metal binding, interacting with the intestinal immune system, and acting as a diffusion barrier for nutrients and macromolecules (McCauley and Guasch, 2015). The obtained results are in accordance with Adewole *et al*., (2021) who observed that supplemented broilers diet with organic acid contribute better to the intestinal health including villi length and increased numbers of goblet cells. Normal histological conformation of liver and kidneys were also noticed in groups A and C compared to the control group (B), that did not receive the Biocid® product, which outweigh the assumption that Biocid® administration may improve the digestive and excretory functions in broilers which ultimately enhance the overall health condition of the birds. For the impacts on the lymphoid organs, the results in Figure 2 agree with those of Ghazalah *et al*. (2011), who found that the relative weights of primary lymphoid organs (spleen, bursa of Fabricius, and thymus) were significantly (p < 0.01) improved by supplementation of organic acids. Similarly, the presences of normal histological structure coupled with lack of any pathological alterations of the immune organs in groups (A and C) indicated that the addition of organic acids in the diet and drinking water of the broiler chicks conferred better immune response and disease resistance besides beneficial immunological advances (Al-Mutairi *et al*., 2020).

Biocid® administration in diet significantly increased total Coliform and Lactobacillus counts but there was no significant difference in total bacterial count between the treatment groups. The present study may display the potential effect of Biocid® on gut microbiota. From the current results, it is firstly apparent that Biocid® administration via diet only (Group C) significantly reduces the Coliform count rather than through feed and drinking water (Group A), although it is significantly reducing the Lactobacillus counts. However, comparing Coliform/Lactobacillus ratio between groups A and C revealed that the ratio was nearly 1 which means that whether dual administration via drinking water + feed or only though feed yielded the same results. Although Coliform count was significantly higher in group A, but it did not cause any pathological alterations may be due to the concurrent increase in Lactobacillus count and the Coliform/Lactobacillus ratio was nearly 1 which mean both bacteria manifest their potential effects equally. These findings are in partial agreement with those reported in Thanh *et al*. (2009) who showed that addition of organic acids in broiler diets decreased the colonization of pathogenic bacteria, and in agreement with Attia *et al*. (2023) who observed that supplementation of organic acids increased the Lactobacillus count in Japanese quails. However, another study by Ghazalah *et al*. (2011) showed that organic acid addition did not change Lactobacillus count, but decreased Coliforms count in broiler chickens as observed in group (C) which received Biocid® only in diet. In addition, Seifi et al. (2015) showed that Lactobacillus and Coliforms were not altered by the addition of organic acid to broiler diets. Ustundag and Ozdogan (2019) found that organic acid added to quail diets increased Coliform in male plus decreasing total bacteria counts in females, implying a gender-specific response. These discrepancies might be attributed to the route and timing of administration, the poultry species used and the experimental design. The impact of organic acids on the gut bacterial population may need further clarification, but we cannot assume that Biocid® administration improve gut health in broiler.

Nevertheless, the responses of broiler chickens to excess dietary organic acids or amino acids have shown considerable inconsistency. While some results showed positive effects, others showed no effect or even negative consequences based on several factors. Regarding organic acids, one factor could be the difference in other dietary constituents and their chemical characteristics such as buffering capacity which may affect the level of acidification that occurs with the addition of organic acids (Kim *et al*., 2015). Similarly, experimental settings such as the sanitation degree of the environment may clarify the dissimilar results in the literature. Organic acids may influence the microbial population in the GIT, hence, that the antimicrobial outcomes of organic acids would be more distinct when birds are subjected to less sanitary conditions (Kil et al., 2011). Moreover, feed palatability is likely affected by the sources and amounts of dietary organic acids, and consequently appears to influence the efficacy of dietary organic acids. Similarly, excess dietary amino acids to growing chickens cause toxic effects such as depressions in growth, decreases in feed intake and increase mortalities. These negative impacts may be due to amino acid imbalance, for example, additional dietary Lys causes an increase in the Arg requirement, and chicks receiving additional Lys may show signs of an Arg deficiency because Arg lessens Lys toxicity (Maynard *et al*., 2022).

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, Biocid® administration in diet and drinking water may be initially considered as a growth promoter in the broiler chicken as in this study they had preliminary positive results on the performance. No significant mortalities were observed between the treatment groups although group (C) did not receive the regular vaccination of broilers. Furthermore, it provides positive impact on histology of small intestine and immune organs, thereby facilitating the nutrient absorption, growth performance and immune response in broiler chicken. Further studies should be conducted to confirm the efficacy of Biocid® as a growth promoter on production in different poultry species and under different environmental circumstances.

ACKNOWLEDGMENT

The authors would like to thank Al-Ahram Mining Company and Al-Hoda Company for agricultural development for their valuable contribution to this work.

NOVELTY STATEMENT

This study is the first trial to test the efficacy of Biocid®, a newly formulated compound, as a potential growth promoter in broilers. The chemical constituents of Biocid®, majorly organic acids, amino acids and other compounds, exerted a significant effect on productive and blood parameters and intestinal structures when administered through diet and drinking water or through diet only.

AUTHOR'S CONTRIBUTION

Dalia Hamid Mansour and Yousry Ibrahim conceived the presented idea. All authors (Except Yousry Ibrahim) performed the experiment. Mohamed El-Shabrawy Ghanem and Marwa Hassan verified the analytical methods. Ibrahim Hegab analyzed the findings of this work. All authors discussed the results and contributed to the final manuscript.

Conflict of interest

The authors affirm that they do not have any competing financial interests or personal relationships that may have influenced the findings presented in this paper.

REFERENCES

Abdou SM, Ismail SA, Ahmed AM, Shaheen HM (2023). Physicochemical comparison between the broiler and layer carcasses and their meat. J. Adv. Vet. Res., 13(6): 985-989.

Adewole DI, Oladokun S, Santin E (2021). Effect of organic acids-essential oils blend and oat fiber combination on broiler chicken growth performance, blood parameters, and intestinal health. Anim. Nutr. 7(4): 1039-1051. https://doi.org/10.1016/j.aninu.2021.02.001

Adil S, Banday T, Bhat GA, Mir MS, Rehman M (2010). Effect of dietary supplementation of organic acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. Vet. Med. Int., 2010: 479485. https://doi.org/10.4061/2010/479485

Alagawany M, Elnesr SS, Farag MR, Tiwari R, Yatoo MI, Karthik K, Michalak I, Dhama K (2020). Nutritional significance of amino acids, vitamins and minerals as nutraceuticals in poultry production and health. A comprehensive review. Vet. Q., 41(1): 1-29. https://doi.org/10.1080/01652176.2020.1857887

Al-Fatah MA (2020). Probiotic modes of action and its effect on biochemical parameters and growth performance in poultry. Iran. J. Appl. Anim. Sci., 10: 9–15.

Al-Mutairi HMS, Hussein EOS, Jar El-Nabi AR, Swelum AA, Abd El-Hack ME, Taha AE, Al-Mufarrej SI (2020). Does the consumption of acidified drinking water affect growth performance and lymphoid organs of broilers? Sustainability, 12: 3093. https://doi.org/10.3390/su12083093

Amer SA, Farahat M, Gouda A, Abdel-Wareth AAA, Younis EM, Elshopakey GE, Baher WM, Saleh GK, Davies SJ, Attia GA (2023). New insights into the effects of microbial muramidase addition in the diets of broiler chickens. Animals (Basel). 13(8): 1356. https://doi.org/10.3390/ani13081356

Attia YA, Abo El-Maaty HM, Alhotan RA, Bovera F, Sherif SK (2023). Coated organic acids with essential oils in Japanese quail's fed restricted during the 2nd week of age: Effects on performance, carcase traits, blood profile, antioxidants status, and caeca microbiota. Ital. J. Anim. Sci., 22(1): 816–828. https://doi.org/10.1080/1828051X.2023.2244973

Attia YA, Al-Harthi MA, Shafi ME, Abdulsalam NM, Nagadi SA, Wang J, Kim WK (2022). Amino acids supplementation affects sustainability of productive and meat quality, survivability and nitrogen pollution of broiler chickens during the early life. Life (Basel). 12(12): 2100. https://doi.org/10.3390/life12122100

Attia YA, Bovera F, Wang J, Al-Harthi MA, Kim WK (2020). Multiple amino acid supplementations to low-protein diets: Effect on performance, carcass yield, meat quality and nitrogen excretion of finishing broilers under hot climate conditions. Animals (Basel). 10(6): 973.

Rahman MA, Parvin MS, Sarker RR, Islam MT (2012). Effects of growth promoter and multivitamin-mineral premix supplementation on body weight gain in broiler chickens. J. Bangladesh Agric. Univ., 10: 245–248. https://doi.org/10.3390/ani10060973

Awad WA, Ghareeb K, Abdel-Raheem S, Böhm J (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poult. Sci., 88(1): 49-56. https://doi.org/10.3382/ps.2008-00244

Ayalew H, Zhang H, Wang J, Wu S, Qiu K, Qi G, Tekeste A, Wassie T, Chanie D (2022). Potential feed additives as antibiotic alternatives in broiler production. Front. Vet. Sci., 9: 916473. https://doi.org/10.3389/fvets.2022.916473

Banupriya S, Kathirvelan C, Patric Joshua P (2016). Significance of feed acidification in poultry feed. Int. J. Sci. Environ. Technol., 5: 1596–1599.

Baxter MFA, Greene ES, Kidd MT, Tellez-Isaias G, Orlowski S, Dridi S (2020). Water amino acid-chelated trace mineral supplementation decreases circulating and intestinal HSP70 and proinflammatory cytokine gene expression in heat-stressed broiler chickens. J. Anim. Sci., 98(3): skaa049. https://doi.org/10.1093/jas/skaa049

Burton EJ, Scholey DV, Belton DJ, Bedford MR, Perry CC (2020). Efficacy and stability of a novel silica supplement for improving bone development in broilers. Br. Poult. Sci., 61(6): 719-724. https://doi.org/10.1080/00071668.2020.1799328

Chattopadhyay MK (2014). Use of antibiotics as feed additives: A burning question. Front. Microbiol., 5: 334. https://doi.org/10.3389/fmicb.2014.00334

Choi J, Kong B, Bowker BC, Zhuang H, Kim WK (2023). Nutritional strategies to improve meat quality and composition in the challenging conditions of broiler production: A review. Animals (Basel). 13(8): 1386.

Al-Garadi MA, Al-Baadani HH, Alqhtani AH (2022). Growth performance, histological changes and functional tests of broiler chickens fed diets supplemented with *Tribulus terrestris* powder. Animals (Basel). 12(15): 1930.

Jha R, Das R, Oak S, Mishra P (2020). Probiotics (direct-fed microbials) in poultry nutrition and their effects on nutrient utilization, growth and laying performance, and gut health: A systematic review. Animals (Basel). 10(10): 1863.

Chowdhury R, Islam KMS, Khan MJ, Karim MR, Haque MN, Khatun M, Pesti GM (2009). Effect of citric acid, avilamycin, and their combination on the performance, tibia ash, and immune status of broilers. Poult. Sci., 88: 1616–1622. https://doi.org/10.3382/ps.2009-00119

Dianawati D, Mishra V, Shah NP (2013). Effect of drying methods of microencapsulated *Lactobacillus acidophilus* and *Lactococcus lactis* ssp. cremoris on secondary protein structure and glass transition temperature as studied by Fourier transform infrared and differential scanning calorimetry. J. Dairy Sci., 2013: 96. https://doi.org/10.3168/jds.2012-6058

Eissawy MM, Ahmed AM, Fares IM, Hafez TA, Ahmed NI (2023). Bacteriological quality of fresh broiler chickens traded in the markets. J. Adv. Vet. Res., 4: 627-630.

Fonseca A, Kenney S, Van Syoc E, Bierly S, Dini-Andreote F, Silverman J, Boney J, Ganda E (2024). Investigating antibiotic free feed additives for growth promotion in poultry: Effects on performance and microbiota. Poult. Sci., 103(5): 103604. https://doi.org/10.1016/j.psj.2024.103604

Ghazalah AA, Abd-Elsamee MO, Moustafa KEME, Khattab MA, Rehan AEAA (2021). Effect of nanosilica and bentonite as mycotoxins adsorbent agent in broiler chickens' diet on growth performance and hepatic histopathology. Animals, 11: 2129. https://doi.org/10.3390/ani11072129

Ghazalah I, Attal AM, KoutElkloub M, El-Moustafa R, Shata FH (2011). Effect of supplementation of organic acids on performance, nutrients digestibility and health of broiler chicks. Int. J. Poult. Sci., 10: 176–184. https://doi.org/10.3923/ijps.2011.176.184

Haines M, Parker H, McDaniel C, Kiess A (2015). When rooster semen is exposed to *Lactobacillus* fertility is reduced. Int. J. Poult. Sci., 14: 541. https://doi.org/10.3923/ijps.2015.541.547

Johnson CA, Duong T, Latham RE, Shirley RB, Lee JT (2020). Increasing amino acid density improves growth performance and processing yield in Cobb 700 × MV broilers. J. Appl. Poult. Res., 29(2): 465-478. https://doi.org/10.1016/j.japr.2020.02.004

Karpiński TM, Ożarowski M (2024). Plant organic acids as natural inhibitors of foodborne pathogens. Appl. Sci., 14: 6340. https://doi.org/10.3390/app14146340

Khan RU, Chand N, Ali A (2016). Effect of organic acids on the performance of Japanese quails. Pak. J. Zool., 48: 1799–1803.

Khan RU, Naz S, Raziq F, Qudratullah Q, Khan NA, Laudadio V, Tufarelli V, Ragni M (2022). Prospects of organic acids as safe alternative to antibiotics in broiler chickens diet. Environ. Sci. Pollut. Res. Int., 29(22): 32594-32604. https://doi.org/10.1007/s11356-022-19241-8

Khan SH, Iqbal J (2015). Recent advances in the role of organic acids in poultry nutrition. J. Appl. Anim. Res., 44(1): 359–369. https://doi.org/10.1080/09712119.2015.1079527

Kil DY, Kwon WB, Kim BG (2011). Dietary acidifiers in weanling pig diets: A review. Rev. Colomb. Cienc. Pecu., 24: 231-247.

Kim JW, Kim JJ, Kil D (2015). Dietary organic acids for broiler chickens: A review. Rev. Colomb. Cienc. Pecu., 2015: 28. https://doi.org/10.17533/udea.rccp.v28n2a01

Krysiak K, Konkol D, Korczyński M (2021). Overview of the use of probiotics in poultry production. Animals (Basel). 11(6): 1620. https://doi.org/10.3390/ani11061620

Ma J, Wang J, Mahfuz S, Long S, Wu D, Gao J, Piao X (2021). Supplementation of mixed organic acids improves growth performance, meat quality, gut morphology and volatile fatty acids of broiler chicken. Animals (Basel), 11(11): 3020. https://doi.org/10.3390/ani11113020

Martínez Y, Altamirano E, Ortega V, Paz P, Valdivié M (2021). Effect of age on the immune and visceral organ weights and cecal traits in modern broilers. Animals (Basel), 11(3): 845. https://doi.org/10.3390/ani11030845

Maynard CW, Mullenix GJ, Maynard CJ, Lee JT, Rao SK, Butler LD, Hiltz JZ, Orlowski SK, Kidd MT (2022). Failure of excess leucine to impact live performance and carcass traits in male and female cobb Mv × 500 broilers during a 15- to 32-day grower period. J. Appl. Poult. Res., 2; 100242. https://doi.org/10.1016/j.japr.2022.100242

McCauley HA, Guasch G (2015). Three cheers for the goblet cell: Maintaining homeostasis in mucosal epithelia. Trends Mol. Med., 21: 492–503. https://doi.org/10.1016/j.molmed.2015.06.003

Nasr J, Kheiri F (2011). Increasing amino acids density improves broiler live weight. Int. J. Poult. Sci., 10: 523-526. https://doi.org/10.3923/ijps.2011.523.526

Natsir MH, Hartutik OS, Widodo E, Widyastuti ES (2017). Use of acidifiers and herb-acidifier combinations with encapsulated and non-encapsulated intestinal microflora, intestinal histological and serum characteristics in broiler. AIP Conf. Proc., 1844(5): 020012. https://doi.org/10.1063/1.4983423

Nhara RB, Marume U, Nantapo CWT (2024). Potential of organic acids, essential oils and their blends in pig diets as alternatives to antibiotic growth promoters. Animals (Basel). 14(5): 762.

Mahmoud FF, Ahmed EA, Ahmed AM, Ahmed NI (2022). Control of aflatoxin residues in broiler chicken using saccharomyces cerevisiae fortified ration. J. Adv. Vet. Res., 4: 409-414. https://doi.org/10.3390/ani14050762

Nielsen SS, Alvarez J, Bicout DJ, Calistri P, Canali E, Drewe JA, Garin-Bastuji B, Gonzales Rojas JL, Schmidt CG, Herskin MS, Miranda Chueca MÁ, Padalino B, Pasquali P, Roberts HC, Spoolder H, Stahl K, Velarde A, Viltrop A, Winckler C, Tiemann I, de Jong I, Gebhardt-Henrich SG, Keeling L, Riber AB, Ashe S, Candiani D, García Matas R, Hempen M, Mosbach-Schulz O, Rojo Gimeno C, Van der Stede Y, Vitali M, Bailly-Caumette E, Michel V (2023). Welfare of broilers on farm. EFSA J., 21(2): e07788. https://doi.org/10.2903/j.efsa.2023.7788

Pappas AC, Zoidis E, Theophilou N, Zervas G, Fegeros K (2010). Effects of palygorskite on broiler performance, feed technological characteristics, and litter quality. Appl. Clay Sci., 49: 276–280. https://doi.org/10.1016/j.clay.2010.06.003

Peric L, Zikic D, Lukic M (2009). Application of alternative growth promoters in broiler production. Biotech. Anim. Husb., 25: 387–397. https://doi.org/10.2298/BAH0906387P

Pirgozliev V, Mansbridge SC, Rose SP, Mackenzie AM, Beccaccia A, Karadas F, Ivanova SG, Staykova GP, Oluwatosin OO, Bravo D (2019). Dietary essential oils improve feed efficiency and hepatic antioxidant content of broiler chickens. Animal, 13(3): 502–508. https://doi.org/10.1017/S1751731118001520

Rafiq K, Tofazzal, Hossain M, Ahmed R, Hasan MM, Islam R, Hossen MI, Shaha SN, Islam MR (2022). Role of different growth enhancers as alternative to in-feed antibiotics in poultry industry. Front. Vet. Sci., 8: 794588.

Herigstad B, Hamilton M, Heersink J (2001). How to optimize the drop plate method for enumerating bacteria. J. Microbiol. Meth., 44(2): 121-129. https://doi.org/10.3389/fvets.2021.794588

Saleh N, Fathalla SI, Nabil R, Mosaad AA (2011). Clinicopathological and immunological studies on toxoids vaccine as a successful alternative in controlling clostridial infection in broilers. Anaerobe, 17(6): 426-430. https://doi.org/10.1016/j.anaerobe.2011.04.019

Seifi S, Sayrafi R, Khoshbakht R, Gilani A (2015). Effects of dietary acetic acid on intestinal microbiota, serum components, internal organs and performance of broilers. Glob. J. An. Sci. Res., 3: 536–543.

Tarek AE, Eva T, Ibrahim H, Mohamed K, Darina C (2022). Recent advances in the role of feed restriction in poultry productivity: Part I- performance, gut development, microbiota and immune response. J. World's Poult. Sci., 78(4): 971-988. https://doi.org/10.1080/00439339.2022.2097149

Thanh NT, Loh TC, Foo HL, Hair-Bejo M, Azhar BK (2009). Effects of feeding metabolite combinations produced by Lactobacillus plantarum on growth performance, faecal microbial population, small intestine villus height and faecal volatile fatty acids in broilers. Br. Poult. Sci., 50(3): 298–306. https://doi.org/10.1080/00071660902873947

Ustundag AO, Ozdogan M (2019). Effects of bacteriocin and organic acid on growth performance, small intestine histomorphology, and microbiology in Japanese quails (*Coturnix coturnix japonica*). Trop. Anim. Health Prod., 51(8): 2187–2192. https://doi.org/10.1007/s11250-019-01931-0

Wang Y, Xu Y, Xu S, Yang J, Wang K, Zhan X (2021). *Bacillus subtilis* DSM29784 alleviates negative effects on growth performance in broilers by improving the intestinal health under necrotic enteritis challenge. Front. Microbiol., 2021: 12. https://doi.org/10.3389/fmicb.2021.723187

Xu YQ, Guo YW, Shi BL, Yan SM, Guo XY (2018). Dietary arginine supplementation enhances the growth performance and immune status of broiler chickens. Livest. Sci., 209: 8–13. https://doi.org/10.1016/j.livsci.2018.01.001

Ministry of Agriculture
AGRICULTURAL RESEARCH CENTER
Soils, Water & Environment Res. Institute
Soils, Water & Environment Unit
Analysis & Studies Component

وزارة الزراعة
مركز البحوث الزراعية
معهد بحوث الأراضي والمياه والبيئة
وحدة الأراضي والمياه والبيئة
مكون التحاليل والدراسات

تم توريد مبلغ / خمسمائة جنيها بإيصال رقم (٣١٦٧) بتاريخ

السادة/ شركة الأهرام للتعدين.

تحية طيبة ... وبعد

فيما يلي نتائج التحليل تقدير نسبة الأحماض الأمينية في عينة البايوسيد والمقدمة بمعرفتكم :

Amino Acid (ppm)	
L. Alanine	797.77
L. Glycine	217.83
L. Glutamic	5097.88
L. Serine	1339.33
L. Valine	364.25
L. Leucine	647.35
L. Iso leucione	751.70
L.Cysteine	214.57
L. Proline	289.54
L. Methionine	1496.87
L. Aspartic	609.18
L.Phenylalanine	675.92
L. Arginine	156.71
L. Tyrosine	1599.89
L. Threonine	1213.20
L. Histidine	430.24
L.Tryptophane	25.57
L. Cystein	8.34

برجاء الإحاطة وشكرا ....

رئيس مكون التحاليل والدراسات
أ.د. أحمد طاهر

رئيس مجلس إدارة وحدة الأراضي والمياه والبيئة
أ.د. حمدى الحسيني خليفة

Address: Cairo Univ. Street, Giza, Egypt

Supplementary Certificate 1: Concentrations of different amino acids in Biocid® compound.

Ministry of Agriculture
AGRICULTURAL RESEARCH CENTER
Soils, Water & Environment Res. Institute
Soils, Water & Environment Unit
Analysis & Studies Component

وزارة الزراعة
مركز البحوث الزراعية
معهد بحوث الأراضي والمياه والبيئة
وحدة الأراضي والمياه والبيئة
مكون التحاليل والدراسات

تم توريد مبلغ / خمسمائة جنيها بإيصال رقم (٣١٦٧) بتاريخ

السادة/ شركة الأهرام للتعدين.

تحية طيبة ... وبعد

فيما يلي نتائج التحليل تقدير نسبة الأحماض العضوية في عينة البايوسيد والمقدمة بمعرفتكم :

Organic Acid (ppm)	
Tartaric	383.39
Citric	14390.25
Maleic	772.62
Oxalic	1251.37
Succinic	6941.25
Fumaric	45839.73

برجاء الإحاطة وشكرا ....

رئيس مكون التحاليل والدراسات
أ.د. أحمد طاهر

رئيس مجلس إدارة وحدة الأراضي والمياه والبيئة
أ.د. حمدى الحسيني خليفة

Address: Cairo Univ. Street, Giza, Egypt

Supplementary Certificate 2: Concentrations of different organic acids in Biocid® compound.