Japanese Journal of Veterinary Research 73(1): 1–9, 2025

JJVR

REGULAR PAPER Experimental Research

Effect of zinc bacitracin on various hematochemical and production attributes of broiler chickens

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Received for publication, March 27, 2024; accepted, November 6, 2024

Abstract

The poultry industry is always at a risk due to high feed prices and frequent disease outbreaks. A global trend of using Antibiotic Growth Promoters (AGPs) for prophylaxis, improvement production, and enhanced growth have entered the poultry industry. Being banned in European countries, the AGPs are still extensively being used in South Asian's poultry industry which has higher bacterial infections as compared to the European countries. Zinc bacitracin, an AGP, at sub-therapeutic concentration has shown advantageous results in poultry. In this study zinc bacitracin was made in CASVAB University of Balochistan labs and applied for the trial. In this study birds were distributed in three groups, group 1 used feed with no feed additive, group 2 used feed with 20 mg zinc bacitracin/kg and group 3 used 40 mg zinc bacitracin/kg in feed as feed additive. Body weight gain was observed as 513.7 grams (g) as compare to control group of birds which was 424.3 (g), which is highly significant and significant with group 2. Final body weight was observed 1250.1 (g) in group 3 significant with group 2 observed 1200.0 (g) and highly significant with group 3 which was 1100.2 (g). Mortality rate in group 3 was observed 1.8 which is highly significant $P \leq 0.05$ with group 3 and significant with group 2. We also observed significant result in group 3 as compare to group 2 and 1, regarding to blood leukogram, hemogram and various serum chemistry attributes.

Key Words: FCR, Feed intake, Weight gain, Zinc bacitracin

Introduction

Poultry sector is the most dynamic sector of the livestock sectors in Pakistan. An estimated 1.5 million people in Pakistan are employed in it owing to this large sub sector. The last decade has witnessed a 7.5% annual growth in poultry hence placing Pakistan at eleventh position among the large poultry producers of the world[21)]. Comparing domestic/rural poultry with commercial poultry, Economic Survey of Pakistan (2020-21) has revealed an increase of 1.5% in rural poultry as compared to 10% increase in commercial poultry for the last year 2019-2020[31)]. All the governments

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doi: 10.57494/jjvr.73.1_1

have always been highly supportive to this industry with developments attained in disease surveillance, diagnostics, intensive hi-tech-production systems, value addition, disease prevention and better management practices. When intensive poultry introduced the production of poultry i.e. good breeds, biosecurity improved, as well as preventive health measures improved. Poultry production improved and brought dramatic changes. In the developing countries the production system is very restricted due to the high cost of infrastructure to protect biosecurity for the different types of birds, the quality of rearing of hybrid chicks cost, providing balanced feed and best veterinary care[4,23]. The poultry industry is always at a risk due to high feed prices and frequent disease outbreaks. Furthermore, pathogenic and virulent microorganisms which lodge themselves in the Gastro Intestinal Tract (GIT) of the poultry such as *E. coli*, *Salmonella spp.* and *Clostridium perfringens* cause gastrointestinal disturbances, digestive inefficacy, poor meat quality, high mortality and various food-borne diseases. Resultantly, a global trend of using Antibiotic Growth Promoters (AGPs) for prophylaxis, improvement production, and enhanced growth have entered the poultry industry[10,11,13]. An extensive work has globally been reported on advantages and disadvantages of AGPs[4,7,9,37]. However, an increased bacterial resistance and meat residues of AGPs, many of the European countries have banned their use resulting in new founded research interest in finding alternatives[8]. Though being banned in European countries, the AGPs are still extensively being used in South Asian's poultry industry which has higher bacterial infections as compared to the European countries. Zinc bacitracin, an AGP, at sub-therapeutic concentration has shown advantageous results in rabbits[12,32], large ruminants[20] and poultry[3,5,19]. Modification of gut flora, enhanced digestibility, increased production, and increased production attributes are a few of the benefits associated with the use of zinc bacitracin. There is no such study related to use of zinc bacitracin in poultry chicken. Our study has therefore been devised with an important objective to assess certain hematochemical and production attributes in zinc bacitracin-fed broiler chickens.

Materials and Methods

Availability of zinc bacitracin

Zinc bacitracin was produced from *Bacillus licheniformis* isolated from milk, in the laboratories of Center for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan Quetta.

Study animals and management

One day-old chicks of broiler (n= 785) (Hubbard classic strain) with a mean (± SE) body weight of (42.3 ± 0.5g) were procured from a commercial hatchery. The birds were reared in February to March 2022 at the Government poultry farm for a 45-days trial. Standard husbandry practices were used for rearing the birds. Floor was disinfected with limestone. Feeders and waterers were washed and disinfected. For the purpose of bedding, saw-dust was used. Day-old chicks were introduced to preheated brooders for 8 days. Vitamin E (Selvite E®) product of Bio Lab purchased from Lahore Pakistan and glucose were added to drinking water in order to reduce stress. The farm temperature was kept at 95ºF in 1st week (1st 7 days), then decreased by 5ºF every week till it reached down to 75ºF. All the birds were well vaccinated against diseases like Newcastle and Bronchitis disease (on 3rd day and 25th day), and against Infectious Bursal Disease (on 13th day and 20th day). Water and feed was *ad libitum* provided to the birds. Our study was approved by Biosafety committee of Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan and Bioethics Committee of Livestock and Dairy Development Department Balochistan in a meeting held on January 10, 2022 Letter No. 598. As CASVAB and Livestock Department signed some MOUs and agreements that's why we are in close departmental relations with each other.

Animal groups and diet

The birds of all groups were acclimatized on basal diet with no supplementation or feed additive for eight days. After a mortality of 35 chicks, remaining 750 chicks were assigned three groups having three replicates on the basis of diet (n= 250 birds/group) *viz*. Group 1: Control group fed basal diet free of zinc bacitracin, group 2: Experimental group fed zinc bacitracin (20 mg/kg) added in vitamin mineral mixtures and group 3: Experimental group fed zinc bacitracin (40 mg/kg) added in vitamin mineral mixtures. Zinc bacitracin was added to the mixture of vitamin and mineral before mixing in the experimental diets (Table 1).

Table 1. Composition of the basal diet

Ingredients	Inclusion level (%)
Corn	40
Rice Tis	10
Maize Gluten 30%	6
Soybean Meal	8
Fish Meal	6
Rice Polishing	11
Limestone Powder	7
Canola Meal	10
Dicalcium Phosphate	1.5
Vitamin mineral Premix + Amino acid	0.5
Total	100
Crude Protein	16
Energy	2795 kcal

kcal: kilocalorie

Production attributes

Various production attributes *viz*. initial body weight (IWG), body weight gain (BWG), final body weight (FBW), feed intake (FI), feed conversion ratio (FCR), water/feed ratio (W/F) and European Production Index (EPI) were determined in the study[33]. Weight gain and feed intake were observed and recorded on daily basis. Mortality rate (MR) was recorded and the weights of dead different birds were used to measure corrected FI, FCR and EPI. For EPI, following formula was used, as prescribed earlier.

$$EPI = \frac{(\text{Average grams gained / day} \times \%\text{Survival rate})}{\text{Feed conversion} \times 10}$$

[a]Premi/kg feed: Cholecalciferol (vitamin D3) 2200 U, Riboflavin (vitamin B2) 5.5 mg, Vitamin A (retinol) 8300 U, D-calcium pantothenic acid (vitamin B5) 15 mg, Niacin (vitamin B3) 36 mg, Choline 500 mg, Folic acid (vitamin B9) 0.5 mg, Vitamin B1 was 1 mg, Pyridoxine was added 2.2 mg, Biotinin feed was 0.05 mg, Vitamin K was found 2 mg in feed formula. Vitamin E was 8 U while cobalmin (Vitamin B12) was 0.02 mg. Moreover in the feed Manganese was 80 mg, Zinc in the feed was 60 mg, Iron 60.0 mg, Copper 5.00 mg, Cobalt 0.2 mg, Iodine 1.00 mg, Selenium 0.15 mg, Amino Acids/kg in the feed: Lysine was 0.72 g, Methionine 0.143 g, Threonine 0.35 g in the feed of birds. The vitamin mineral premix contained 20 mg and 40 mg of zinc bacitracin for groups 2 and 3, respectively throughout the study.

Blood collection and processing

The birds (n= 15 from each group) were bled thrice during the 45-day experiment at day 0, 21 and 45 through aseptic methods. Two aliquots of blood were ascertained i.e. unclotted blood in EDTA vacutainers and clotted in serum extraction vacutainers. In the laboratory, blood was centrifuged at 3000 G for 15 minutes (Sigma Aldrich, MZ50, Germany) and serum was accordingly harvested. Serum was frozen at -25°C till final analyses. Whole blood was tested for hematological attributes within 8 hours of collection using veterinary hematology analyzer (Rayto, China). On-site thin blood smears were also prepared and were fixed with methanol (SDL, Pakistan). The slides were stained using Field's stain (SDL, Pakistan) and were studied under microscope (Labomed, USA) (100X) for differential leukocyte count "DLC"[21]. The hematological parameters included *viz*. concentration of hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC) and total leukocyte count (TLC). The Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated using the prescribed formulae. Regarding serum biochemical profile, triglycerides

Table 2. Mean (±SE) values for various production attributes of broilers fed different levels of zinc bacitracin

Attributes	Treatment groups		
	Control	ZB20	ZB40
Initial body weight (g)	42.3±0.5	43.3±0.5	43.7±0.5
Body weight gain (g/week)	424.4±23.4	504.0±15.0a	513.7±77.0a
Final body weight (g)	1100.2±32.5	1200.0±47.5a	1250.1±78.2a
Mortality rate (%)	3.8±0.05	2.5±0.5a	1.8±0.4a
Feed intake (g/bird)	2001.0±92.7	2048.7±78.2a	2100.7±52.3a
Feed conversion ratio (kg/kg)	2.8±0.5	3.7±0.7a	4.8±0.4a
Water/feed ratio (L/kg)	1.7±0.4	1.9±0.4a	2.7±0.9a
European production index (units)	152.0±11.0	189.4±13.2a	205±15.2a

a; significant difference at $P \leq 0.05$ of treatment groups with the control group, ZB: zinc bacitracin, g: gram, kg: kilogram, L: liter

Table 3. Mean (±SE) values for various hematological attributes of broilers fed different levels of zinc bacitracin

Attributes	Treatment groups		
	Control	ZB20	ZB40
HEMOGRAM			
Hemoglobin concentration (g/L)	7.4±1.4	8.4±2.0a	10.4±1.7a
Total erythrocyte count (1012/L)	2.3±0.4	3.4±0.7a	3.7±0.8a
Packed Cell volume (%)	32.0±1.2	38.4±1.7a	39.2±1.9a
Mean corpuscular volume (fL)	99.0±2.5	120±3.5a	121.0±2.9a
Mean corpuscular hemoglobin (pg)	29.2±1.2	34.2±2.5a	38.4±2.9a
Mean corpuscular hemoglobin concentration (g/L)	28.2±1.4	33.2±2.7a	34.5±2.4a
LEUKOGRAM			
Total leukocyte count (109/L)	11.2±1.4	12.4±1.4a	10.0±1.4a
Neutrophils (%)	38.1±1.7	41.0±2.4	40.0±1.7
Lymphocytes (%)	49.0±1.7	51.1±2.0	51.2±1.7
Eosinophils (%)	2.4±0.7	1.9±0.9	1.8±0.7
Basophils (%)	1.7±0.7	1.6±0.7	1.7±0.8
Monocytes (%)	1.2±0.1	1.2±0.1	1.3±0.1

a; significant difference at $P \leq 0.05$ of treatment groups with the control group, ZB: zinc bacitracin, g: gram, kg: kilogram, L: liter
fL: femtoliter, pg: picogram

(TGs), total cholesterol (TC), high-density lipoproteins (HDL), low-density lipoproteins (LDL) and total proteins (TP) were determined using spectrophotometer (APEL PD 303S, Japan) and commercial diagnostic kits (Spinreact®, Spain) following the manufacturer's instructions.

Statistical analyses

It was conducted through Social Science (SPSS for 12 Windows version, USA). Mean values (±SE) were calculated for all the production and hematochemical attributes. Difference between the three groups Repeated Measures Analysis of Variance (MANOVA), followed by Bon ferroni post-hoc test. Significance was considered at $P \leq 0.05$.

Results

Amongst the studied attributes, the BWG, FBW, FI, FCR, W/F and EPI observed significantly ($P \leq 0.05$) higher in group 3 fed 40 mg/kg of zinc bacitracin. Mortality rate was found significantly ($P \leq 0.05$) lower in this group as compared to the counterpart two groups (Table 2).

Group 1, was fed with no feed additive in the feed, group 2 was fed with 20 mg zinc bacitracin as feed additive per kilogram in feed while group 3 was fed with 40 mg zinc bacitracin as feed additive in the birds feed. In this table the results of 40 mg feed additive were more significant as compare to other 2 groups like body weight gain and final body weight of birds found high as compare to other groups $P \leq 0.05$. We also found less mortality

Table 4. Mean (±SE) values for various serum chemistry attributes of broilers fed different levels of zinc bacitracin

Attributes	Treatment groups		
	Control	ZB20	ZB40
Triglycerides (mg/dL)	124.0±7.8	134.4±6.2a	120.0±5.4a
Total cholesterol (mg/dL)	140.4±7.8	131.4±6.4a	120.0±4.7a
High-density lipoproteins (mg/dL)	58.0±4.5	52.1±3.8a	40.7±2.4a
Low-density lipoproteins (mg/dL)	62.0±5.5	54.2±3.4a	71.4±5.9a
Total proteins (g/dL)	2.7±0.7	3.0±0.9a	4.7±1.0a

a; significant difference at $P \leq 0.05$ of treatment groups with the control group, ZB: zinc bacitracin, mg: milligram, dL: deciliter, g: gram

rate in group 3 the same significant results were found in FCR, Water feed ratio and EPI of group 3 (Table 3).

Group 1, was fed with no feed additive in the feed, group 2 was fed with 20 mg zinc bacitracin as feed additive per kilogram in feed while group 3 was fed with 40 mg zinc bacitracin as feed additive in the birds feed. Results of hemoglobin concentration, total erythrocyte count, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and total leukocyte count level were significantly higher ($P \leq 0.05$) in treatment 1 and 2 groups compared to control group. As a whole group 3 results are significantly higher in Hemogram section. In Leukogram section the results of attributes were close to each other but as whole results of group 3 were slightly higher to group 1. It was evident that there was significant ($P \leq 0.05$) increase in hemogram (Hb, TEC, PCV, MCV, MCH and MCHC) of the birds fed zinc bacitracin with best results shown at 40 mg. Amongst the leukogram attributes, only TLC count was effected significantly higher ($P \leq 0.05$) being lowest for group 3 fed 40 mg of zinc bacitracin.

Group 1, was fed with no feed additive in the feed, group 2 was fed with 20 mg zinc bacitracin as feed additive per kilogram in feed while group 3 was fed with 40 mg zinc bacitracin as feed additive in the birds feed (Table 4). It was noticed that TC and HDL were significantly higher ($P \leq 0.05$) increased whereas LDL and TP were significantly ($P \leq 0.05$) decreased in group 3 fed with 40 mg of zinc bacitracin. The TGs showed a significant ($P \leq 0.05$) increase in group 2 (fed 20 mg zinc bacitracin) but significantly lower or decreased for group 3 (fed 40 mg zinc bacitracin).

Discussion

In this study zinc bacitracin was produced in the labs of our department. *Bacillus licheniform* is which was isolated from milk was selected as source for antibiotic production. In the production process we adopted the cheapest methods to compete the Chinese products which are dominant on the local markets of Pakistan. In future our goal is marketing the product in Pakistan. In Pakistan the use of zinc bacitracin and other AGPs are on large scale due to endemic conditions of bacterial infections in poultry sector the present research work is a novel one being reported from Pakistan which was aimed towards assessment of zinc bacitracin-fed broiler chickens through alterations in various hematochemical and production attributes. Results of the present study clearly indicate that feeding 40 mg/kg of zinc bacitracin to broiler chicken had beneficial effects on all the studied attributes owing to a generalized better health of birds. As there is a paucity of research work on similar aspects from Pakistan, hence the results have been logically discussed with studies which have emanated globally and with those reported for other AGPs. Amongst production attributes, the BWG, FBW, FI, FCR, W/F and EPI were higher in zinc bacitracin-fed birds with a lower mortality rate in the present study. Similar results have been reported from other studies in which certain other AGPs were fed to the broiler chickens. Supplementation of yeast cell

walls tended to increase BW, FCR, FI and EPI in poultry and decreased mortality rate[35]. Prebiotics-fed groups have also presented similar results[1]. On the contrary, there are many studies which have resulted that taking AGPs do not affect the FI in chicken[6,14]. Another report has showed that prebiotics supplementation in feed had same effects on weight gain (263 vs. 264 g/bird) and FCR (1.40 vs. 1.40) in broiler compared with enramycin[27]. However, yet another study reported that broiler birds fed diets which have antibiotics like zinc bacitracin, enramycin and furazolidone, had best weight gain (851, 863 and 862 vs. 821 g/bird), FCR (1.47, 1.48 and 1.49 vs. 1.53) than those on diet added with prebiotics during starter phase[24].

Comparison of our results with those published from Pakistan revealed that mostly mannan oligosaccharides and other prebiotics have been tried for poultry birds, however, zinc bacitracin has not been used and tried[24,27]. Better production attributes of the study birds in our work may plausibly be attributed to better health conditions of intestinal lumen, elevated intestinal beneficial micro flora and better absorption of digestible nutrients[35,36]. Ultimately, the better health condition of study birds result in a lower mortality rate as depicted through our results.

Study and interpretation of hematological attributes have long been marked as one of the best diagnostic and management tools to assess general health condition of a living organism[16,17,30]. These attributes not only depict the physiological and nutritional status but also provides an insight into the pathological/clinical aspects of the animal under question. The present study incorporated various hematological parameters which are in vogue for assessment of health. Regarding our results on hematological parameters, our results showed that there was a significant increase in hemogram (Hb, TEC, PCV, MCV, MCH and MCHC) of the birds fed zinc bacitracin with best results shown at 40 mg. Amongst the leukogram attributes, only TLC count was the lowest for birds supplemented with 40 mg of zinc bacitracin. It has already been established that the RBC parameters (Hb, TEC, PCV, MCV, MCH and MCHC) are a true depiction of the health status of an animal amongst all hematological parameters. Increase in these attributes indicates a higher erythrogenesis (primary erythrocytosis) and hence circulatory well-being of the animal[15,26]. An increased level of these RBC parameters in zinc-bacitracin birds of our study indicates better health status of animals as correlated with better productive attributes of this study as ascertained through prior studies[2,38]. Amongst the hematological attributes, the TLC can detect hidden infections in an animal. An elevated level indicates a stressful or a pathological status of the said animal/bird. Diagnostic and prognostic ability of TLC has widely been studied with the conclusion that leukocytosis (increased TLC) could be an indication of infection, bacteremia, parasitemia, and toxemia[19,39]. The lower TLC for zinc bacitracin-fed birds in our study again indicates stress-free and healthy status of the study birds fed with zinc-bacitracin. Similar results have been reported by other researchers as well[24,34]. The variables of serum chemistry of our study include tests of interest in a routine clinical pathology laboratory. The results of serum chemistry in our study showed that TC and HDL were increased significantly while LDL and TP were observed significantly decreased or low in the birds fed with 40 mg of our product (zinc bacitracin)[28]. Birds taken bacitracin in tested dose individually or together in drinking water for five successive days showed a non-significant enhancement in serum total protein, albumin, total, γ, ß and α globulin coupled with a non-significant decline in ratio of albumin globulin at first day post administration in another study[28]. In the same way, zinc bacitracin induced a non-significant elevation in plasma protein profile levels[18]. Moreover, zinc bacitracin in the feed of broilers chickens was observed safe as the function of liver, tests and protein picture, were not altered[22]. In addition, bacitracin supplement in ration mixed a non-significant elevation in total protein, albumin, total globulin, γ globulin, ß and α globulin[25]. In recent past, tour results have been supported by another trial study the results showed that the using the feed additive induced non-significant enhancement in serum total

protein, albumin, total protein, and albumin in broiler chickens[1]. Another study supporting us stated that growth promoters like bacitracin and lincomycin, induced a non-significant increase in serum total protein, albumin, total, α, ß and γ globulin in healthy broilers[29]. In a nutshell, it is concluded that the broiler chicken supplemented with 40 mg/kg of zinc bacitracin show positive health aspects in terms of production (BWG, FBW, FI, FCR, W/F and EPI), hematological (TEC, Hb, PCV, MCV, MCHC and MCHC) and serum chemistry (TC, HDL, LDL and TP) attributes. We recommend supplementation of zinc bacitracin at a dose rate of 40 mg/kg in poultry chicken for beneficial productive results. We further recommend that other dosages for this AGP and other AGPs may also be tested in future research endeavors.

Conflict of Interest

The authors have no conflict of interest.

References

1) Abdel-Hafeez HM, Saleh ESE, Tawfeek SS, Youssef IMI, Abdel-Daim ASA. Effects of probiotic, prebiotic, and synbiotic with and without feed restriction on performance, hematological indices and carcass characteristics of broiler chickens. Asian-Australas J Anim Sci 30, 672–682, 2017. doi: 10.5713/ajas.16.0535.
2) Ahsan-ul-Haq, Tabassum Ch. M, Ahmad F, Shafi J, Ashraf M, Javed M, Shahid-ur-Rehman. Effect of dietary acidification with citric acid on carcass characteristics, haemogram and serum metabolite values of broiler chicken. Pakistan journal of life and social science 12, 36–41, 2014.
3) Alp M, Kocabağli N, Kahraman R, Bostan K. Effects of dietary supplementation with organic acids and zinc bacitracinon ileal microflora, pH and performance in broilers. Turkish journal of veterinary and animal sciences 23, 451–456, 1999.
4) Ashraf S, Bhatti SA, Kamran Z, Ahmed F, Rahman SU. Assessment of refined functional carbohydrates as substitutes of antibiotic growth promoters in broilers: Effects on growth performance, immune responses, intestinal micro-flora and carcass characteristics. Pakistan veterinary journal 39, 157–162, 2019. doi: 10.29261/pakvetj/2019.040.
5) Attia YA, Bovera F, Abd El-Hamid AE, Tag El-Din AE, Al-Harthi MA, El-Shafy AS. Effect of zinc bacitracin and phytase on growth performance, nutrient digestibility, carcass and meat traits of broilers. J Anim Physiol Anim Nutr (Berl) 100, 485–491, 2016. doi: 10.1111/jpn.12397.
6) Baurhoo B, Ferket PR, Zhao X. Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations, and carcass parameters of broilers. Poult Sci 88, 2262–2272, 2009. doi: 10.3382/ps.2008-00562.
7) Bedford M. Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimise subsequent problems. World's Poult Sci J 56, 347–365, 2000. doi: 10.1079/WPS20000024.
8) Casewell M, Friis C, Marco E, McMullin P, Phillips I. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. J antimicrob Chemother 52, 159–161, 2003. doi: 10.1093/jac/dkg313.
9) Cervantes HM. The future of antibiotic growth promoters in poultry production. Paper presented at the 24th World's Poultry Congress, 2012.
10) Crisol-Martínez E, Stanley D, Geier MS, Hughes RJ, Moore RJ. Understanding the mechanisms of zinc bacitracin and avilamycin on animal production: linking gut microbiota and growth performance in chickens. Appl Microbiol Biotechnol 101, 4547–4559, 2017. doi: 10.1007/s00253-017-8193-9.
11) Elkomy AA, Farag E, Elgharbawy EI,

Elbadawy M. Comparative studies on the effects of lincomycin and bacitracin on hematobiochemical and immunological parameters in broiler chickens. International journal of phaemacology and toxicology 7, 1–5, 2019. doi: 10.14419/IJPT.V7I1.28033.

12) El-Deep MH, Amber KA, Eid YZ, Aboelenin SM, Soliman MM, Sakr MS, Dawood MAO. The influence of chicken egg lysozyme or zinc-bacitracin antibiotic on the growth performance, antibacterial capacity, blood profiles, and antioxidative status of rabbits: A comparative study. Animals (Basel) 11, 1731, 2021. doi: 10.3390/ani11061731.
13) Enberg RM, Hedemann MS, Leser TD, Jensen BB. Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. Poult Sci 79, 1311–1319, 2000. doi: 10.1093/ps/79.9.1311.
14) Eseceli H, Demir E, Değirmencioğlu N, Bilgic M. The effects of Bio-Mos® mannan oligosaccharide and antibiotic growth promoter performance of broilers. Journal of animal and veterinary advances 9, 392–395, 2010. doi: 10.3923/javaa.2010.392.395.
15) Farooq U, Ahmad N, Ahmad I, Mahmood SA, Andrabi SMH, Idris M. Effect of seasonal variations on hematochemical profile of Cholistani service bulls. Journal of applied animal research 45, 85–89, 2017. doi: 10.1080/09712119.2015.1125351.
16) Farooq U, Ijaz A, Ahmed N, Rehman H, Zaneb H. Haematologic profile revisited: Adult Cholistani breeding bulls as a model. Journal of animal and plant sciences 22, 835–839, 2012.
17) Farooq U, Samad HA, Khurshid A, Sajjad S. Normal reference hematological values of one humped camels (Camelus dromedarius) kept in Cholistan desert. Journal of animal and plant sciences 21, 157–160, 2011.
18) Gouda A, Amer SA, Gabr S, Tolba SA. Effect of dietary supplemental ascorbic acid and folic acid on the growth performance, redox status, and immune status of broiler chickens under heat stress. Trop Anim Health Prod 52, 2987–2996, 2020. doi: 10.1007/s11250-020-02316-4.
19) Govaert GAM, Bosch P, IJpma FFA, Glauche J, Jutte PC, Lemans JVC, Wendt KW, Reininga IHF, Glaudemans AWJM. High diagnostic accuracy of white blood cell scintigraphy for fracture related infections: Results of a large retrospective single-center study. Injury 49, 1085–1090, 2018. doi: 10.1016/j.injury.2018.03.018.
20) Hillerton JE, Bryan MA, Beattie BH, Scott D, Millar A, French N. Use of antimicrobials for food animals in New Zealand: updated estimates to identify a baseline to measure targeted reductions. N Z Vet J 69, 180–185, 2021. doi: 10.1080/00480169.2021.1890648.
21) Hussain J, Rabbani I, Aslam S, Ahmad HA. An overview of poultry industry in Pakistan. Worlds Poult Sci J 71, 689–700, 2015. doi: 10.1017/S0043933915002366.
22) Ismail IB, Al-Busadah KA, El-Bahr SM. Oxidative stress biomarkers and biochemical profile in broilers chicken fed zinc bacitracin and ascorbic acid under hot climate. American journal of biochemistry and molecular biology 3, 202–214, 2013. doi: 10.3923/AJBMB.2013.202.214.
23) Jamali MB, Soomro HJ, Halepoto AH, Hashmi MA, Shaikh FM. Problems faced by the poultry industry in Pakistan. Australian journal of business and management research 1(8), 96–100, 2011. doi: 10.52283/NSWRCA.AJBMR.20110108A12.
24) Kamran Z, Mirza MA, Ahmad S, Samad HA, Sohail MU, Saadullah M. Performance of broiler chickens fed mannan oligosaccharides as alternatives to antibiotics from one to twenty-two days of age. Journal of animal and plant sciences 23, 1482–1485, 2013.
25) Manafi M, Khalaji S, Hedayati M, Pirany N. Efficacy of *Bacillus subtilis* and bacitracin methylene disalicylate on growth performance, digestibility, blood metabolites, immunity, and intestinal microbiota after intramuscular inoculation with *Escherichia coli* in broilers. Poult Sci 96, 1174–1183, 2017. doi: 10.3382/ps/pew347.
26) McMullin MF. The classification and diagnosis of erythrocytosis. Int J Lab Hematol, 30,

447–459, 2008. doi: 10.1111/j.1751-553X.2008.01102.x.

27) Mohammed MA, Hassan HMA, El-Barkouky EMA. Effect of mannan oligosaccharide on performance and carcass characteristics of broiler chicks. Journal of agriculture and social sciences 4, 13–17, 2008.
28) Mohamed MI, Maareck YA, Abdel-Magid SS, Awadalla IM. Feed intake, digestibility, rumen fermentation and growth performance of camels fed diets supplemented with a yeast culture or zinc bacitracin. Anim Feed Sci Technol 149, 341–345, 2009. doi: 10.1016/j.anifeedsci.2008.07.002.
29) Naveenkumar S, Karthikeyan N, Narendra Babu R, Veeramani P, Sivarama Krishnani S, Srinivasan G. Effect of calcium propionate and coated sodium butyrate as an alternative to antibiotic growth promoters on the serum profile of commercial broiler chicken. International journal of chemical studies 6(1), 36–39, 2018.
30) Njidda AA, Isidahomen CE. Hematological parameters and carcass characteristics of weanling rabbits fed sesame seed meal (Sesamum indicum) in a semi-arid region. Pakistan veterinary journal 31, 35–39, 2011.
31) Finance Division, Government of Pakistan. 2019. Agriculture. In: Pakistan Economic Survey 2018–19. http://www.finance.gov.pk/survey/chapters_19/2-Agriculture.pdf [accessed on April 8, 2020].
32) Pinheiro V, Mourão JL, Alves A, Rodrigues M, Saavedra MJ. Effect of zinc bacitracin on the performance, digestibility and caecal development of growing rabbits. In: Proceedings of the 8th world rabbit congress, September 7–10, 2004, Puebla, Mexico. pp. 942–947, 2004.
33) Qubih TS, Bayon OS, Shareef AM. Correlation between broiler aflatoxicosis and European production efficiency factor. Iraqi journal of veterinary sciences 22, 49–55, 2008. doi: 10.33899/ijvs.2008.5670.
34) Rafeeq M, Rashid N, Tariq MM, Tareen RB, AsadUllar, Mustafa Z. Evaluation of alternatives to antibiotic feed additives in broiler production. Pak J Zool 49, 1063–1069, 2017. doi: 10.17582/journal.pjz/2017.49.3.1063.1069.
35) Santin E, Maiorka A, Macari M, Grecco M, Sanchez JC, Okada TM, Myasaka AM. Performance and intestinal mucosa development of broiler chickens fed diets containing Saccharomyces cerevisiae cell wall. J Appl Poult Res 10, 236–244, 2001. doi: 10.1093/japr/10.3.236.
36) Sugiharto S, Yudiarti T, Isroli I, Widiastuti E. The physiological responses to dietary administration of zinc bacitracin and Bacillus mixture on low-weight day-old chicks. Poultry science journal 6, 51–62, 2018. doi: 10.22069/PSJ.2018.14251.1301.
37) Thema K, Mlambo V, Snyman N, Mnisi C M. Evaluating alternatives to zinc-bacitracin antibiotic growth promoter in broilers: Physiological and meat quality responses. Animals (Basel) 9, 1160, 2019. doi: 10.3390/ani9121160.
38) Tollba AAH. Reduction of broilers intestinal pathogenic micro-flora under normal or stressed condition. Egyptian poultry science journal 30, 249–270, 2010.
39) Yu C-W, Juan L-I, Wu M-H, Shen C-J, Wu J-Y, Lee C-C. Systematic review and meta-analysis of the diagnostic accuracy of procalcitonin, C-reactive protein and white blood cell count for suspected acute appendicitis. Br J Surg 100, 322–329, 2013. doi: 10.1002/bjs.9008.