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COMPARATIVE STUDY TO EVALUATE ANTIBACTERIAL FEED ADDITIVE AND PROBIOTIC ON THE PERFORMANCE OF BROILER CHICKEN

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Abstract

The present study was carried out to evaluate the effect of antibiotic feed additives and probiotics on the productive performance of broiler chicken. For this purpose, 280-day-old broiler chicks were randomly divided into seven treatment groups. The birds were offered basal starter and finisher diet (Control) or the same basal diet supplemented with zinc bacitracin @ 250 and 500mg kg^{-1} (ZnB1 & ZnB2) alone and in combination (AB+ZnB1 & AB+ZnB2) with commercial blend of antibacterial feed additive (AB) 500mg kg^{-1}; probiotic was offered to birds through gavage once every week @ 3x10^{8}cfu per bird. Supplementation of feed additives and probiotics improved the growth performance of broiler chicken in comparison to the control ($p<0.05$). Supplementation of ZnB1 and ZnB2 alone did not show better growth in comparison to the commercial blend of antibiotics; similarly combination of zinc bacitracin with antibiotic did not exhibit any additive effect ($p>0.05$). Feed consumption was noted significantly different among treatment groups ($p<0.05$). Haematological and serum biochemical parameters observed were noted as insignificant among treatment groups ($p>0.05$); however, minor numeric differences within normal ranges were present. In conclusion, the results of the present study suggest that the supplementation of antibacterial additive can improve the growth performance of broiler chickens and probiotics given through water can also be an option to replace antibacterial growth promoter feed additives.

Keywords: *Broiler chicken, growth, probiotics, zinc bacitracin*

INTRODUCTION

The commercial production of poultry in Pakistan started in 1960s, since than it has played a vital role in bringing the gap between the demand and supply of animal protein and its contribution in the GDP is about 1.3 percent. At present poultry industry is the second largest industry providing employment over 1.5 million people after textile industry in Pakistan (1). According to World Health Organization (WHO) standard per capita animal protein consumption per day is 27 g/day; while in Pakistan this consumption is 17 g/day (2), this gap in animal protein availability and increase in human population indicates that the poultry industry has immense opportunity to flourish.

The intensive poultry production system is facing some serious challenges like, high feed costs, inadequate cold chain, transportation, infrastructure and high vulnerability to diseases out breaks (2). Beside these challenges one of the most serious challenges of this industry is the use of antibiotics as growth promoters (AGPs). In the past the antibiotics were used for the prophylaxes purposes intensively in the animals farming to improve the growth and health of animals (3). The dietary antibiotic not only improves the growth and feed conversion efficiency, but also controls enteric disorders (4). However due to the negative effect of AGPs; such as, cross resistance and carry over effect resulted in the ban over the use of AGPs (5). In some parts of the world use of antibiotics as growth promoters has already been banned and they are striving for the search of alternative to AGPs (6). In 1986 Sweden ban certain growth promoting antibiotics such as avopracin and virginiamycin. Initially European Union (EU) banned the use of antibiotics as growth promotors in 1999 on the basis of precautionary principles the four antibiotics were Spiromycin, Tylosin and Virginimycin (7; 8). These antibiotics were banned due to political, consumer opinion and scientific concerns. In 2012 the world health organizations (WHO) declared the antimicrobials in food as a global threat; furthermore, WHO stated that the healthy animals should only receive antibiotic to prevent the diseases (9).

Antibiotics are widely used as remedial agents and are considered as last resort against diseases for human beings. The discovery of antibiotic resistant bacterial strain alarmed the situation and most of the criticism over the use of same antibiotics in animal health and production can be witnessed. Under these circumstances unconventional resources that can replace AGPs are the need of the time. Certain prebiotics and probiotics are extensively tested and used throughout the globe. The addition of Zinc bacitracin to the broiler feed can play important role to enhance growth performance and utilization of feed (10). Similarly certain probiotics have been found to enhance the production performance. There has been variation in results about the effects of probiotics supplemented diets as a growth promoters and immune associated organs of chickens (11). Different production system and environmental conditions play critical role. Hence present study was carried out with the objective to evaluate the productive performance of broiler chicken supplemented probiotics, zinc bacitracin either solo or in combination with commercial blend of antibiotic growth promoter.

MATERIALS AND METHODS

EXPERIMENTAL LAYOUT AND TREATMENTS

The present study was conducted at the experiment station of CASVAB, University of Balochistan, Quetta, Pakistan. Two hundred and eighty (n=280) day-old broiler chicks were divided into seven groups, forty (n=40) chicks in each group, further divided into four replicate of ten (n=10) chicks each. Dietary treatments were control (basal diet), probiotic (*Bacillus clausii*) @ 1ml / bird once a week, ZnB1 (Zinc bacitracin @ 250mg / kg feed), ZnB2 (Zinc bacitracin @ 500mg / kg feed), AB (commercial blend of antibiotics containing streptomycin and procaine penicillin @ 500mg / kg), AB+ZnB1 (anti-biotic @ 500mg / kg + zinc bacitracin 250mg / kg) and AB+ZnB2 (anti-biotic @ 500mg / kg + zinc bacitracin @ 500mg / kg). The broiler chickens were offered measured quantity of feed twice daily and recorded any refusal on weekly basis. Fresh water was provided ad libitum. Experiment lasted for 42 days.

BIRD HUSBANDRY

The chicks were housed in the floor pens and optimal environmental conditions were provided. Vaccination was carried out against Newcastle Disease (ND) and Infectious Bursal disease (IBD) as per standard schedule. Chicks were allowed a free access to fresh water. The experimental groups were allotted seven dietary treatments. Single basal broiler starter and finisher feed was offered to all the treatment groups.

PREPARATION OF ZINC BACITRACIN

For the present experiment zinc bacitracin was prepared at Physiology laboratory of CASVAB, University of Balochistan, Quetta using the previously published protocol (12) with some modification. Briefly, the strain of *Bacillus subtilis* was grown in the bacteriological media for 24 hour at 35ºC and pH 7 with continuous shaking. Later on, the media was centrifuged to collect the supernatant and Zinc chloride was added and allowed to precipitate for 24 hours. Later, the tubes were centrifuged to obtain Zinc adsorbed bacitracin. The precipitate obtained were allowed to dry at the room temperature and stored in dry containers until further experimentation.

PREPARATION OF BACTERIAL GROWTH MEDIA

The bacterial growth media (Broth and agar) was prepared in the physiology laboratory CASVAB. To make one liter of bacterial growth media meat peptone 10g, beef extract 10g, yeast extract 5g, ammonium citrate 2g, dextrose 20g, tween solution 1g, sodium acetate 5mg, $MgSO_4$ 0.1g, $MnSO_4$ 0.05 g, K_2HPO_4 2g and agar 2g, were added to water to make volume one liter; adjusted pH at 6.5± 0.5. Prepared media was then autoclaved at 121°C for 20 minutes. The media was then transferred in the petri dishes and incubated at 37°C for 24 hours to ensure sterility.

SEPARATION OF ZINC BACITRACIN FROM THE BACTERIAL CULTURE

Cultured media with optimal bacterial growth was poured in sterilized falcon tubes (30ml) and centrifuged at 5000 rpm for 10 Minutes, collected the supernatant and discarded the bacterial biomass. Zinc chloride (1g/10ml) was added and incubated it for 24hrs at 37°C. Zinc choloride and bacitracin solution shifted to falcon tubes; centrifuge it at 5000 RPM for 10 minutes. Zinc bacitracin precipitated at the bottom of falcon tubes recovered with the help of R/O Water by shaking. Product recovered then transferred to the beaker and kept it in the hot air dryer and allowed R/O water to evaporate overnight and dried zinc bacitracin collected in the powder form.

PREPARATION OF PROBIOTIC

Probiotic (Bacillus Clausii) was suspended in phosphate buffer saline and make the dilution of 1.0 macfarland standard containing approximately 3x108cfu per ml.

GROWTH PERFORMANCE

To evaluate growth performance of broiler chicken weekly average body weight gain and average feed intake of the treatment groups were recorded. Feed conversion Ratio (FCR) and average daily gain (ADG) were calculated from the data collected.

HEMATOLOGY AND SERUM BIOCHEMISTRY

At the end of the experiment, at day 42 two (n=2) chicks from each replicate were randomly taken and blood sample were collected to evaluate hematology parameters. For serum blood samples were collected and centrifuged @ 2000*g for 10 minutes and serum collected and stored at -20ºC until serum biochemistry analysis was carried out.

RELATIVE ORGAN WEIGHT

At the end of the experiment two (n=2) broilers chicken from each replicate were randomly taken, live weight measured and humanly slaughtered and weight of internal organs viz. heart, liver, gizzard, stomach, intestine and carcass were recorded and relative weight were calculated.

DATA ANALYSIS

For data processing and analysis MS Excel and statistical software (Statistical Package for Social Sciences SPSS 16 for Windows) were used. Data regarding all the parameters collected during the experiment was subjected to analysis by using one way analysis of variance (ANOVA). Duncan's Multiple Range (DMR) test was used to determine the significant differences among treatment groups. All the data were presented as mean ± standard error of mean (Mean±SE). Statistical model applied is as: $Yij = \mu + Ti + eij$.

RESULTS

The result of growth performance of broiler chicken during starter phase (0-3 weeks) is depicted in Table I. Live body weight (LBW), weight gain (WG) and feed conversion ratio (FCR) were observed significant among treatment groups ($p<0.05$). Highest WG (465.11g) and ADG (22.15g) was noted in AB+ZnB1 treatment group, followed by (510.25g) LBW and ADG (22.05g) was found in AB+ZnB1 respectively; whereas lowest LBW and ADG (475.7g & 21.40g) was noted in control group ($p<0.05$). During starter phase treatment group supplemented antibiotic alone and in combination with Zinc bacitracin was noted showed better growth performance in comparison to control.

The result of growth performance of broiler chicken during finisher phase (3-6 weeks) is presented in Table II. LBW, WG and FCR were observed significantly different among treatment groups ($p<0.05$). Highest WG (1894.2g) and ADG (90.20g) was noted in probiotic treatment group, followed by (1812.7g) LBW and ADG (86.31g) was found in AB+ZnB1 respectively; whereas lowest LBW and ADG (1735.0g & 82.62g) was noted in control group ($p<0.05$). During finisher phase treatment group supplemented antibiotic alone and in combination with Zinc bacitracin was noted showed better growth performance in comparison to control, but no significant difference prevailed among them ($p>0.05$).

Table I. Growth parameters of the broiler chicken supplemented with feed additives during starter phase 0-3 weeks

Treatments	Live body weight (g)	Weight gain (g)	Feed intake (g)	Feed conversion ratio (g/g)	Average daily gain (g)
Control	475.70±23.71^{b}	449.50±24.33	611.65±17.68	1.37±0.07	21.40±1.15
Probiotic	498.26±19.42^{ab}	451.50±19.11	609.70±12.74	1.36±0.08	21.50±0.91
ZnB1	500.25±13.17^{b}	452.68±12.81	613.12±12.66	1.35±0.01	21.55±0.61
ZnB2	495.50±5.07^{ab}	449.00±5.11	618.08±7.83	1.37±0.08	21.38±0.24
AB	510.25±20.59^{a}	463.10±20.57	637.70±6.21	1.38±0.05	22.05±0.97
AB+ZnB1	511.82±4.28^{a}	465.18±14.33	645.12±11.95	1.38±0.02	22.15±0.20
AB+ZnB2	497.75±6.12^{b}	452.98±15.85	629.00±11.29	1.38±0.01	21.57±0.27

AB: antibiotics, ZnB: Zinc bacitracin, *a, b, c Different superscript in the same column indicate significant difference ($p<0.05$)

Table II. Growth parameters of broiler chicken supplemented with feed additive during finisher phase 3-6 weeks

Treatments	Weight gain (g)	Feed intake (g)	Feed conversion ratio (g/g)	Average daily gain (g)
Control	1735.0±64.98^{b}	2491.0±8.87^{b}	1.44±0.04^{a}	82.62±3.09^{b}
Probiotic	1894.2±47.48^{a}	2486.0±4.63^{bc}	1.31±0.03^{b}	90.20±2.26^{a}
ZnB1	1807.2±26.50^{ab}	2470.8±5.43^{c}	1.36±0.02^{ab}	86.05±1.26^{ab}
ZnB2	1769.0±10.58^{ab}	2439.2±5.66^{d}	1.37±0.06^{ab}	84.23±0.50^{ab}
AB	1798.8±51.25^{ab}	2494.8±5.35^{b}	1.39±0.03^{ab}	85.65±2.44^{ab}
AB+ZnB1	1812.7±18.22^{ab}	2527.8±8.79^{a}	1.39±0.01^{ab}	86.31±0.86^{ab}
AB+ZnB2	1787.2±23.40^{ab}	2423.2±2.52^{d}	1.35±0.01^{ab}	85.10±1.11^{ab}

AB: antibiotics, ZnB: Zinc bacitracin, *a, b, c, d Different superscript in the same column indicate significant difference ($p<0.05$)

The result of growth performance of broiler chicken during experiment (0-6 weeks) is presented in in Table III. LBW, WG and FCR were observed significantly different among treatment groups ($P<0.05$). Highest WG (1894.2g) and ADG (90.20g) was noted in probiotic treatment group, followed by (1812.7g and 86.31g). LBW and ADG was found in AB+ZnB1 respectively; whereas lowest LBW and ADG (1735.0g & 82.62g) was noted in control group ($p<0.05$). Overall better feed conversion ($p<0.05$) was noted in probiotic group (1.32) followed by Zn+B1 and AB+ZnB2 (1.36). Whereas highest FCR was noted in the control (1.42). During finisher phase, treatment group supplemented antibiotic alone and in combination with Zinc bacitracin noted better growth performance in comparison to control, but no significant difference prevailed among them ($p>0.05$). The result of relative organ weight of broiler chicken is presented in Table IV. No significant difference in relative weights of organ was noted among treatment groups ($p>0.05$); though, some numerical differences were noted.

Table III. Growth parameters of broiler chicken supplemented with feed additive during experimental phase 0-6 weeks

Treatments	Live body weight (g)	Weight gain (g)	Feed intake (g)	Feed conversion ratio (g/g)	Average daily gain (g)
Control	2160.8±43.74^{c}	2114.6±43.03^{b}	3102.6±26.29^{bc}	1.42±0.02^{a}	52.01±1.02^{b}
Probiotic	2392.5±36.82^{a}	2345.8±36.87^{a}	3095.7±10.55^{bcd}	1.32±0.01^{b}	55.85±0.87^{a}
ZnB1	2307.5±33.50^{a}	2259.9±33.09^{ab}	3083.9±12.75^{cd}	1.36±0.01^{a}	53.80±0.78^{ab}
ZnB2	2264.5±12.20^{bc}	2218.0±12.07^{b}	3057.3±8.12^{cd}	1.37±0.02^{ab}	52.80±0.28^{b}
AB	2309.0±32.41^{ab}	2261.8±33.26^{ab}	3132.4±6.61^{ab}	1.38±0.02^{a}	53.85±0.76^{ab}
AB+ZnB1	2272±19.90b^{bc}	2225.35±20.36^{ab}	3172.9±20.73^{a}	1.39±0.01^{a}	52.98±0.48^{ab}
AB+ZnB2	2285.0±27.91^{b}	2240.2±27.37^{b}	3052.2±10.72^{d}	1.36±0.01^{ab}	53.33±0.65^{b}

AB: antibiotics, ZnB: Zinc bacitracin, *a, b, c, d Different superscript in the same column indicate significant difference ($p<0.05$)

Table IV. Relative organ weight of broiler chicks supplemented with feed additives (g/100g body weight) at day 42

Groups	Carcass	Abd. Fat	Liver	Gizzard	Heart	Spleen	Pancreas	Intestine
Control	71.85±0.37	1.73±0.065	2.14±0.058	2.68±0.016	0.59±0.004	0.13±0.011	0.19±0.012	4.23±0.04
Probiotic	72.17±0.20	1.73±0.087	2.12±0.038	2.65±0.035	0.58±0.008	0.14±0.009	0.20±0.008	4.25±0.045
ZnB1	71.68±0.34	1.73±0.085	2.18±0.059	2.70±0.020	0.61±0.011	0.15±0.008	0.22±0.011	4.23±0.063
ZnB2	71.27±0.16	1.78±0.060	2.12±0.045	2.71±0.012	0.61±0.001	0.15±0.01	0.20±0.008	4.25±0.061
AB	71.45±0.13	1.78±0.062	2.18±0.048	2.70±0.013	0.61±0.01	0.14±0.006	0.20±0.01	4.23±0.060
AB+ZnB1	71.08±0.12	1.74±0.057	2.15±0.037	2.71±.016	0.62±0.01	0.14±0.01	0.22±0.011	4.23±0.042
AB+ZnB2	70.43±0.34	1.67±0.082	2.19±0.058	2.72±0.02	0.65±0.008	0.15±0.006	0.23±0.015	4.15±0.015

AB: antibiotics, ZnB: Zinc bacitracin,

Hematology and serum biochemical indices are presented in Table V and VI. The results showed no significant difference among treatments either in hematology or serum biochemistry (P>0.05); however, minor numeric differences within normal ranges were noted among treatment groups.

Table V. Hematological parameters of the broiler chicken supplemented with feed additives

Treatments	RBC (1x10⁶)	WBC (1x10³)	Hb (g/dl)	Hematocrit (%)
Control	4.20±0.04	31.12±0.65	10.75±0.25[a]	32.75±0.47
Probiotic	3.80±0.08	28.25±0.62	11.60±0.46[b]	31.65±0.75
ZnB1	4.15±0.04	32.62±0.42	10.84±0.38[a]	29.32±0.28
ZnB2	3.65±0.06	34.50±0.61	10.93±0.35[a]	29.47±0.47
AB	3.82±0.04	30.37±0.74	11.39±0.35[b]	29.68±0.47
AB+ZnB1	4.22±0.08	28.87±0.65	10.52±0.56[a]	29.37±0.47
AB+ZnB2	4.17±0.06	32.62±0.55	10.47±0.49[a]	31.59±0.75

a, b Different superscripts in same column indicate significant difference (P<0.05)

Table VI. Serum biochemical parameters of the broiler chicken supplemented with feed additives at day 42

Treatments	T. Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	AST (IU/l)	ALT (IU/l)	LDH (IU/l)
Control	3.36±0.03	1.59±0.02	1.77±0.17	245.32±5.68	31.15±2.30	948.65±7.22
Probiotic	3.68±0.06	1.62±0.05	2.08±0.10	239.19±7.89	33.20±1.70	929.92±6.41
ZnB1	3.78±0.07	1.59±0.07	2.19±0.12	233.54±7.21	32.96±1.23	933.18±5.23
ZnB2	3.46±0.03	1.62±0.03	1.84±0.15	238.54±4.45	29.87±1.12	931.53±4.71
AB	3.45±0.08	1.69±0.04	1.86±0.10	241.44±4.87	31.83±1.42	942.81±6.49
AB+ZnB1	3.44±0.02	1.59±0.06	1.85±0.11	238.54±6.32	29.55±1.64	939.20±5.81
AB+ZnB2	3.38±0.08	1.68±0.04	1.70±0.14	233.27±5.73	32.76±1.32	930.66±8.36

DISCUSSION

Antibiotics have played pivotal role in the development and growth of poultry sector. Use of antibiotics at sub therapeutic doses in the feed as a growth enhancer initially started during 1950s (13). There are about twenty seven classes of antibiotics are used in animal production. Among these nine are exclusively utilized in animal whereas other eighteen classes are used both in human and animals (14). With increased concern over the use of antibiotic growth promoter in certain countries because of isolation of antibiotic resistant bacterial strains some countries have already ban the use of certain classes of antibiotics as growth promoter in animal and poultry production; very small quantities of AGP's may enter food chain through meat and eggs (15). This ban can only be implemented in developed countries as developing and poor countries are struggling for the provision of food to the masses and physical structure required for the proper hygienic conditions is not up to mark and ban over the use of antibiotics may further deteriorate the food security and availability of protein to the masses. In the mean while probiotics has emerged as possible green substitute to antibiotic growth promoter has shown considerable useful effects in humans and birds current years (16, 17). In the present experiment broiler chicken supplemented zinc bacitracin alone and in combination with procaine penicillin and streptomycin showed better growth performance in comparison to control. Treatment group ZnB1 and AB supplemented zinc bacitracin @250mg/kg and commercial antibiotic blend showed 6.78% and 6.85% respectively better LBW was recorded in comparison to control. Similarly probiotic supplementation also exhibit 10.72% increased live body weight and 7.38% ADG in comparison to control. However, zinc bacitracin in combination with antibiotic blend did not showed interaction or any significant additive effect. Comparison between probiotic and antibiotic groups revealed insignificant growth performance in terms of ADG and FCR in the probiotic group with numeric increase from AB and AB+ZnB1. In several previous studies antibiotics as growth promoters has reported improved growth performance of broiler chicken in comparison to control (18,19,20). The mode of action may involve in the AGP's may be alteration in the bacterial population characteristics of the GIT either by eradicating or by subduing their growth (21). The intestine is a defense barrier to stop the entry of pathogenic microbes into the body through intestinal mucus layer, using valuable microbiota and immunoglobulin A (IgA) produced by the host to maintain optimal nutrient absorption in the intestine (22).

The most common types of probiotic microorganisms used are lactic acid bacteria and bifido-bacteria; even though other microbes are also used (23). Lactobacillus and Enterococcus species are the colonizing microbes whereas Bacillus and Saccharomyces species are the example of non-colonizing species. In the present study *Bacillus Clausii* was used as a probiotic. Supplementation of probiotic through gavage produced significant growth output in the form of live body weight and ADG in comparison to control as well as AB, ZnB1 and ZnB2. It seems that combination of zinc bacitracin with penicillin and streptomycin did not performed as per expectation. Probiotics play multi-functional role in the intestine. They are involved in the environment modification of the intestine so that making it difficult for the other bacerial strain to flourish. Sugiharto et al. (24) used zinc bacitracin alone and in combination with probiotic (bacillus mixture) and revealed probiotic had no significant effect on breast meat yield; while AGP supplementation showed some improvement in growth performance. Thema et al. (25) used probiotic (*Bacillus subtilus*) in comparison to organic acid and reported comparative results with comment AGPs can be replaced. Contrary to present study results Letlole et al. (26) reported supplementation of zinc bacitracin @ 0.5g/kg had no positive effect of growth performance of broiler; he further reported that supplementation had negative effect on intestinal structure. Similarly Attia et al. (27) used zinc bacitracin alone and in combination with phytase revealed no effect on body weight gain, decreased feed intake and poor FCR. The heterogeneous results from different parts of the world indicate difference in the environmental conditions have a profound effect on the effectiveness of antibacterial growth promoters including zinc bacitracin. This may be due to already existing ideal hygienic conditions in the production process may not support antibacterial to express their effect through improved growth (28). In the present study growth performance of broiler chicken was improved considerably. Many researchers in previous studies revealed that probiotic improved growth performance with better feed conversion (29,30,31). In a study Abdel-Raheem et al. (32) used *Saccharomyces cerevisiae* and reported higher final body weight and FCR in probiotic group as compared to control group. In other experiment Toghyani et al., (33) reported probiotics increased the broiler chicks body weight at 28 and 42nd day and have at par effect like antibiotic. The improvement in growth supplemented probiotic may be attributed to limiting pathogenic food borne microorganism in the intestinal environment resulting in decreased harmful bacterial metabolites in the intestine thus enhanced gut functions and digestive enzymes secretions.

In the present study restricted feeding regime was opted and same amount of feed was offered to all treatment groups during the experiment and refusal was measured at the end of each week. The results showed a significant difference in feed consumption. Highest feed consumption was noted in AB+ZnB1. However, higher intake did not translated in to better growth in comparison to probiotic. This might be due to AGP dose given, this could be justified as the AB+ZnB2 perform better than AB+ZnB1. Feed intake is an important factor in broiler production process and there are many factors like caloric value of feed, environmental temperature, feed particle size, visual appearance, intestinal viscosity, saliva production and toxicity of feed ingredients can impact feed consumption (4).

CONCLUSION

The result of the present study suggests that under optimal farm conditions supplementation of antibacterial feed additives and probiotic can improve growth performance of the broiler chicken. To avoid social concern regarding the drug resistance bacterial population in the food chain probiotic could be a feasible and economical option.

Conflicts of Interest:

Authors have no conflict of interest.

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