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RESEARCH ARTICLE

EFFECT OF DIETARY SUPPLEMENTATION OF ANTIBIOTIC GROWTH PROMOTERS VS SYNBIOTICS ON GROWTH PERFORMANCE, CARCASS CHARACTERISTICS AND IMMUNE RESPONSE OF BROILER CHICKENS

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ABSTRACT

A study was to conducted to evaluate the effects of dietary supplementation of antibiotic growth promoters (AGPs) – (Oxytetracycline OTC and Chlortetracycline CTC) and synbiotics on growth performance, carcass characteristics and immune response of broiler chickens. A total of 240 day-old broiler chicks were randomly assigned to six dietary treatments each consisting of eight replicates and each replicate having 5 birds and reared up to 42 days of age. The dietary treatments were (T_1) control group with basal diet, (T_2) basal diet supplemented with oxytetracycline-OTC (at 100mg/kg), (T_3) basal diet supplemented oxytetracycline-OTC (at 200 mg/kg) (T_4) basal diet supplemented with chlortetracycline-CTC (at 100 mg/kg), (T_5) basal diet supplemented chlortetracycline-CTC (at 200 mg/kg) and (T_6) basal diet supplemented with synbiotics(at 250 mg/kg). The birds were provided with *ad-libitum* feed and drinking water during the entire experimental period. Weekly feed intake, body weight gain, feed conversion ratio, carcass traits and immune response were recorded. The body weight gain of broilers were significantly ($P<0.05$) higher in the dietary groups on supplementation of OTC, CTC or synbiotics. Supplementation of AGPs or synbiotics did not have any significant ($P>0.05$) influence on cumulative feed consumption and feed conversion ratio of broilers during pre-starter and finisher phases and during overall period (0-42 d).There was no significant ($P>0.05$) influence of AGPs or synbiotics on any of the carcass parameters studied in the present investigation. There was no significant ($P>0.05$) difference in CBH response and log_2ND titre valuesamong the treated groups but numerically higher CBH response was noticed in all treatment groups compared to control.The control and synbiotic groups had higher log_2 titre than different antibiotic fed groups but present study revealed that synbiotics can also be used as alternative to antibiotic growth promoters in broiler production.

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INTRODUCTION

The poultry industry in India has undergone a major shift in structure and operation during the last two decades. It has transformed from a mere backyard activity into a major commercial activity with growing presence of large integrated players and successful implementation of contract poultry farming on a large scale. Poultry is one of the world's fastest growing sources of meat. The modern production unit can produce market ready broiler chickens in less than six weeks.

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This development arose from genetic selection, improved feeding and health management practices involving usage of antibiotics as therapeutic agents to treat bacterial diseases in intensive farming systems. They may also be used as prophylactic agents in the water of healthy birds and as growth promoters at sub-therapeutic concentrations in feed. The feed cost accounts around 70-80 percent of the total cost of broiler production. Antibiotic feed additives as growth and health promoters supplemented to poultry diets to stabilize the gut microflora, improve performance and prevent some specific intestinal diseases and for optimizing productivity (Waldroup *et al*., 1995; Hashemi and Davoodi, 2011). The economic and health advantages of using antibiotics have revolutionized intensive poultry and livestock production. It has been reported that antibiotic use is more frequent in broilers approaching market age (42 days) that results in microbial resistance to antibiotics and is now a worldwide problem in human and veterinary practices (Hussain *et al*., 2013). To promote growth, protect well-being and maximize the genetic prospective of modern broiler and layer hybrids non antibiotic growth promoter feed additives have been included in poultry diets. Synbiotics is a combination of probiotics and prebiotics. This product could improve the survival of the probiotic organism because of its specific substrate is available for fermentation. This could result in advantages to the host through the availability of the live microorganism. The combination of a pre- and probiotic in one product has been shown to confer benefits beyond those of either on its own. A way of potentiating the efficacy of probiotic preparations may be the combination of both prebiotics and probiotics as synbiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract. Since there is ongoing concern on the use of antibiotics in poultry diet and subsequent presence in human food chain and the fear of antibiotic resistance, the present study was aimed to study the effect of antibiotic growth promoters (OTC,CTC) and synbiotics (combination of probiotic and prebiotics) on growth, feed consumption, FCR, mortality, carcass characteristics and immune response of broiler chickens and also to study synbiotics as an alternative to the AGPs in the performance of broilers.

MATERIALS AND METHODS

Place of work

A biological trial was conducted at Poultry Experimental Station, Instructional Livestock Farm Complex (ILFC), College of Veterinary Science, Rajendranagar, Hyderabad. Laboratory analysis of the collected samples was conducted utilizing the facilities of ICAR-Directorate of Poultry Research, Rajendranagar, Hyderabad and ICAR- National Research Centre on Meat, Chengicherla, Hyderabad.

Biological experiment

A total of 240 day old male broiler chicks (Vencobb-400) were obtained from Venkateswara hatcheries Pvt. Ltd, Hyderabad. The chicks were wing banded, weighed individually and randomly distributed into ten groups with eight replicates for each group and five birds per each replicate. The birds were reared for 42 days in stainless steel battery brooders following standard management and vaccination schedule. Feed and water were provided *ad libitum* during the entire experimental period. The average maximum and minimum ambient temperature during the 6-week of experimental period ranged from 37.2°C to 41.3°C (average 39.25°C) and 21.7-24.8°C (average 23.8°C), respectively.

Experimental diets

Sixisonitrogenous, isocaloric diets were formulated for corn-soya based broiler pre-starter (0-14days), starter (15-28days) and finisher (29-42days) periods. The control diet (T_1) was prepared with corn-soya diets without antibiotics (shown in Table no. 1), for remaining treatment groups (T_2-T_5) were formulated by corn-soya diets with different levels of antibiotics (oxytetracyclines@(100mg/kg, 200mg/kg), chlortetracyclines@(100mg/kg, 200mg/kg) and synbiotics @250mg/kg (T_6) for 42 days as shown in Table 2.

Table 1. Basal diet composition of pre-starter, starter and finisher diets

Ingredient (%)	Pre-starter	Starter	Finisher
Maize	56.60	57.80	61.49
Oil	2.32	3.28	3.90
Soybean meal	36.30	34.20	30.00
Stone grit	1.65	1.60	1.85
DCP	1.85	1.90	1.65
Salt	0.40	0.40	0.40
DL- Methionine	0.23	0.19	0.16
Lysine	0.19	0.14	0.06
Manganese sulphate	0.03	0.03	0.03
Zinc sulphate	0.02	0.02	0.02
Ferrous sulphate	0.02	0.04	0.04
Copper sulphate	0.0035	0.0035	0.0035
[1]Vitamin AB2D3K	0.02	0.02	0.02
Vitamin B complex	0.03	0.03	0.03
Coccidiostat	0.05	0.05	0.05
Choline	0.10	0.10	0.10
Tylosine	0.05	0.05	0.05
Toxinbinder	0.10	0.10	0.10
Total	100	100	100
Nutritional composition			
ME, K.cal/kg	2950	3050	3154
Crude protein	22.49	21.5	19.53
Calcium	1.00	1.00	1.00
Available phosphorus	0.45	0.45	0.4
Lysine	1.3	1.2	1.00
Methionine	0.55	0.5	0.45
Total S-amino acids	0.9	0.83	0.76
Ether extract	3.48	5.72	6.93
Crude fiber	3.45	3.32	3.12

Vitamin premix provided per kg diet: Vitamin A, 20000IU; Vitamin D_3, 3000IU; Vitamin E, 10mg; Vitamin K, 2mg; Riboflavin, 25mg; VitaminB_1, 1mg; Vitamin B_6, 2mg; Vitamin B_{12}, 40mcg and Niacin, 15 mg.
*Calculated values

Table 2. Formulation of Antibiotic growth promoters and synbiotics in broiler diet

Treatment Group	Treatment diets
T1	CONTROL (without OTC,CTC, Synbiotics)
T2	Basal diets with OTC @100mg/kg
T3	Basal diets with OTC @200mg/kg
T4	Basal diets with CTC @100mg/kg
T5	Basal diets with CTC @200mg/kg
T6	Basal diets with Synbiotics @ 250mg/kg

Performance Parameters

The body weights of individual birds were recorded at weekly interval, and average body weight gain was calculated. Feed consumption of birds of each replicate was recorded at weekly intervals and feed consumption per bird per week was calculated. Daily mortality was recorded and due importance was given to mortality while calculating feed consumption and FCR.

Carcass Characteristics

At the end of experiment (42[nd] day) one bird from each replicate (8 birds per treatment) was randomly selected and starved from the feed for 12 h and the same birds were slaughtered by cervical dislocation to study the slaughter parameters. The parameters studied were total carcass dressing yield, breast yield and individual organ weights like liver, heart, gizzard, abdominal fat and immune related organs weight (bursa, thymus, spleen).

Table 3. Composition of synbiotics (prebiotic + probiotic) used in the diet

Item	Probiotic	Prebiotic
composition	Each gram contains 100 billion CFU/kg *Lactobacillusacidophilus, Lactobacillusbulgaricus, Lactobacillusplantarum, Streptocofaecium, Bifidobacteriumbifidus*, and *S. cerevisiae*	MOS (naturally derived from extracts of yeast cell walls) 14-16%.
Dosage	250 mg/kg of feed	

Table 4. Effect of antibiotic growth promoters (OTC and CTC) and synbiotics on growth performance of male broiler Chickens

Item	Control (T1)	OTC-100ppm (T2)	OTC-200ppm (T3)	CTC-100ppm (T4)	CTC-200ppm (T5)	Synbiotics (T6)	P value
Body weight gain							
0-2 weeks	314.4	300.6	311.5	307.3	314.2	309.8	0.216
3-4 weeks	694.0^{b}	784.7^{a}	809.2^{a}	802.4^{a}	814.4^{a}	810.4^{a}	0.000*
4-6 weeks	852.5	902.2	864.1	876.2	858.3	869.7	0.246
0-6 weeks	1860.9^{c}	1987.5^{ab}	1984.8^{ab}	1985.9^{ab}	1987^{ab}	1989.9^{ab}	0.031*
Feed intake (g/bird)							
0-2 weeks	389.4	384.6	397.8	387.9	384.0	391.1	0.606
3-4 weeks	1040.9^{c}	1101.4^{b}	1156.2^{a}	1141.0^{ab}	1173.2^{a}	1127.0^{ab}	0.001*
4-6 weeks	1470.6	1549.3	1467.9	1486.9	1499.5	1470.8	0.468
0-6 weeks	2900.9	3035.3	3021.9	3015.8	3056.7	2988.9	0.346
FCR							
0-2 weeks	1.24	1.28	1.28	1.27	1.22	1.26	0.835
3-4 weeks	1.50^{a}	1.40^{ab}	1.43^{ab}	1.42^{b}	1.45^{ab}	1.40^{b}	0.020*
4-6 weeks	1.73	1.73	1.71	1.78	1.77	1.69	0.112
0-6 weeks	1.56	1.53	1.52	1.52	1.54	1.50	0.115
Mortality	7.5	5.0	2.5	2.5	-	-	-

means bearing different superscripts in the same row differ significantly (*P<0.05).FCR=Feed conversion ratio

Table 5. Effect of antibiotic growth promoters (OTC and CTC) and synbiotics on carcass traits (%) of broiler male Chicken (1-6 weeks of age)

Item	Control (T1)	OTC-100ppm (T2)	OTC-200ppm (T3)	CTC-100ppm (T4)	CTC-200ppm (T5)	Synbiotics (T6)	P value
Dressing yield(%)	67.17	67.56	67.49	66.41	67.62	67.91	0.975
Breast yield (%)	21.73	21.81	20.93	20.91	22.26	22.15	0.497
Giblet (%)	5.26	4.64	4.75	5.00	4.94	4.98	0.734
Abdominal fat (%)	1.60	1.23	1.42	1.55	1.48	1.44	0.697

means bearing different superscripts in the same row differ significantly (*P<0.05).

Table 6. Effect of antibiotic growth promoters (OTC and CTC) and synbiotics on immune response of broiler male chicken (1-6 weeks of age)

Item	Control (T1)	OTC-100ppm (T2)	OTC-200ppm (T3)	CTC-100ppm (T4)	CTC-200ppm (T5)	Synbiotics (T6)	P value
Immune organs							
Spleen	0.124	0.124	0.114	0.111	0.116	0.135	0.156
Bursa	0.068	0.065	0.056	0.081	0.063	0.066	0.538
Thymus	1.420	1.330	1.570	1.100	1.230	1.480	0.567
Immune response							
CBH(PHA-P)	0.840	0.873	0.952	0.905	1.077	0.910	0.949
NDV (log2 HI titer)	7.57	5.57	6.43	6.00	5.57	7.57	0.972

Means bearing different superscripts in the same row differ significantly (P<0.05). CBH (PHA-P): Cutaneous basophil hypersensivity (Phytohaemagglutinin antigen) HI: Haemagglutination Inhibition NDV: Newcastle disease virus

Immunological Studies

The immune status of the animals was assessed involving both humoral and cell mediated immune response.

Humoral Immune response to NDV

The effect of dietary supplementation of antibiotic growth promoters on humoral immunity in birds was estimated by measuring antibody titer to Newcastle disease (ND) vaccine (antibody production against ND virus).Broilers were vaccinated against ND by ocular route at 7th and 28th day of age with Lasota strain (ND Lasota Vac-500; IndivaxPvt., Ltd., Hyderabad, India) At 40th days of age, blood was collected and serum was separated. Subsequently antibiotic specific for NDV were detected in sera of chicks by haemagglutination inhibition (HI) test and were expressed as log2 titers (Allan *et al.,* 1978).

Cell mediated immune response

The effect of dietary supplementation of antibiotic growth promoters on Cell mediated immune (CMI) response was assessed by measuring in vivo the cutaneous basophil hypersensitivity (CBH) to phytohaemagglutin-P (PHA-P).On 40th day of experiment, one bird from each replicate in each dietary treatment were used for testing .thickness of web between the third and fourth inter-digital space of left and right feet was measured by micrometer (Corrier and Deloach, 1990).

Statistical Analysis

The data obtained in this study were analyzed statistically in SPSS software (version 16.0) as per the methods outlined by Snedecor and Cochran (1989). The significance between the treatment groups was analyzed by one-way ANOVA test. P value statistical significance was declared at 5 percent.

RESULTS AND DISCUSSION

The performance of broilers in terms of body weight gain, feed intake and FCR during pre- starter(0-14 d), starter (15-28 d) and finisher period (29-42 d) is presented in Table 4.The performance of broiler chicks did not differ significantly due to dietary supplementation of either AGPs or synbiotics during the pre- starter and finisher phase. However the performance parameters such as body weight gain, feed intake and FCR differ significantly during starter phase (15-28 d).

Body weight gain

The broilers fed the control diets (without AGPs and without synbiotics) gained significantly lower weight compared to all other dietary groups. The dietary addition of either AGPs or synbiotics resulted in higher weight gain compared to control. The highest gain was noticed in the dietary groups supplemented with synbiotics, OTC and CTC @200mg/kg diet. The results of the present study indicated that OTC, CTC and synbiotics had improved the body weight and weight gain of broilers irrespective of doses. Similar findings were also reported in broilers fed with OTC or *Lactobacillus* (LC) during 0-42d of age (Zulkifli *et al*., 2000; Kalavathy *et al*., 2003), virginamycin or *Bacillus coagulans* (Cavazzoni *et al*., 1998) and zinc bacitracin or *L.acidophillus* (Abdul Rahim *et al*., 1999). In contrast, Yeo and Kim (1997) reported that only broilers fed prebiotics improved the average daily weight gain during first 3 week period compared to control or CTC treatments.

Feed consumption

The highest amount of feed consumption was noticed in the dietary group supplemented with CTC @200mg/kg diet being significantly higher compared to synbiotics supplemented group. Lowest feed consumption by the birds was recorded in the control group. The feed consumption was comparable between control and synbioticgroup. But the feed consumption in the OTC 200mg/kg diet,OTC 100 mg/kg, CTC 100 or 200 mg/kg diet supplemented group was significantly higher compared to control group. The present study showed no significant (P>0.05) influence of AGPs and synbiotics on feed consumption in broilers during 0-42 d of age. The amount of feed consumed by the birds were numerically similar ranging between 2901 to 3057 g. Similar observations were noticed in the study of Kalavathy*et al*. (2003); Miles *et al*. (2006); Gunal*et al*. (2006) where OTC and prebiotics had no effect on feed intake.In contrast to our findings Razieh (2010) reported a significant (P<0.05) influence on feed intake in broilers (0-42d) due to AGP and AGPs showed lowest feed intake than synbiotics and control group. But during the starter (15-28d) phase there was significant (P<0.05) difference on feed intake due to dietary addition of AGPs or synbiotics irrespective of dosage. This findings thus suggested that long term use of antibiotics can produce a better effect than short term usage. However, the same was not observed in the present investigation.

Feed conversion Ratio

The FCR was significantly poor in the birds fed the control diets. Dietary supplementation of AGPs or synbiotics significantly improved the FCR compared to the control group. No difference in FCR could be observed between AGP and synbiotic supplemented group. The results pertaining to Feed conversion ratio (feed intake /weight gain) of broilers of 0-42 d of age showed that there was no significant (P>0.05) difference due to addition of AGPs (OTC & CTC) or synbiotics. Supplementation of AGPs and synbiotics in broilers had elicited similar FCR as reported in earlier findings of Duwa (2013); Miles (2006) and Razieh. (2010). But during starter phase (15-28 d) there is significant influence on FCR in different treatments of AGPs or synbiotics. In contrast to present report there was significant interaction among antibiotics or synbiotics on FCR in broilers in earlier reports of Zulkifli*et al*. (2000) and Kalavathy *et al*. (2003). This may be due to inclusion of AGPs interact with gut microflora, thereby suppress harmful microorganisms and favours *Lactiobacillus* to increase the surface area of intestinal villi for better absorption of feed at early age (Markovic, 2009).

Mortality / Livability

Mortality was not influenced by dietary supplementation of either AGPs or synbiotics alone or in combination. The mortality rate in all the dietary treatments was within the acceptable range (<5%). However several researchers reported reduced mortality percentage of broilers with supplementation of AGPs and synbiotics or both. This might be due to the continuous feeding of antibiotics or synbiotics which suppressed the undesirable microorganisms leading to improved health status (build-up resistance) and ultimately improved growth and overall performance. The lower mortality in the present study could be due to better management facility and it has been reported that antibiotics \and/or synbiotics could reduce mortality in poor management or stress conditions (Anderson *et al*., 1999).

Carcass Traits

The data on carcass parameters such as dressed yield, breast yield, giblet yield and abdominal fatof different dietary treatments are presented in Table 5. The results showed that all dietary groups failed to exert any significant (P>0.05) influence on carcass traits like dressing yield, breast yield, giblet (liver, heart and gizzard) and abdominal fat. In agreement with the results of this study, other reports (Razieh, 2010; Landy *et al*., 2011; Mathivanan *et al*., 2006) demonstrated that addition of AGPs (avilanmycin 100mg/kg diet or flavophospholipol @4.5mg /kg) had no significant influence on carcass yield, breast and thigh weight but on other hand, treatments had significant effect on abdominal fat percentage. Antibiotic, probiotics and synbiotic groups showed lower abdominal fat percentage than control group. These results are also in agreement with Kumprecht *et al*. (1997) and Mohamed *et al*. (2008), who found significant reduction (P<0.05) in abdominal fat by supplementation of prebiotics

(MOS). No significant differences, pathological lesion and damages were noticed for heart, gizzard, liver, pancreas, caecum and small intestine.

Immune Response

The results of relative weight of immune organs, humoral immune response and cell mediated immune response and of different dietary treatments of were shown in Table No. 6.

Immune organs

Immune organs like spleen, bursa and thymus are important organs for immunity development. In the present study, the dietary supplementation of OTC, CTC and synbiotics had no significant influence (P>0.05) on the relative weights of spleen, bursa and thymus at 42 d of age. The findings of Ankari *et al*. (1996) suggest that the administration of antibiotics (0.05 g/kg OTC) significantly (P<0.05) decrease the size of bursa of Fabricius and thymus but not spleen. It is suggested that the prolonged administration of antibiotics to chickens may induce an immunosuppressant effect. In support to this results Panda *et al*., (2000) observed lack of difference in the weight of spleen and bursa in probiotic groups. Contrary to these findings Rama Rao *et al*. (2004) reported that higher lymphoid organs (bursa and spleen) weights in broiler fed probiotics, acidifiers and antibiotics.

Humoral immune response

The humoral immunity was evaluated in terms of antibody response to ND virus at 42 days of age using HA assay. In the present findings, the mean $\log_2$ titer values are higher in control and synbiotic groups but there was no significant difference (P>0.05) observed between treatment groups. Similar results were found in the findings of Zulkifli (2000); Panda *et al*. (2005) where supplementation of probiotic did not have any significant (P>0.05) effect on antibody production against SRBC. This may be due to in the no increase in weights of lymphoid organs which support the increase in immune response in broiler birds. In contrast to our findings Khaksefidi and Ghoorchi (2006); Lilbrun*et al*.(2000) observed significantly higher antibody titers against ND virus in probiotic or prebiotics (Bio-Mos.) supplemented groups than control. The exact mechanisms that mediate immune modulatory activities of probiotics, prebiotics was not clear, the possible reason of increased level of antibody titer against ND in the birds may be due to increased activity of neutrophils in blood after vaccination, which could play a major role in body immunity production (Guo *et al*., 2009). The reduced gut P^H and pathogenic bacteria in intestine and increased weight of lymphoid organs might have acted indirectly to enhance the immune competence in broilers.

Cell mediated immune response

Cell mediated immunity was evaluated in terms of CBH response at 42 of age by injecting PHA-P into digits. There was no significant (P>0.05) difference in CBH response among the treatment groups but higher CBH response was noticed in all test diets compared to control except T3. These results are in agreement with Panda *et al*. (2000), who observed that cutaneous basophilic hypersensivity (CBH) responses to inoculation with phyto-hemagglutinin did not differ significantly in probiotic supplemented diets in White Leghorn layers in either 24 or 40 wks. of age.

Conclusion

A biological experiment conducted did not show any significant increase in feed consumption, FCR, mortality, percentage of carcass yield and immune response by the dietary inclusion of antibiotic growth promoters or synbiotic compared with unsupplemented control in commercial broiler chicken. But there was significant difference in body weight of broiler fed with AGPs or synbiotics compared to control diets. The synbiotic treatment decreased the feed: Gain ratios and mortality percentage as compared to control. Therefore, these might be promising alternatives for antibiotic growth promoters, as pressure to eliminate antibiotic growth promoters in animal feed increases.

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