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EFFECT OF DIFFERENT COMBINATIONS OF ANTIBIOTIC GROWTH PROMOTERS ON THE PHYSIOLOGICAL INDICES OF BROILER CHICKEN

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Abstract

The present research was conducted to evaluate the effect of growth promoter antibiotics Oxytetracyclin and Tylosin Phosphate, alone and in combination, to study the physiological indices of broiler chicken. For this purpose, 405 day-old broiler chicks were randomly divided into nine treatment groups in 3^2 factorial arrangements. The results showed that the use of Oxytetracycline and Tylosin Phosphate as an antibiotic growth promoter feed supplement resulted in a significantly higher count of red blood cells in treatment groups in comparison to control ($P<0.05$). While white blood cells, lymphocytes and other hematology constituents were noted to rise significantly among treatment groups ($P>0.05$). The results of serum biochemistry parameters revealed that Oxytetracycline and Tylosin Phosphate improved hemoglobin concentration of treatment groups in comparison to the control ($P<0.05$). However, no significant difference was observed among groups in mean corpuscular volume, hematocrit and platelets ($P>0.05$). In conclusion it can be suggested that the use of Oxytetracyclin and Tylosin Phosphate as antibiotic growth promoter have no adverse effect on hematology and serum biochemical parameters observed in the present study.

Keywords: *Antibiotics, Constituents, Hematocrit, Hemoglobin, Oxytetracyclin, Supplement, Tylosin Phosphate*

INTRODUCTION

Poultry production is one of the most important industries of the livestock sector; bridges between demand and supply of animal protein with an investment of more than 750 billion rupees and this industry has been growing at an impressive rate of approximately 7.5% per annum over the last decade. At the same time, it is employing more than 1.5 million people in Pakistan. The Ministry of National Food Security and Research has reported that in Pakistan total commercial poultry production is about 1,486.09 million in number out of which broiler comprises 1,407.73 million in number with the production of 1681.64 million tons of chicken meat (1). Pakistan is now placed in 11th position among the largest poultry producers in the world and has ample space for further improvement.

An increase in investment in intensive poultry farming is in full swing. But at the same time poultry industry is under immense pressure of disease and low productive efficiency resulting in huge financial losses. These hurdles can cause a severe reduction in poultry products; however, these problems can be addressed by considering multiple solutions. Supplements and additives are normally used in poultry feed (2) and several types of additives like probiotics, enzymes, immune modulators (3, 4) and another type of additives (5,6) have been tested in all parts of the world (7, 8). Antibiotics have long been used as feed additives as the first choice (2, 9, 10). However, in recent past few industrialized countries have imposed ban on the use of certain antibiotics as growth promoters to prevent the spread of antibiotic resistance in the human population (11). No doubt phytogenic and other feed additives as growth promoters are gaining acceptance (12). Their inconsistent efficiency nature could result in huge losses to the industry and the lack of documented reliable findings there extensive use might be associated with other unidentified harmful factors. Countries like Pakistan, where food security is a burning issue, cannot afford to completely ban the use of antibiotics as growth promoters in poultry feed additives.

Different classes of antibacterial drugs are used as growth promoters in broiler production (2). Among these Oxytetracyclin (OTC) and Tylosin are commonly used antibiotic growth promoters (AGP) that have been used in poultry production (13, 14).

Antibiotic growth promoters AGPs in the diet of broiler improve growth performance (15- 17), immune response (18) physiology (19) and intestinal morphology (6, 20- 25). Oxytetracycline (OTC), a derivative of Streptomyces fungus, is an important tetracycline (TC). It is synthetically produced and has a comparatively low market price and ease of availability. It is easy to administer through drinking water or feed as well as widely used to treat and control diseases (26, 27). Keeping in view these factors present study was carried out to evaluate the effect of antibiotics and their combinations on the immune response hematology and serum biochemistry.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

Four hundred and five (405) day-old chicks were divided randomly into 9 groups having 45 birds in a group with 3 replicates of 15 broiler chicks each that were reared for 42 days.

FEEDING AND AGP's SUPPLEMENTATION PLAN

A controlled feeding regimen was implemented in this study, involving two daily feeding sessions (morning and evening). Commercial feed, obtained from Gawadar Feed Mill Lahore, was provided to the broiler chicks. Specifically, starter feed was administered from the 1st to the 14th day, grower feed from the 15th to the 22nd day, and finisher feed from the 23rd to the 42nd day. Oxytetracycline (OTC) and Tylosin Phosphate (TP) were incorporated as antibiotic growth promoters (AGPs) in the feed. Two dosage levels, 0.5g/kg and 1g/kg, of Oxytetracycline and Tylosin Phosphate were employed in a 3x2 factorial design to investigate their impact on the hematological and serum biochemical parameters of broiler chickens.

HOUSING AND HUSBANDRY CONDITIONS

The chicks were raised in an environment with a floor covered in rice husk litter, which had a thickness of 2-3 inches. To prevent chicks from foraging during the initial three days, the floor was covered with paper. The temperature in the shelter was initially set at 95° F upon the arrival of the chicks and was maintained at this level for the first week. Subsequently, the ambient temperature was gradually decreased by 5° F each week until it reached 75° F.

During the first 21 days, each chick was allocated a brooding space of ½ square foot. From the 22nd to the 42nd day, the floor space was increased to 1 square foot per chick. Vaccinations against Newcastle and infectious bursal disease were administered on the 7th and 14th days, respectively. Booster doses were provided on the 23rd and 25th days.

BROILER PHYSIOLOGICAL PERFORMANCE EVALUATION

The physiological performance of broiler chickens involved the evaluation of hematological and serum biochemistry parameters, which serve as valuable indicators for assessing the influence of antibiotic

growth promoters on avian health. To gain deeper insights into the physiological status and ailments affecting broiler chickens, an array of hematological and serum biochemical examinations were carried out. These assessments offered a comprehensive evaluation of the health condition and enabled a meticulous analysis of the impacts of antibiotic growth promoters on the overall well-being of the birds.

HEMATOPHYSIOLOGY

On the 42nd day of the experimental period, blood specimens were collected for the evaluation of the physiological parameters in broiler chickens. The ensuing examinations were performed: quantification of erythrocytes (RBCs), determination of hemoglobin (Hb) concentration, enumeration of leukocytes (WBCs), and tabulation of platelet (PLT) or thrombocyte counts. To prevent coagulation, blood specimens were collected using vacutainers containing ethylene diamine tetra-acetic acid (EDTA) as an anticoagulant agent at a concentration of 1-2 mg/ml (3, 28). Blood samples were drawn from six birds, with two randomly selected from each replicate, through the brachial vein, yielding approximately 3 ml of blood per sample. These blood samples were analyzed within 2 hours of collection employing a hematology analyzer (Medonic M-series) following the company's established standard operating procedures (SOPs) for hematology and hematophysiological parameters. Additionally, leukocytes were manually enumerated (29) utilizing a hemocytometer (Neubauer chamber, Germany) for all treatment groups.

ESTIMATION OF HEMOGLOBIN CONCENTRATION LEVEL

The quantification of hemoglobin concentration was carried out employing a hematology analyzer on blood samples collected in the presence of the anticoagulant EDTA. The incorporation of EDTA in the samples was instrumental in averting coagulation and preserving the samples' structural integrity. The hematology analyzer was employed following established standard procedures, ensuring precise determination of hemoglobin concentration. This process yielded crucial insights into the physiological status of hemoglobin concentration in broiler chickens.

ERYTHROCYTES (RBCs) COUNT

Erythrocyte quantification entailed the utilization of blood samples collected with EDTA as an anticoagulant. Analysis was carried out employing a hematology analyzer to ensure accurate RBC counting. The addition of EDTA to the blood samples effectively prevented clotting, thereby facilitating precise RBC enumeration using the hematology analyzer in accordance with established protocols.

LEUKOCYTES (WBCs) COUNT

Leukocyte counting was executed manually via the use of a hemocytometer Neubauer chamber (Germany), involving blood samples collected with EDTA as an anticoagulant. A dilution was prepared by combining 2.5 ml of white blood cell diluting liquid with 0.05 ml of the blood sample. The presence of leukocytes or white blood cells in the blood sample was identified by observing the blue-colored nuclei under a microscope (Olympia BX41, Japan). This manual counting method ensured the accurate assessment of leukocyte count, contributing to the evaluation of broiler chicken physiological conditions.

PLATELET COUNT

Platelet count serves as a vital indicator of hemostatic function and thrombocyte levels in broiler chickens. For platelet count determination, blood samples were collected from the chickens and mixed with the anticoagulant EDTA to inhibit clotting factors. Subsequently, platelet counts were measured using a hematology analyzer. This thrombocyte counting analysis furnished valuable insights into the broiler chickens' hemostatic function and overall physiological health.

EVALUATION OF BROILER SERUM BIOCHEMICAL PARAMETERS

Serum samples collected on the 42nd day of the trial were employed to assess the physiological status of broiler health. Serum isolation involved the collection of approximately 3-4 ml of blood from the brachial vein of each broiler. After collecting the blood, it was transferred into vacutainers containing clot

activators, which were then placed in an inclined position for 25-30 minutes to facilitate clot formation. Subsequent centrifugation of the vacutainers at 1400 revolutions per minute (rpm) for 5 minutes using a bench-top centrifuge led to serum separation. The separated serum was meticulously collected in cryogenic vials and stored at -20°C until further analysis.

To assess the effects of oxytetracycline (OTC) and Tylosin Phosphate (TP) on broiler hepatic and renal physiology, serological tests were conducted employing a clinical biochemistry analyzer (Microlab 300). Specifically, renal function (urea, creatinine) and liver function (alanine aminotransferase, aspartate aminotransferase) tests were executed using commercially available kits on the 42nd day of the trial. These serum biochemical analytical tests furnished insights into the health status and physiological performance of the broilers' liver and kidneys.

EVALUATION OF BROILER RENAL PHYSIOLOGY

ANALYSIS OF BLOOD UREA NITROGEN CONCENTRATION (mg/dl)

Serum urea levels in broiler chickens were assessed using Innoline® kits manufactured by Merck Specialties (Pvt.) Ltd, France. The determination of urea levels was conducted in milligrams per deciliter (mg/dl) according to the manufacturer's protocols. The Innoline® kits were specifically designed to ensure accurate and validated measurement of urea levels in serum samples.

ANALYSIS OF CREATININE LEVEL (mg/dl)

The quantification of creatinine levels in broiler serum samples was executed utilizing Innoline® kits, also produced by Merck Specialties (Pvt.) Ltd, France. These kits are tailored to provide precise measurements of creatinine concentration in milligrams per deciliter (mg/dl).

HEPATIC PHYSIOLOGY AND ENZYMATIC PERFORMANCE

ALANINE AMINOTRANSFERASE LEVEL (ALT -U/L)

The determination of alanine aminotransferase (ALT) levels in broiler serum samples was accomplished using Innoline® kits from Merck Specialties (Pvt.) Ltd, France. The enzymatic performance level of ALT activity was measured in units per liter (U/L).

ASPARTATE AMINOTRANSFERASE LEVEL (AST -U/L)

Aspartate aminotransferase (AST) levels in broiler serum were ascertained employing Diasys® GmbH kits, manufactured by Diasys® GmbH, Germany. The measurement of AST activity was expressed in units per liter (U/L).

RESULTS

The effects of adding Oxytetracycline and Tylosin Phosphate to treatment groups alone or in combination on hematology and serum biochemical parameters are presented in Tables I and II.

Table I. Hemato-physiological health performance of broiler supplemented with AGP's on 42nd day (Mean ±SE)

Treatments	ERTs × 10^{12}/l	LEUKs × 10^{9}/l	LYM %	MID %	GRAN %	LYM# × 10^{9}/l	MID# × 10^{9}/l	GRAN# × 10^{9}/l
T1	2.61±0.10^{c}	76.40±1.80	83.10±1.60	8.03±0.42	9.61±0.91	63.10±1.30	5.61±0.50	6.60±1.00
T2	2.65±0.15^{b}	71.73±0.49	84.80±1.34	7.60±0.25	7.40±1.37	63.57±0.77	5.32±0.39	4.97±1.12
T3	2.56±0.11^{b}	76.55±1.05	84.20±2.04	7.93±0.45	7.98±1.29	64.30±3.14	5.83±0.42	7.55±1.38
T4	2.98±0.04^{a}	74.23±2.77	84.28±2.03	8.53±0.45	8.23±1.28	62.37±3.13	6.47±0.57	5.92±1.04
T5	2.53±0.09^{b}	71.90±3.38	81.62±2.21	7.63±0.46	9.43±1.84	58.90±3.29	5.67±0.35	6.88±1.25
T6	2.50±0.23^{b}	72.78±2.02	84.42±0.50	8.05±0.16	7.10±0.46	60.68±1.01	5.60±0.14	5.73±0.47
T7	3.08±0.04^{a}	77.28±2.48	84.02±1.30	8.15±0.24	8.55±0.95	61.43±1.06	6.20±0.39	7.22±0.65
T8	2.98±0.11^{a}	70.78±1.07	83.13±1.74	8.43±0.53	7.60±1.17	62.40±2.92	6.12±0.68	5.85±0.93
T9	3.10±0.12^{a}	77.00±2.50	83.10±1.20	8.10±0.23	8.60±0.80	61.41±1.10	5.80±0.50	7.00±1.00

Different super script in a column shows significant difference (P<0.05).

T1 (Control), T2 (OTC 0.5g/Kg feed), T3 (OTC 1g/Kg feed), T4 (TP 0.5g/Kg feed), T5 (OTC+TP 0.5g/Kg+0.5g/Kg feed), T6 (OTC+TP 1g/Kg+0.5g/Kg feed), T7 (TP 1g/Kg feed), T8 (OTC+TP 0.5g/Kg+1g/Kg feed) and T9 (OTC+TP 1g/Kg+1g/Kg feed). ERTs (Erythrocytes), LEUKs (Leukocytes), LYM (Lymphocytes, monocytes, neutrophils, eosinophils, basophils, and macrophages). MID (Combined value of the other type of leukocytes not classified as lymphocytes and granulocytes) GRAN (Granulocytes)

The results showed significant difference (P<0.05) in RBCs in treatment groups comparing to control group. However, other parameters WBC, lymphocytes (LYM) and granulocytes (GRAN) revealed non-significant difference (P>0.05) between AGPs supplemented treatment groups in comparison to control group at 42nd day. Lowest RBC count (2.50±0.23) was observed in T6 group; while, among the AGPs supplemented groups T4=2.98±0.04, T8=2.98±0.11, T9= 3.10±0.12 and (T7) 3.08±0.04 had the highest RBCs count at 42nd day. The results revealed that RBCs were significantly different in AGP supplemented treatment groups and control (P<0.05). Significant difference (P<0.05) in Hb content was noted among AGP and control groups. However, other parameters HCT, MCV, MCH, MCHC, RDW and PLT showed non-significant difference among either AGP groups or control group (P>0.05). Among the AGP supplemented groups T4 was noted having highest Hb (11.60±0.64), followed by (T7) 10.85±0.46, (T8) 10.78±0.46 and T9 (10.70±0.20) at 42nd day with no significant difference (P>0.05) in broiler hematology. Lowest Hb was noted in control group T1 (10.00±0.40) at 42nd day showed significant difference from AGP treatment groups. The results of serum biochemical indices i.e. Urea, Creatinine, ALT and AST (Table III) of broiler chicken provided AGP in comparison to control group and among treatment groups showed insignificant effect (P>0.05).

Table II. Hemato-physiological values of broiler chicken on 42nd day against the effect of AGP's (Mean ±SE)

Treatments	Hb (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW-CV (%)	RDW (fl)	PLT × 10³/l
T1	10.00±0.40[c]	38.40±1.40	154.00±3.20	42.20±0.70	28.10±0.30	9.50±0.60	39.90±2.10	56.20±6.70
T2	9.60±0.44[b]	37.05±1.98	159.05±5.87	45.02±0.98	29.05±1.21	11.08±1.18	39.35±2.29	55.80±5.56
T3	9.62±0.51[b]	39.72±1.76	163.20±8.09	44.15±1.36	27.18±0.68	8.72±0.21	38.87±1.93	61.35±5.68
T4	11.60±0.64[a]	38.77±2.47	153.63±4.40	42.80±1.21	28.03±0.34	10.37±0.55	39.13±1.95	59.48±9.73
T5	9.30±0.16[b]	34.07±1.67	155.02±1.10	43.52±0.32	28.22±0.29	8.92±0.34	37.03±0.27	63.45±2.46
T6	8.85±0.17[b]	34.90±1.02	155.20±7.00	44.93±0.75	28.37±0.61	10.05±1.29	35.23±0.59	73.88±5.41
T7	10.85±0.46[a]	37.80±1.12	151.53±3.42	43.92±1.55	27.55±0.47	9.88±0.51	37.87±0.60	58.10±6.31
T8	10.78±0.46[a]	38.45±2.57	152.65±3.14	42.83±1.09	28.37±0.87	10.00±1.12	36.90±1.77	58.00±6.78
T9	10.70±0.20[a]	37.60±1.30	150.60±3.00	43.13±1.04	27.56±0.70	9.30±0.70	36.03±0.50	61.00±3.70

Different super script in a column shows significant difference (P<0.05).
T1 (Control), T2 (OTC 0.5g/Kg feed), T3 (OTC 1g/Kg feed), T4 (TP 0.5g/Kg feed), T5 (OTC+TP 0.5g/Kg+0.5g/Kg feed), T6 (OTC+TP 1g/Kg+0.5g/Kg feed), T7 (TP 1g/Kg feed), T8 (OTC+TP 0.5g/Kg+1g/Kg feed) and T9 (OTC+TP 1g/Kg+1g/Kg feed).
Hb (Hemoglobin), HCT (Hematocrit), MCV (Mean corpuscular volume), MCH (Mean corpuscular hemoglobin), MCHC (Mean corpuscular hemoglobin concentration), RDW-CV (Red cell distribution width), RDW (Red cell distribution), PLT (Platelets), g/dL (Gram per deciliter), pg (Serum Pepsinogen), fl (Femtoliter)

Table III. Serum biochemical analysis of broiler on 42nd day to evaluate the chemo-physiological performance of kidney and liver against supplementation with AGP's (Mean ±SE)

Treatments	ALT (U/L)	AST (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
T1	22.9±1.12	283.2±8.56	3.6±0.21	3.67±0.41
T2	20.93±4.4	257.27±1.8	3.63±0.09	3.53±0.12
T3	27.63±2.47	273.03±6.01	3.73±0.24	3.27±0.19
T4	21.33±1.51	282±6.92	4.07±0.22	3.63±0.26
T5	23.53±4.75	283.13±9.06	3.87±0.12	2.87±0.49
T6	24.53±4.41	273.23±10.72	4.1±0.29	3.6±0.15
T7	27.43±2.26	269.43±5.68	3.73±0.12	3.93±0.28
T8	29.7±2.69	283.2±8.49	3.9±0.2	3.93±0.09
T9	27.17±1.79	267±14.42	3.87±0.38	4.2±0.17

Different super script in a column shows significant difference (P<0.05).
T1 (Control), T2 (OTC 0.5g/Kg feed), T3 (OTC 1g/Kg feed), T4 (TP 0.5g/Kg feed), T5 (OTC+TP 0.5g/Kg+0.5g/Kg feed), T6 (OTC+TP 1g/Kg+0.5g/Kg feed), T7 (TP 1g/Kg feed), T8 (OTC+TP 0.5g/Kg+1g/Kg feed) and T9 (OTC+TP 1g/Kg+1g/Kg feed). ALT (Alanine transaminase) and AST (Aspartate aminotransferase)

DISCUSSION

Most of the antibiotic growth promoters (AGPs) are used for prophylactic purposes (30) to improve the cellular physiology, hemato-physiology, immune-physiology, intestinal morphology, and efficient feed conversion, ultimately resulting in better growth performance (31). Variations in the normal functioning of intestinal microorganisms can influence the immunity and physiology of birds. Broiler management practices, including housing hygiene, pathogenic infestations, feed composition, and the presence of AGPs, can also impact the intestinal microbiota and broiler chicken physiology (13). Therapeutic antibiotics,

especially oxytetracycline (OTC), are commonly used in animal production for human food consumption. Tetracyclines, including OTC, act as growth promoters and have a positive impact on gut physiology, which, in turn, influences broiler growth rates (32), immune responses (13), and resistance against various pathogens.

Hematology is an essential tool for evaluating the health and diagnosing diseases in living beings. It plays a vital role in assessing the health condition of an individual. Changes in various hematological parameters can indicate physiological, pathological, and nutritional disturbances. Moreover, these parameters are valuable for evaluating the effects of nutritional supplements in feed and for detecting infections. For instance, a sudden increase in white blood cells serves as a good indicator of infections in the body, as these white blood cells act as the body's first line of defense against pathogens (33). In the present study, the supplementation of AGPs to broiler chickens showed insignificant differences among treatment groups in hematology parameters, except for RBCs. However, numerical differences were observed. The present findings are consistent with those of a previous study (34) that reported non-significant changes, except for RBCs and Hb, in AGP-supplemented groups. Another study (35) reported reductions in hematological values with the use of these antibiotics. Conversely, (36) found that Hb and RBCs were significantly higher in AGP-supplemented groups compared to the control group. Toghyani et al., (2010) also reported higher levels of RBCs and Hb in AGP-treated groups compared to the control group. Hb levels in AGP-treated groups were higher than in the control group (37).

In the present study, supplementation of AGP had non-significant effects on ALT, AST, urea, and creatinine levels in broiler chickens. These findings are supported by the results of (38) who used Flavomycin. Similarly, it was found that creatinine, uric acid, ALP, and AST showed non-significant differences between control and AGP-supplemented groups (34, 39, 40).

In recent years, the use of antibiotic growth promoters (AGPs) has come under increased scrutiny due to concerns about antibiotic resistance and the potential transfer of resistant bacteria from animals to humans (41). This has led to regulatory changes and a shift towards more responsible antibiotic use in animal agriculture (42). As a result, there is a growing interest in alternative strategies such as probiotics, prebiotics, synbiotics, and phytogenic feed additives to promote gut health, improve nutrient utilization, and enhance broiler performance (43, 44).

Probiotics, which are beneficial live microorganisms, have gained attention as potential replacements for AGPs. They can positively influence gut microbiota, enhance immune responses, and contribute to improved growth performance (45). Prebiotics, on the other hand, are non-digestible food ingredients that selectively stimulate the growth and activity of beneficial gut bacteria, which can have a similar positive impact on broiler physiology (46). Synbiotics, which combine probiotics and prebiotics, offer a synergistic approach to support gut health and overall bird well-being (47), additionally, the effect of antioxidants on growth of neonate calves has also been reported (48).

Furthermore, phytogenic feed additives derived from herbs, spices, and other plant materials have demonstrated antimicrobial and anti-inflammatory properties that can benefit broiler chickens (49). These natural compounds have the potential to modulate gut microbiota, improve nutrient absorption, and promote better health without the concerns associated with antibiotic resistance. Therefore, future research should explore the effectiveness of these alternative strategies and their impact on broiler hemato-physiology, immune-physiology, and growth performance in the context of responsible and sustainable poultry production (50).

In conclusion, the use of antibiotic growth promoters in broiler production has been a common practice with the aim of enhancing growth and health. However, there is a growing need to explore alternative approaches that promote broiler physiology without the associated risks of antibiotic resistance. Probiotics, prebiotics, synbiotics, and phytogenic feed additives offer promising avenues for future research. These alternatives have the potential to positively influence hematological and serum biochemical parameters, immune responses, and overall broiler health. As the poultry industry continues to evolve, a focus on responsible and sustainable practices is essential, and these alternative strategies provide a path forward in achieving those goals.

CONCLUSION

The study suggests that AGPs can improve Hb and RBCs levels in broiler chicken without any significant modification in hematology and serum biochemical indices.

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