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Use of Growth Promoter in Feed: Tylosin Phosphate and Oxytetracycline Di-Hydrate Show Synergistic Effect on the Haematological Parameters and Biochemical Components of Broiler Chicken Blood

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ISS and SHS conceived and designed the study. SHS, MEK and MZM executed the experiment. NK and ISS analysed the data and wrote the paper. NK, ISS and MMT critically reviewed the paper and all authors collectively approved the final version.

Key words
Oxytetracycline di-hydrate, Tylosin phosphate, Antibiotic growth promoter, Broiler chicken, Blood, Liver and kidney

ABSTRACT

The antibiotic growth promoters (AGPs) are provided in poultry feeds as an additive on sub-therapeutic level. The *in vivo* study was designed to evaluate the effect of oxytetracycline di-hydrate (OXY) and tylosin phosphate (TP) on different blood physiological and biochemical parameters in broiler chicken. AGPs supplement alone and in combinations were provided as pre-starter (days 1-12), starter (days 13 to 24) and finisher (day 25 to 42) feed. Standard broiler rearing temperature was provided in the shed. Results showed that the OXY and TP had significant ($P<0.05$) effect on physiology of blood parameters, mainly on hemoglobin (Hb) and erythrocytes (RBCs) in all treatment groups treated till day 42 compared with the control group. Remarkably, the AGPs showed a positive impact on the liver enzymes such as alanine aminotransferase (ALT) and aspartate aminoransferase (AST) on day 42. A non-significant effect was seen on hematological and biochemical profile of liver and kidney of broiler chicken on days 14th and 28th. In conclusion, the study showed significant synergistic effect of TP and OXY on the Hb, RBC and liver enzymes such as ALT and AST that ultimately lead to promote the growth and can be used safely at sub-therapeutic levels in food and/or water of broiler chicken. The result further determined that the longer use (6 weeks) of AGPs had no harmful effect on blood, liver and kidney in broiler chicken and can be used after withdrawal period.

INTRODUCTION

Antibiotics are biological elements, synthesized by microorganisms, used to inhibit the growth of harmful microbes. The use of antibiotics in animal and poultry diets is in practice since decades. In addition to anti-microbial activity, antibiotics are used as growth-promoters and to cure or reduce the occurrence of contagious disease, which results in physiological improvement (Choi *et al*., 2018; Ashraf *et al*., 2019). Studies have determined the use of antibiotics during rearing of broiler chicken to improve weight gain and produce more meat (Sheikh *et al*., 2020). Estimation of biochemical and hematological parameters are decent indicators to evaluate the health status of animals and play important role in diagnosis and treatments of poultry diseases (Gilani *et al*., 2018). The supplementation/ selection of suitable AGPs in feed has a positive effect on gastro-intestinal tract development and microbial flora at different stages of development in broiler chicken (Miles *et al*., 2006).

Several studies have described the use of antibiotic such as tetracyclines, bactrican, penicillin and streptomycin as additives in poultry and livestock. Bambermycins, procaine penicillin, neomycin sulfate, chlortetracycline is some of the antibiotics used at sub-therapeutic level in poultry (Barcelo, 2007). It was determined that the use of antibiotics in broiler chicken improve the immune system and physiological parameters of blood (Lee *et al*., 2012). Bai *et al*. (2019) have reported that environmental factors also affect different metabolites such as triglycerides, protein, and glucose in broiler chicken. The supplementation of suitable AGPs in broiler feed has a

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positive effect on gastrointestinal tract (GIT) development and microbial flora in broiler chicken (Miles *et al*., 2006; Yadav and Jha, 2019).

In most of the earlier studies single antibiotic were used as AGPs in poultry, which showed no effect or less effect on growth. Furthermore, it also showed harmful effect on visceral organs by disturbing the metabolites. In this study we have used AGPs alone and in combination with different concentration and for different durations on different physiological parameters of blood including kidney and liver.

METERIALS AND METHODS

Broiler chicks maintenance and collection of blood samples

A total of 432, one-day old broiler chicks, purchased from International Poultry Karachi, Pakistan were maintained in a shed at 95°F for one weak and then temperature was reduced 5°F on weakly basis. Chicks divided into nine groups, each of 48 chicks, 3 replicates of sixteen broiler chicks and vaccinated against Newcastle disease virus (ND) (Salisbury) and Infectious bursal disease (IBD) (Salisbury) that was followed by booster doses. Feed purchased from Gawadar Oils and Feed limited, supplemented with AGPs in different combination and fed on for 42 days as shown in Table I. Drinking water was provided *ad libitum*.

At the end of experiment periods chicken were sacrificed and blood samples were collected under a sterile condition from the jugular vein. A part of this sample was allowed to clot for separating serum for biochemical studies, while the remaining sample was added in a collection tube containing anticoagulant EDTA (ethylene diamine tetra acetic acid) for hematological studies.

Hematological and biochemical analyses

For hematological profiles the blood was analyzed by hematology analyzer (Medonic M-series) according to the manufacturer instructions.

For biochemical analysis, the serum was analyzed on Microlab-300 (Innolin, Martin Dow Marker Specialties (pvt) Ltd. for estimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine according to the standard protocols available in the commercial kits (Haque *et al*., 2017).

Statistical analysis

The data was organized in Microsoft Excel. Statistical analysis was carried out using program SPSS 21. Analysis of variance (ANOVA) with interaction technique, followed by post hoc Duncan multiple range (DMR) test were applied.

RESULTS

Effect of AGP on hematological parameters

Oxytetracycline di-hydrate in combination with Tylosin phosphate increases the hemoglobin concentration. Table I shows the effect of TP and OXY on hematological parameters of broilers. Use of 0.5 mg/kg TP at sub-therapeutic level has reduced the RBCs (2.56×10^{12}/L) and Hb (9.62 g/dL) levels. These effects became more prominent and the Hb was dropped to 8.85 g/dL by increasing concentration upto 1.0 mg/kg. Interestingly, similar effect was seen, when the OXY (2.0 mg/kg) was used solely as AGPs, and the Hb concentration was dropped to 9.30 g/dL including RBCs compared to control group. The non-significant ($p>0.05$) impact was seen on hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC). Study revealed that use of both antibiotics in a proper concentration had impact on the blood physiological parameters and increases Hb level and RBC count. Interestingly, there is no significant ($p>0.05$) impact on leucocytes, differential leucocytes count (DLC), and platelets (Data not shown). Furthermore, chicken treated with OXY and TP for days 14 and 28 showed non-significant effect on the hematological profile (Data not shown). Overall, result showed that the use of TP had significant ($p<0.05$) impact on the physiological parameters of blood such as Hb and erythrocytes.

Combination of Oxy (1.0 mg/kg) and TP (0.5 mg/kg) have increased the Hb (11.60 g/dL) and RBCs (2.98 10^{12}/L) level significantly ($p<0.05$) compared to the control group (10.20 g/dL). However, the combined effects with increased concentration of (Oxy1.0 mg/kg, TP 1.0 mg/kg) have reduced the hemoglobin concentration (Hb 10.65 g/dL). Similarly, a positive ($p<0.05$) effect was seen when OXY 2.0 mg/kg was used in combination with TP 0.5 mg/kg and 1.0 mg/kg, respectively. The data showed that the standard concentration and proper combination of antibiotic is necessary to use them as antibiotic growth promoter. The synergistic effect of OXY 1.0 mg/kg and TP 0.5 mg/kg is a favorable combination to use as antibiotic growth promoter in broiler chicken.

Effect of AGP on renal function

Table II shows effect of longer use of AGPs on sub-therapeutic levels on renal function tests of broilers. Interestingly, no significant ($p>0.05$) impact was found on the renal parameters, suggesting that the use of OXY and TP at sub-therapeutic level for a longer period (6 week) has no side effect on kidney performance of broiler chicken.

Table I. Effect of different concentration of oxytetracycline di-hydrate and tylosin phosphate administered for a total period of 42 days on the hematological parameters of broiler chicken.

Treatments	Hb (g/dL)	RBCs (x10^{12}/L)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Control	10.20±0.43^{c}	2.60±0.08^{c}	38.42±1.36	153.73±3.19	42.18±0.69	28.22±0.40
OXY-0+TP-0.5	9.62±0.51^{b}	2.56±0.11^{b}	39.72±1.76	163.20±8.09	44.15±1.36	27.18±0.68
OXY-0+TP-1.0	8.85±0.17^{b}	2.50±0.23^{b}	34.90±1.02	155.20±7.00	44.93±0.75	28.37±0.61
OXY-1.0+TP-0	9.60±0.44^{b}	2.65±0.15^{b}	37.05±1.98	159.05±5.87	45.02±0.98	29.05±1.21
OXY-1.0+TP-0.5	11.60±0.64^{a}	2.98±0.04^{a}	38.77±2.47	153.63±4.40	42.80±1.21	28.03±0.34
OXY-1.0+TP-1.0	10.65±0.22^{a}	3.06±0.14^{a}	37.55±1.28	149.58±2.90	44.20±1.03	27.50±0.74
OXY-2.0+TP-0	9.30±0.16^{b}	2.53±0.09^{b}	34.07±1.67	155.02±1.10	43.52±0.32	28.22±0.29
OXY-2.0+TP-0.5	10.78±0.46^{a}	2.98±0.11^{a}	38.45±2.57	152.65±3.14	42.83±1.09	28.37±0.87
OXY-2.0+TP-1.0	10.85±0.46^{a}	3.08±0.04^{a}	37.80±1.12	151.53±3.42	43.92±1.55	27.55±0.47

Control (Basal diet, Without AGPs); Oxytetracycline di-hydrate (OXY)-0+ Tylosin phosphate (TP)-0.5 (50 mg/kg); OXY-0+TP-1.0 (100 mg/kg); OXY-1.0+TP-0 (100 mg/kg); OXY-1.0+TP-0.5 (100 + 50 mg/kg each); OXY-1.0+TP-1.0 (100 + 100 mg/kg each); OXY-2.0+TP-0 (200 mg/kg); OXY-2.0+TP-0.5 (200 + 50 mg/kg each); OXY-2.0+TP-1.0 (200 + 100 mg/kg each). Hb, hemoglobin; RBC, red blood cells; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration. ^{abc} superscripts shows significance (P<0.05).

Table II. Effect of different concentration of oxytetracycline di-hydrate and tylosin phosphate (mg/dl) administered for a period of 14, 28 and 42 days on the renal profile of broiler chicken.

Treatments	Day 14th		Day 28th		Day 42nd	
	Creatinine	Urea	Creatinine	Urea	Creatinine	Urea
Control	0.24±.02	1.50±.22	0.30±.02	1.17±.17	1.11±0.03	2.67±0.33
OXY-0+TP-0.5	0.26±.01	2.00±.52	0.34±.02	1.67±.33	1.34±0.26	3.00±0.58
OXY-0+TP-1.0	0.27±.01	1.83±.31	0.33±.01	1.50±.22	1.34±0.05	3.33±0.42
OXY-1.0+TP-0	0.26±.02	2.00±.52	0.31±.01	1.67±.21	1.48±0.04	3.17±0.54
OXY-1.0+TP-0.5	0.28±.01	2.33±.42	0.35±.02	1.83±.31	1.65±0.22	3.67±0.49
OXY-1.0+TP-1.0	0.26±.01	1.83±.31	0.35±.02	2.17±.40	1.66±0.26	4.00±0.45
OXY-2.0+TP-0	0.26±.02	1.67±.33	0.32±.01	1.33±.21	1.43±0.14	3.50±0.43
OXY-2.0+TP-0.5	0.28±.01	2.00±.37	0.35±.03	1.83±.54	1.66±0.20	3.83±0.48
OXY-2.0+TP-1.0	0.27±.01	1.67±.33	0.36±.02	2.00±.26	1.67±0.18	4.17±0.48

For details of treatment see Table I.

Table III. Effect of different concentration of oxytetracycline di-hydrate and tylosin phosphate (U/L) administered for a period of 14, 28 and 42 days on the liver function of broiler chicken:

Treatments	Day 14th		Day 28th		Day 42nd	
	AST	ALT	AST	ALT	AST	ALT
Control	342.83±9.51	32.50±1.67	342.83±9.51	32.50±1.67	396.67±21.08^{c}	28.33±1.50^{c}
OXY-0+TP-0.5	361.33±23.40	32.83±3.20	361.33±23.40	32.83±3.20	450.33±1.96^{b}	35.00±2.14^{b}
OXY-0+TP-1.0	391.67±23.08	38.83±2.36	391.67±23.08	38.50±1.95	447.50±2.95^{b}	35.67±2.29^{b}
OXY-1.0+TP-0	350.33±6.81	39.67±1.69	350.33±6.81	38.00±1.65	451.83±5.11^{b}	35.83±1.45^{b}
OXY-1.0+TP-0.5	392.00±17.00	39.67±1.20	397.33±19.95	39.17±.95	490.83±12.93^{a}	40.00±1.86^{a}
OXY-1.0+TP-1.0	398.67±15.06	38.50±1.95	398.67±15.06	38.83±2.36	485.33±10.96^{a}	39.67±1.58^{a}
OXY-2.0+TP-0	397.33±19.95	33.83±3.28	391.67±16.97	33.83±3.28	445.33±3.66^{b}	35.00±1.79^{b}
OXY-2.0+TP-0.5	391.67±16.97	39.17±.95	392.00±17.00	39.50±2.09	487.83±12.94^{a}	38.67±1.50^{a}
OXY-2.0+TP-1.0	398.33±14.61	39.50±2.09	398.33±14.61	39.67±1.20	496.67±16.03^{a}	42.67±1.63^{a}

^{abc} superscripts shows significance (P<0.05). For details of treatment see Table I. AST, aspartate amin-transferase; ALT, alanine aminotransferase.

Effect of AGPs on liver function

The antibiotic growth promoter (AGPs), Oxytetracycline di-hydrate and Tylosin phosphate interrupt liver function enzymes. Table III shows effect of the AGPs, OXY and TP on liver function enzymes of broiler chicken. The effect of AGPs was analysed alone and in combination with different concentration. The *in vivo* study revealed a significant ($p<0.05$) effect of using antibiotics, showing an increase in liver enzymes. Results showed that the individual use of TP (0.5 mg/kg) and OXY (1.0 mg/kg) has increased the level of AST and ALT by 450.33 U/L and 35.0 U/L respectively compared to the control (AST 396 U/L, ALT 28 U/L). Similar, individual effect was seen, when the concentration of TP was increased upto 1.0 mg/kg and Oxy was increased to two fold (2.0 mg/kg).

Remarkably, a strong effect was seen when OXY was used in combination with TP. The synergistic effect of Oxy 1.0 mg/kg and TP 0.5 mg/kg increased the AST and ALT to 490.83 U/L and 40.0 U/L, respectively. An increase level of liver enzymes was observed when the concentration of TP was increased upto 1.0 mg/kg in the presence of Oxy 1.0 mg/kg. This effect persisted even when the concentration of Oxy was increased upto 2.0 mg/kg in combination with TP 1.0 mg/kg. Our results showed that the TP and Oxy have significant synergistic effect on liver enzymes when used in different concentration. Furthermore, the effect of AGPs on liver enzymes were realized only on day 42nd, while no effect was seen on days 14th and 28th, compared to the control group (Table III).

DISCUSSION

The use of antibiotic during rearing of broiler chicken is common and usually supplemented in feed and water to improve weight gain and meat production. In veterinary practice the antibiotics are used at sub-therapeutic level to enhance the growth in broiler chicken (Barcelo, 2007), which ultimatly protect the poultry from infections (Chattopadhyay, 2014; Lee *et al*., 2012). Several studies have been carried out using antibiotic as growth promoter such as bambermycins, procaine, penicillin, neomycin sulfate, chlortetracycline (Barcelo, 2007). Among various AGPs, TP and OXY play key role in promoting growth (Shah *et al*., 2021). TP shows a bacteriostatic activity against Gram-positive bacteria and can be administered safely in veterinary and poultry feeds as an additive. Furthermore, TP can be supplemented in broiler chicken at sub-therapeutic levels without disturbing meat quality and texture (Costa *et al*., 2007; Shah *et al*., 2021). In addition, due to broad spectrum action against acute and chronic microbial infections, OXY and TP are frequently used in veterinary practice (Berrada *et al*., 2010).

Shah *et al*. (2021) have concluded that the use of AGPs at sub-therapeutic level have beneficial effect on the growth performance of broiler chicken. A better growth performance was determined in AGPs treated groups, prebiotic and probiotic groups without effecting blood physiological parameters (Roshanfekr and Mamooee, 2009). It was described that the use of AGPs in feed showed significant impact, enhance growth and improve physiological properties such as an increase in Hb and red blood cells (RBCs) (Kondera *et al*., 2020). Use of AGPs increased RBCs and Hb levels (11.01×mg/100ml) when compared to the control group (10.6×mg/100ml) (Ashraf *et al*., 2019; Toghyani *et al*., 2010; Ambili *et al*., 2013).

In the current study, chicken treated with OXY and TP for days 14th and 28th showed non-significant effect on the hematological profile (data not shown). However, the results coordinate with the study of Toghyani *et al*., (2010) and Ambili *et al*. (2013), when AGPs were used in combination. The results showed that the use of both antibiotics had an impact on the blood physiological parameters and an increased Hb level and RBC count were measured. Remarkably, a combination of Oxy (1.0 mg/kg) and TP (0.5 mg/kg) had significantly ($p<0.05$) increased hemoglobin (11.60 g/dL) and RBCs (2.98 10^{12}/L) level compared to the control group (10.20 g/dL).

Interestingly, data showed that the use of 0.5 mg/kg TP alone had reduced the RBCs (2.5610^{12}/L) and Hb (9.62 g/dL) level. This effect became prominent and the Hb was dropped further when the concentration was increased upto 1.0 mg/kg. Similar, effect was seen when OXY was used alone. The outcomes of present study correlated with the previous studies that the supplementation of OXY and TP have significant effects ($p<0.05$) on RBCs and Hb in all nine groups. Remarkably, use of Oxy (1.0 mg/kg) and TP (0.5 mg/kg) had increased significantly ($p<0.05$) the Hb and RBCs levels. However, this effect was released when the concentration of TP (Oxy1.0 mg/kg, TP 1.0 mg/kg) was increased and no effect was seen on HCT, MCV, MCH, MCHC, RDW and platelets count.

The results of Gilani *et al*. (2018) correlates with the results of this study, which showed a non-significant changes ($p>0.05$) on most of the blood parameters except RBC and Hb. In addition, the AGPs showed no significant effect on leucocytes and differential counts such as lymphocytes (LYM), granulocyte (GRAN). Interestingly, this non-significance effect was measured at all 3 points of investigation. However, in contrast to our study, the tilmicosin used as AGPs have shown impact on WBC including other parameters (Elsayed *et al*., 2014).

In addition, results of our study revealed that the use of OXY and TP had no harmful effect on kidney and normal levels of urea and creatinine were measured at

all 14th, 28th, and 42nd days. ALT and AST are important liver enzymes measured from blood serum. Bhatti and Dil (2005) determined effect on different enzyme activity in broilers supplemented with antibiotic. The flavomycin have shown non-significant impact on glucose, albumin, alkaline phosphatase, ALT and AST levels (Attia *et al*., 2011). Similarly, Fitriana *et al*. (2020) analyzed, impact of tylosin and enrofloxacin on liver and renal organs supplemented in the diet. The correlates with our study, showing non-significant effect on liver enzymes like ALT, AST at days 14th and 28th. Overall, non-significant effect was determined on hematological profile such as Hb, WBC, RBC, and platelets and biochemical that is urea, creatinine, AST, and ALT on day 14th and 28th of experiment. The combined effect of OXY and TP even not observed. However, significant effect of OXY and TP were determined on Hb, RBCs, AST and ALT on the 42nd day of experiment when AGPs were used solely and in combination.

CONCLUSIONS

In conclusion, the results showed a significant effect of TP and OXY on Hb and RBCs, AST and ALT in broiler. The study further suggested that the use AGPs in combination have a stronger effect on blood physiology that ultimately lead to promote the growth and can be used safely at sub-therapeutic levels in broiler. The result further determined that the longer use (6 week) of antibiotic has no harmful effect on broiler chicken.

Statement of conflict of interest

The authors have declared no conflict of interest.

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