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Original Article

In vivo analysis the effect of antibiotic growth promoters (AGPs), Oxytetracycline di-hydrate and Tylosin phosphate on the intestinal microflora in broiler chicken

Análise *in vivo* do efeito de antibióticos promotores de crescimento (AGPs), di-hidrato de oxitetraciclina e fosfato de tilosina na microflora intestinal em frangos de corte

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Abstract

The study was aimed to analyse the effects of antibiotic growth promoters (AGPs), Oxytetracycline di-hydrate and Tylosin phosphate on the intestinal microflora in broiler chicken. The AGPs were provided in different concentrations solely or in combinations for 42 days of rearing. Faecal samples were collected from the intestine (duodenum, jejunum and caeca) of broiler chicken on 14th, 28th and 42nd days of trial. Samples were cultured on different selective medium and bacterial identification was performed by different biochemical and molecular diagnostic tools. Results showed a significant effect of AGPs on the growth of pathogenic microorganisms such as *Escherichia coli* and *Clostridium perfringens* in the intestine. Interestingly, an impaired growth was observed for both bacterium showing a significant effect (P<0.05) of AGPs on *E. coli* and *C. perfringens* on day 14th, 28th, and 42nd. This effect was observed solely and in combination while using AGPs. Data further showed that the effect was more prominent in combination and with an increase concentration of AGPs. Remarkably, no impairment was seen on the growth of *L. reuteri* at different sites of intestine and duration (14th, 28th, and 42nd days). The results showed that the use of AGPs in diet has no harmful effect on beneficial bacteria, however, an impaired growth was seen on the harmful bacteria. It is suggested that a combination of AGPs (OXY-1.0+TP-0.5) is economical and have no harmful effect on the broiler chicken. The use of AGPs in a recommended dose and for a specific period of time are safe to use in poultry both as growth promoter and for the prevention of diseases.

Keywords: antibiotic growth promoter (AGPs), *E. coli*, *C. perfringens*, *L. reuteri*, broiler chicken.

Resumo

O estudo teve como objetivo analisar os efeitos dos antibióticos promotores de crescimento (AGPs), di-hidrato de oxitetraciclina e fosfato de tilosina na microflora intestinal de frangos de corte. Os AGPs foram fornecidos em diferentes concentrações isoladamente ou em combinações por 42 dias de criação. Amostras fecais foram coletadas do intestino (duodeno, jejuno e ceco) de frangos de corte no 14º, 28º e 42º dias de ensaio. As amostras foram cultivadas em diferentes meios seletivos e a identificação bacteriana foi realizada por diferentes ferramentas de diagnóstico bioquímico e molecular. Os resultados mostraram um efeito significativo dos AGPs no crescimento de microrganismos patogênicos como *Escherichia coli* e *Clostridium perfringens* no intestino. Curiosamente, um crescimento prejudicado foi observado para ambas as bactérias, mostrando um efeito significativo (P <0,05) de AGPs em *E. coli* e *C. perfringens* nos dias 14, 28 e 42. Este efeito foi observado apenas e em combinação com o uso de AGPs. Os dados mostraram ainda que o efeito foi mais proeminente em combinação e com um aumento da concentração de AGPs. Nenhum comprometimento foi observado no crescimento de *L. reuteri* em diferentes locais do intestino e duração (14º, 28º e 42º dias). Os resultados mostraram que o uso de AGPs na dieta não tem efeito nocivo nas bactérias benéficas, no entanto, foi observado um crescimento prejudicado nas bactérias nocivas. Sugere-se que uma combinação de AGPs (OXY-1.0+TP-0.5) seja econômica e não tenha efeito prejudicial sobre o frango de corte. O uso de AGPs em uma dose recomendada e por um período de tempo específico é seguro para uso em aves tanto como promotor de crescimento quanto para prevenção de doenças.

Palavras-chave: antibiótico promotor de crescimento (AGPs), *E. coli*, *C. perfringens*, *L. reuteri*, frango de corte.

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1. Introduction

Commercial poultry farming started in early 1960s in Pakistan, which is one of the most active sector in meat production (26.8%), and that contribute 1.40% of the total national Gross domestic product (GDP) during 2016-17 (Achakzai et al., 2020). Feeding broilers with antibiotic growth promotor (AGP) has been documented to increase by weight up to 3.3-8.0%, including effect on growth, immunity and physiology (Wang et al., 2016) Albeit, the AGPs disturb the normal gastro-intestinal microbiota, it is recommended to reduce the infection in broiler chicken (Danzeisen et al., 2011).

Microbiotas in the gastro intestinal tract (GIT) of chicken play important role in absorption and digestion of nutrients, enhance immunity and supports resistance against various infections (Mehdi et al., 2018; Sheikh et al., 2020). In addition, the normal bacterial flora greatly contributes to the beneficial metabolic changes. The *Lactobacillus* enhances the broiler health which results in improving health. In contrast, the pathogenic bacteria *Salmonella* and *Campylobacter* suppress the growth of healthy tissues and cause diseases (Torok et al., 2011). The pathogenic bacteria *E. coli* causes colibacillosis disease. It is estimated that about 30% of the broiler flocks are affected by the disease in the United States (Fancher et al., 2020).

The arrangement and composition of microbiota in GIT influenced by feed and additives used, which influence the occurrence of intestinal pathogens, such as *Salmonella, Campylobacter jejuni* and *Eubacteria*. Studies have determined that the AGPs provides alteration in the microorganisms of GIT (Metzler et al., 2005). Interestingly, in addition to commercial antibiotics, in a study the effect of *Zingiber officinale* (Ginger) is analysed as herbal feed additives in broiler feed. Significant positive impact was observed on cholesterol, triglycerides and gut microbes (Asghar et al., 2021).

The data from previous studies showed that, interplay between AGPs and the intestinal microbiota have positive impact on growth performance. Since the AGPs have long been in practice as growth promoters, still need further investigation for the safer use as growth promotor in the feed of broiler chicken (Choi et al., 2018). Therefore, the purpose of this study is to evaluate the effects of AGPs, that is Oxytetracycline di-hydrate and Tylosin phosphate on the intestinal microflora in broiler chicken.

2. Materials and Methods

2.1. Research center

The research was conducted at the poultry house of Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta.

2.2. Husbandry of broiler chicks

The *in vivo* experiment was carried out on broiler chicken. A day old broiler chicks were purchased from International poultry Multan, Pakistan. The feed was purchased from Gwadar Oils and Feed limited and provided in different combination (Table 1). The pre-starter feed (F1) was provided on day 1st - 12th followed by starter feed (F-2) from day 13th to 24th and the finisher feed was offered from day 25th - 42nd. The trial was performed for 6 weeks (42 days). Optimal broiler rearing temperature was maintained with initial temperature 95 °F for the 1st week which was reduced up to 5 °F weekly. The birds were vaccinated against Newcastle Disease Virus (ND) and Infectious Bursal Disease (IBD) disease.

2.3. Group distribution and AGPs supplementations

In total 432 broiler chicks were divided into nine (09) groups. Each group was comprised of 48 chicks which was triplicates of sixteen broiler chicks. Control group was kept on basal diets and detail of AGP supplementation is described in Table 1.

2.4. Collection of samples

As per group distribution and scheduled experiment protocol the chicks from each group were slaughtered and the digestive tract was detached. Fecal samples from intestine that is duodenum loop, mid-jejunum and caeca were collected and stored separately in disposable polythene bags at -20 °C for detailed analysis.

2.5. Laboratory procedures

2.5.1. Isolation and identification of bacterial isolates

The fecal samples were inoculated on different culture media and initial identification was carried out by Gram-

Table 1. Supplementation of antibiotic growth promotors.

s.no	Groups	Treatments	Dose (mg/kg of feed)
1	Control	Basal diet	Without AGPs
2	OXY-0+TP-0.5	Tylosin Phosphate	50 mg/kg
3	OXY-0+TP-1.0	Tylosin Phosphate	100 mg/kg
4	OXY-1.0+TP-0	Oxytetracycline Di-hydrate	100 mg/kg
5	OXY-1.0+TP-0.5	Oxytetracycline Di-hydrate + Tylosin Phosphate	100 + 50 mg/kg
6	OXY-1.0+TP-1.0	Oxytetracycline Di-hydrate +Tylosin Phosphate	100 + 50 mg/kg
7	OXY-2.0+TP-0	Oxytetracycline Di-hydrate	200 mg/kg
8	OXY-2.0+TP-0.5	Oxytetracycline Di-hydrate + Tylosin Phosphate	200 + 50 mg/kg
9	OXY-2.0+TP-1.0	Oxytetracycline Di-hydrate + Tylosin Phosphate	200 + 100 mg/kg

staining followed by different biochemical tests such as Oxidase test, Indole test, Catalase test, Nitrate-reduction test and Methyl red Voges Proskauer's test. Molecular identification was performed by polymerase chain reaction (PCR).

2.5.2. Molecular identification by PCR

DNA extraction was performed from fresh bacterial culture. Bacterial colonies were suspended in 300µl 1% tris-EDTA. Vortexed shortly and incubated for 10 minutes at 95°C in water bath, followed by centrifugation at 6000rpm for 3-4 minutes at room temperature. Supernatant was separated and primer pair *uidA*_F_CCAAAAGCCAGACAGAGT and *uidA*_R_ GCACAGCACATCAAAGAG, primer pair *L. reut*_F CAGACAATCTTTGATTGTTTAG and *L. reut*_R GCTTGTTGGTTTGGGCTCTTC, and primer pair *ClPER*-F AGATGGCATCATTCAAC and *ClPER*-R GCAAGGGATGTCAAGTGT were used for the amplification of *uidA*, *lreut* and *clper* from *E. coli* (Scaletsky et al., 2002), *L reuteri* (Brolazo et al., 2011) and *C. perfringens* (Kikuchi et al., 2002), respectively.

2.5.3. Viable bacterial counting

About 01gram of collected fecal samples from intestine were serially diluted (1:10) in phosphate-buffered saline (PBS). Dilutions of 10^5 and/or 10^6 were inoculated on appropriate selective media and viable bacteria were determined by colony forming unit (CFU) using the following the Formula 1:

$$CFU\ /\ g = \left(No.\ of\ colonies\ x\ dilution\ factor\right)\ /\ Volume\ of\ culture\ plate \quad (1)$$

3. Results

3.1. Molecular characterization of isolated bacteria

3.1.1. Molecular identification of uidA in E. coli

E. coli is frequently associated with food born disease. The *uidA* gene encodes β-D-glucuronidase used for the identification of *E. coli* by PCR. Molecular identification of the bacterial isolates was performed by PCR, using specific gene oligonucleotides. The amplified product of size 623bp of *uidA* gene verified *E. coli* isolated from intestinal microflora of broiler chicken (Figure 1A).

3.1.2. Molecular identification of L. reut

The *L. reuteri* play important role in the regulation in intestinal microflora. The suspected isolates for *Lactobacillus reuteri* were obtained from the intestine of broiler chicken. After being biochemically characterized, the strains were further verified for *lreut* gene. The previously designed primers were applied to amplify the targeted gene of suspected size 303bp. The molecular identification by PCR verified the isolates from *L. reuteri* (Figure 1B).

3.1.3. Molecular identification of ClPER

Clostridium perfringens is β-glucuronidase producing bacteria. Common source of *C. perfringens* can be found on raw meat and poultry in the intestines of animals and in the environment. Genomic research has revealed high diversity in the genome of *C. perfringens*. However, 16S rRNA region is highly conserved showed sequence identity of >99.1%. The isolates from intestine of broiler chickens were verified by amplifying conserved *ClPER* gene (793bp) in *C. Perfringens.* Previously published specific gene primers were used to verify the gene product *ClPER* from *C. perfringens* (Figure 1C).

3.2. Effect of antibiotic growth promotors on Lactobacillus reuteri

L. reuteri is a beneficial bacterium that colonizes at several body sites such as skin, urinary track and gastrointestinal tract. Several beneficial effects of *L. reuteri* have been determined including antimicrobial activity (Mu et al., 2018). The effect of antibiotic growth promotors on the *L. reuteri* was determined in intestine. Remarkably, results showed no harmful effect on the growth of *L. reuteri*. The non-significant effect was observed on days 14th, 28th,

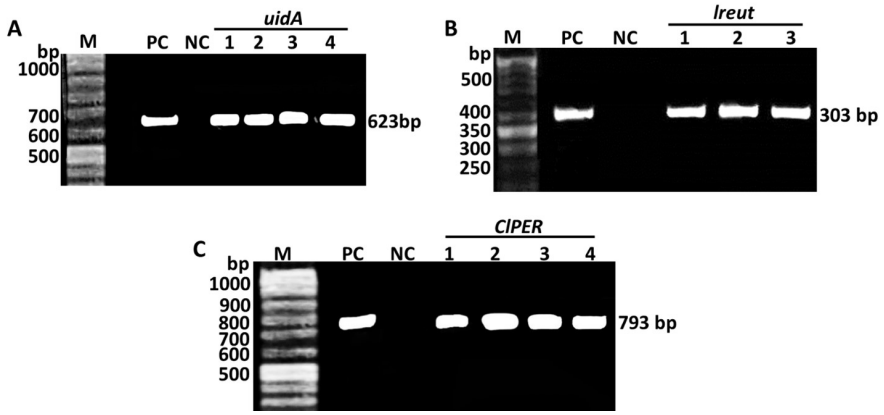

Figure 1 (A-C) Colony PCR of the *uidA, lreut* and *Clper* from *E. coli*, *Lactobacillus reuteri* and *Clostridium perfringens* respectively. Specific oligonucleotides were used to amplify the *uidA* (623bp), *lreut* (303bp) and *ClPER* (793bp) by colony PCR from the isolated bacterial strain of (A) *E. coli*, (B) *Lactobacillus reuteri* and (C) *Clostridium perfringens*. M = DNA leader; PC = Positive control; NC = Negative control; 1-4 = bacterial isolates.

and 42nd (Table 2). Results showed that *L. reuteri* neutralizes the effect of AGPs supplemented in the diet and show no harmful effect on the beneficial bacteria in the intestine of the broiler chicken.

3.3. Effect of antibiotic growth promotors on Escherichia coli

Escherichia coli is a commensal organism and faecal indicator. However, many of the *E. coli* strains are pathogenic and causes mild to severe disease (Godambe et al., 2017). We analysed the effect of AGPs on *E. coli* in the intestine of broiler chicken. Overall, results showed reduced bacterial load of *E. coli* and significant effect (P<0.05) at all three sites of intestine was determined (Table 3). Interestingly, this effect was seen separately and in combination of AGPs. However, the effect was less prominent alone when compared in combination. Furthermore, the effect was more prominent with increase in concentration, that is higher the concentration of AGPs, lower the bacterial load. Furthermore, the data showed that TP is more effective than OXY at all three sites of intestine.

In addition, the effect of AGPs was also analysed for different durations such as days 14th, 28th and 42nd. Reduced number of bacteria were realized on day 42nd compared to days 14th and 28th respectively, which favor longer use of AGPs as growth promoter at sub-therapeutic level in diet. Previous studies have shown that major part of the nutrient or drug absorb in duodenum, which supports our results, that the high concentration of absorbed AGPs in the duodenum inhibits the growth of *E. coli* and less bacterial load was determined compared to Jejunum and Caecum respectively. The results showed that the supplementation of AGPs in the diet for a specific period and concentration reduces the harmful bacteria in the intestine of broiler chicken.

3.4. Effect of antibiotic growth promotors on Clostridium perfringens:

C. *perfringens* is a Gram-positive, spore forming, anaerobic commensal colonizing in the early phase of life in the intestinal tract of animals. The effect of AGPs was analysed for C. *perfringens* in the intestine of broiler chicken. Reduced growth of *C. perfringens* against the AGPs in the intestine showed significant effect (P<0.05) compared to control groups. Results showed reduced number of bacteria in the duodenum, which correlate with the previous studies that major part of the drug is absorbed in the duodenum, which ultimately inhibit the growth of *C. perfringens* (Table 4).

The growth response of *C. perfringens* was determined against AGPs solely and/or in combination. The individual effect of OXY and TP was not that significant on day 14th of treatment. Interestingly, this effect was not intensive, even with increase concentration of AGPs. However, significant (p<0.05) synergic effect was seen against the growth of *C. perfringens*. This effect became more prominent with increased concentration of AGPs and higher synergic effect was seen in duodenum and jejunum in a combination of Oxy 2.0 and TP1.0 followed by OXY1.0 and TP 1.0.

The individual effect of OXY and TP was higher on day 28th and this effect become prominent with increased concentration of AGPs. Remarkably, the AGPs showed a prominent effect at 42nd day of treatment and the significant effect was seen alone and in combination. Higher effect was seen by using OXY1.0 and TP 1.0 in duodenum, followed by Jejunum and Caecum respectively. Results showed that use of AGPs solely and/or in combination have higher effect at 42nd day of treatment and this effect was more prominent in duodenum compared to jejunum and caecum.

In conclusion, the supplementation of AGPs in feed of broiler chicken significantly decreases the bacterial load of pathogenic

Table 2. Effect of Oxytetracycline and Tylosin phosphate on *Lactobacillus reuteri* (log_{10} CFU/g) in Duodenum, Jejunum and Caeca at 14th, 28th, 42nd day.

Treatments	14th day			28th day			42nd day		
	Duodenum	Jejunum	Caeca	Duodenum	Jejunum	Caeca	Duodenum	Jejunum	Caeca
Control	8.48±0.02	7.45±0.10	7.27±0.08	8.71±0.10	7.66±0.12	7.85±0.17	8.92±0.03	8.13±0.14	8.21±0.21
OXY-1.0+TP-0	7.74±0.37	7.65±0.03	7.24±0.06	8.03±0.50	7.49±0.04	7.22±0.06	7.66±0.58	7.35±0.06	7.26±0.12
OXY-0+TP-0.5	8.78±0.01	7.75±0.04	7.63±0.10	8.64±0.01	7.57±0.05	7.34±0.03	8.51±0.02	7.46±0.04	7.19±0.02
OXY-1.0+TP-0.5	8.75±0.07	7.58±0.12	7.45±0.07	8.51±0.04	7.61±0.04	7.27±0.02	8.18±0.08	7.52±0.05	7.21±0.04
OXY-2.0+TP-0	8.68±0.06	7.69±0.05	7.68±0.14	8.56±0.06	7.54±0.05	7.42±0.08	8.44±0.07	7.48±0.07	7.25±0.01
OXY-0+TP-1.0	8.74±0.04	7.72±0.10	7.43±0.06	8.61±0.03	7.56±0.11	7.29±0.04	8.38±0.09	7.57±0.14	7.25±0.07
OXY-2.0+TP-1.0	8.65±0.04	7.59±0.12	7.50±0.04	8.48±0.01	7.53±0.05	7.32±0.06	8.19±0.04	7.53±0.11	7.38±0.07
OXY-2.0+TP-0.5	8.70±0.05	7.55±0.07	7.58±0.06	8.52±0.05	7.51±0.05	7.35±0.07	8.32±0.08	7.46±0.09	7.23±0.04
OXY-1.0+TP-1.0	8.72±0.05	7.57±0.03	7.50±0.09	8.46±0.06	7.54±0.01	7.31±0.07	8.22±0.06	7.39±0.05	7.30±0.07

Table 3. Effect of Oxytetracycline and Tylosin phosphate on *Escherichia coli* (log_{10} CFU/g) in Duodenum, Jejunum and Caeca at 14th, 28th, and 42ndday.

Treatments	14th day			28th day			42nd day		
	Duodenum	Jejunum	Caeca	Duodenum	Jejunum	Caeca	Duodenum	Jejunum	Caeca
Control	5.21±0.02	5.56±0.02	6.63±0.03	6.11±0.03	6.03±0.03	7.28±0.03	7.48±0.03	7.13±0.02	7.60±0.03
OXY-1.0+TP-0	4.50±0.07	5.28±0.02	6.31±0.02	3.99±0.23	5.19±0.03	6.13±0.04	4.77±0.04	4.92±0.03	5.79±0.02
OXY-0+TP-0.5	4.51±0.19	5.04±0.05	5.88±0.02	4.26±0.13	4.93±0.02	5.72±0.02	4.59±0.02	4.86±0.04	5.58±0.03
OXY-1.0+TP-0.5	3.48±0.27	4.72±0.02	4.82±0.03	3.35±0.16	4.68±0.04	4.73±0.02	3.31±0.13	4.34±0.03	4.34±0.57
OXY-2.0+TP-0	4.09±0.03	4.87±0.02	5.33±0.02	3.90±0.02	4.79±0.03	5.26±0.03	3.74±0.03	4.62±0.03	5.08±0.03
OXY-0+TP-1.0	4.27±0.02	4.88±0.02	5.47±0.03	3.89±0.04	4.76±0.03	5.37±0.03	3.81±0.03	4.53±0.03	4.88±0.04
OXY-2.0+TP-1.0	3.10±0.06	4.45±0.14	4.22±0.03	2.78±0.21	3.85±0.14	3.87±0.07	2.28±0.30	3.41±0.08	3.35±0.10
OXY-2.0+TP-0.5	3.23±0.17	4.53±0.03	4.49±0.14	3.15±0.10	4.37±0.03	4.15±0.04	2.74±0.19	4.11±0.05	3.38±0.11
OXY-1.0+TP-0.5	3.32±0.12	4.69±0.10	4.68±0.09	3.30±0.11	4.14±0.14	3.91±0.12	3.26±0.08	3.68±0.13	3.88±0.06

Table 4. Effect of Oxytetracycline and Tylosin phosphate on *Clostridium perfringens* (log_{10} CFU/g) in Duodenum, Jejunum and Caeca at 14th, 28th, 42ndday.

Treatments	14th day			28th day			42nd day		
	Duodenum	Jejunum	Caeca	Duodenum	Jejunum	Caeca	Duodenum	Jejunum	Caeca
Control	7.97±0.35	8.49±0.15	9.76±0.05	8.23±0.17	8.57±0.03	9.94±0.03	8.48±0.31	8.91±0.18	10.83±0.03
OXY-1.0+TP-0	7.08±0.61	7.72±0.20	9.07±0.30	6.66±0.29	7.30±0.35	8.55±0.50	6.05±0.02	6.69±0.09	8.45±0.14
OXY-0+TP-0.5	7.14±0.20	7.61±0.20	9.00±0.23	6.66±0.23	7.29±0.27	8.97±0.16	6.13±0.02	6.47±0.03	8.31±0.65
OXY-1.0+TP-0.5	6.25±0.44	6.54±0.49	7.93±0.24	5.27±0.22	6.28±0.30	7.40±0.60	4.84±0.33	5.93±0.33	6.48±0.02
OXY-2.0+TP-0	7.18±0.02	7.16±0.02	8.58±0.04	6.43±0.30	7.04±0.15	8.59±0.19	5.56±0.05	6.37±0.46	8.02±0.37
OXY-0+TP-1.0	7.19±0.48	7.06±0.02	8.84±0.03	6.10±0.18	7.10±0.04	8.63±0.20	5.78±0.06	6.26±0.25	7.82±0.38
OXY-2.0+TP-1.0	5.72±0.25	6.05±0.25	7.49±0.03	5.09±0.29	6.08±0.04	7.22±0.07	4.64±0.11	4.93±0.45	6.34±0.19
OXY-2.0+TP-0.5	5.89±0.24	6.26±0.25	7.67±0.03	5.19±0.29	6.18±0.04	7.34±0.06	4.65±0.11	5.24±0.30	6.35±0.19
OXY-1.0+TP-0.5	5.79±0.21	6.09±0.26	7.55±0.05	5.09±0.29	6.08±0.04	7.23±0.07	4.76±0.11	5.34±0.30	6.45±0.19

bacteria in the intestine. Interestingly, the supplementation of AGPs does not harm the beneficial bacteria that is *L. reuteri* in the intestine. The data favor the use of AGPs in the diet of broiler chicken which inhibit the growth of harmful bacteria and have no harmful effect on beneficial bacteria, which ultimately lead to promote the growth.

4. Discussion

The Antibiotic growth promoter (AGPs) supplemented in feed of broilers are capable to disturb the metabolism of microbes and can alter certain cellular and metabolic activities of bacterial cells which results in an impaired growth or kills bacteria. Including bacteriostatic and/or bactericidal effects, the AGPs supplemented in feed promote the growth performance of farm animals. The AGPs is involve to inhibit the growth of harmful bacteria in the gastro intestinal tract (GIT) of chicken (Yadav and Jha, 2019), that ultimately enhance the growth performance of broiler chicken. The improvement in growth is due to inhibit the pathogenic bacteria, reduction of microbial anti-metabolites and reduction of feed intake in the gut microbial loop and

enhance the uptake of nutrients due to histological changes in intestine (Brüssow, 2015; Shah et al., 2022).

Earlier studies showed that supplementation of AGPs didn't show a harmful effect on the beneficial bacteria that co-relate to our study showing that Oxy and TP has no harmful effect on the beneficial bacteria that is *L. reuteri* which favors our results that the use of Oxy and TP to promote the growth in poultry. Furthermore, the outcomes of our study were associated with the study of Hamid et al. (2019), which didn't affect the growth of beneficial bacteria. Our study revealed, reduced number of harmful bacteria in the intestine of broiler chicken compared to the control group. The significant ($P<0.05$) effect on pathogenic bacteria of *E. coli* and *C. perfringens* co-relate with previous studies showing significant effect at the microflora of pathogenic bacteria.

In our study we provided the AGPs for 6 weeks and the effect was analysed on days 14th, 28th, and 42nd. Interestingly the effect of AGPs against the pathogenic bacteria was observed at all three intervals according to group distribution. The study of Stutz and Lawton (1984) favor the current results that AGP treated groups had recorded lessen CFU count of *C. perfringens* as compared to the control group. Outcomes of this study is associated with the study of Manafi et al. (2018) that AGPs significantly reduces the *E. coli* and *Salmonella* in AGP supplemented groups at day 42nd. In addition, it was determined that the supplementation of OXY lower the bacterial count in duodenum and jejunum compared with the control group at day 35th (Saeid and Al-Alosi, 2018).

The dietary antibiotics promote the efficient growth of broiler chicken and benefits to the poultry industry and the consumer. Beneficial bacteria can protect the host from pathogenic bacteria by the different competitive mechanism including development of intestinal immune system (Yadav and Jha, 2019). The results of this study were in accord with the findings of Ashraf et al. (2019) that AGP supplemented groups of broiler chicken significantly decline the gut microflora than the control group at 35th day of experiment. It is suggested that group 4 (OXY-1.0+TP-0.5) is suitable combination for the broiler chickens and low dosage may cause lower side effect. The data favor the use of Oxytetracycline and Tylosin phosphate in the diet of broiler chicken which inhibit the growth of harmful bacteria and have no harmful effect on beneficial bacteria in the intestine. The AGPs are safe to use in poultry as growth promotor and for the prevention of diseases, while using within the normal range and for a specific period of time.

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The study was approved from the Research and Ethical Committee (REC) of the University of Balochistan vide letter No 293/CASVAB/UoB, dated March 25th 2021.

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