



# Effects of Antibiotics on Growth Performance, Immune Response, and Intestinal Microflora of Broilers

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## ABSTRACT

This study was conducted to evaluate the effects of flavomycin, lincomycin, and zinc bacitracin on body growth, morphometry of immune organs, Newcastle disease virus (NDV) and avian influenza virus (AIV) antibody response, intestinal microflora, and feed efficiency of broilers. Forty-five day-old broiler chickens were randomly divided into five treatments and housed under identical husbandry conditions. Antibiotic-free poultry feed was procured from a commercial feed mill. The antibiotics were mixed in the feed as per the manufacturer's instructions and offered *ad libitum* to the chickens for the entire study period. The chickens were vaccinated for NDV and AIV. None of the antibiotics adversely affected the development of NDV or AIV hemagglutination inhibition geometric mean titers. Flavomycin and zinc bacitracin did not adversely affect the mean splenic ( $1.27 \pm 0.20$  g and  $1.21 \pm 0.15$  g, respectively), thymic ( $3.42 \pm 0.26$  g and  $3.78 \pm 0.48$  g, respectively), hepatic ( $21.78 \pm 0.83$  g and  $23.15 \pm 0.37$  g, respectively), or bursal ( $1.55 \pm 0.79$  g and  $1.63 \pm 0.21$  g, respectively) body weight ratios. However, lincomycin did adversely affect bursal ( $0.91 \pm 0.12$  g), but not splenic ( $1.21 \pm 0.23$  g), thymic ( $3.52 \pm 0.36$  g), or hepatic ( $23.72 \pm 1.78$  g) body weight ratios. The total viable bacterial counts per gram of feces before and 120 h after medication were significantly different ( $p < 0.05$ ). Interestingly, the feed efficiency of non-medicated, non-vaccinated chickens was equal to the flavomycin-medicated chickens but better than zinc bacitracin and lincomycin-medicated chickens. Additionally, the non-medicated, non-vaccinated chickens were the most economical to raise. Overall growth-promoting antibiotics did not interfere with the broiler's immunity, altered total intestinal microflora counts, or improved feed efficiency.

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## Authors' Contribution

SA and SA conducted the experiments, analyzed data, and wrote the manuscript. MAM planned the study, as well as contributed to the write up. LA ran the statistical analyses. TNP planned the study and provided research supplies and reagents.

## Key words

Flavomycin, Immunity, Lincomycin, viable counts, Zinc bacitracin

## INTRODUCTION

Global consumption of animal proteins is expected to rise by 100% in the next 4 decades (Lillehoj and Lee, 2012). During the last 5 decades, a four-fold surge in poultry production has been recorded worldwide (Godfray *et al.*, 2010). In Pakistan, since the early 1960s, the poultry production is increasing around 10% per year despite infectious disease challenges. Infectious diseases greatly hinder production performances of chickens depending on morbidity and mortality (Akhtar, 1998), besides inflicting heavy economic losses to poultry producers

(Mustafa and Ali, 2005). Therefore, poultry producers in some developed and many developing countries including Pakistan routinely use various antibiotics to stimulate the growth performance of broilers, hereafter referred to as growth-promoting antibiotics (GPAs). The possible mechanism of action of GPAs is through modulation of the immune system and intestinal microflora of broilers leading to improved feed efficiency (Srivastava, 2010; Lillehoj and Lee, 2012). However, many reports suggest that antibiotic treatment may adversely affect the immune system's functions (Al-Ankari and Homeida, 1996). In contrast, some workers reported that feeding antibiotics to poultry could lead to faster maturation of some immune cells (Takahashi *et al.*, 2011). These conflicting reports on the effect of GPAs on chicken's immune organs (Al-Ankari and Homeida, 1996; Dafwang *et al.*, 1996), do not

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provide definitive guidance to the poultry producers.

Vaccination in broilers can cause stress (Hentges *et al.*, 1984) that ultimately negatively affects their growth performance (Landman, 2012). Stress also impairs the body's metabolism, which results in decreased body growth and skeletal muscle development in broilers (Klasing *et al.*, 1990; Klasing and Johnstone, 1991). The antibiotics such as Zinc bacitracin (ZB) are reported to reduce feed efficiency (Abdulkarim and Liebert, 1999). However, if GPAs are augmented with the treatment of probiotic preparations, feed conversion may improve (Abdulrahim *et al.*, 1999). Little or no changes in the composition of gut micro-flora (Corpet, 1999) or their number (Lattemann *et al.*, 1999; Kim *et al.*, 2000) with the use growth promoter antibiotics are reported. ZB treatment has not been reported to affect weight gain, feed consumption, or feed efficiency (Erdogan, 1999). Some antibiotics have been reported to increase antibody response of broilers (Brisin *et al.*, 2008), yet others are not (Zulkifli *et al.*, 2000). Conflicting findings have been reported on the effect of GPAs on feed intake, feed conversion ratio (FCR), or final body weight (Haque *et al.*, 2010). Flavomycin (FN) has been reported to improve the overall performance of chickens facing bacterial infections (Torok *et al.*, 2011). The effect of some antibiotics on growth may be masked under poor hygienic conditions (Srivastava, 2010). Because of the above controversial scientific knowledge, an evaluation of antibiotic's effect on broiler's growth and immune organs was undertaken. The effects of FN, Lincomycin (LN), and ZB on the performance, immune response, and intestinal microflora of broiler chickens were evaluated under experimental conditions.

## MATERIALS AND METHODS

Forty-five day-old broiler chickens were randomly divided into five treatment groups, each having 9 birds. These groups were named as T<sub>1</sub> (Non-medicated, non-vaccinated), T<sub>2</sub> (Non-medicated, vaccinated), T<sub>3</sub> (Flavomycin-medicated, vaccinated), T<sub>4</sub> (Lincomycin medicated, vaccinated), and T<sub>5</sub> (Zinc Bacitracin-medicated, vaccinated). Each group was reared on a littered floor, separately, under identical, standard husbandry conditions in an experimental room located at the Institute of Microbiology, University of Veterinary and Animal Sciences, Lahore (Pakistan). All experiments were conducted using standard ethical procedures in line with the institutional policies to minimize unnecessary pain and discomfort to birds. Feed and water were made available to the experimental chickens, *ad libitum*. At the end of the trial, the birds were humanely slaughtered using the halal method (Guerrero-Legarreta, 2010). The GPAs were

added in the feed as per the manufacturer's instructions: Flavomycin<sup>®</sup> 112.5 g/ton (Flavophospholipol 9 ppm) (Flavomycin<sup>®</sup> 80, Huvepharma); Licomix<sup>®</sup> 100 g /ton (Lincomycin 4.4 ppm) (Lincomix<sup>®</sup>, Pharmacia and Upjohn Company LLC—a subsidiary of Pfizer Inc.); Albac<sup>®</sup> 500 g / ton (Zinc Bacitacin 50 ppm) (Albac<sup>®</sup>, Alpharma, Inc.).

### *Vaccination and weight gain study*

To determine the effect of various treatments on body weight gains all experimental chickens were weighed at the beginning and end of study. The mean weights of the bursa of Fabricius, thymus, spleen, and liver of all chickens in various treatment groups were also recorded at the end of the experiment.

The chickens in different treatment groups except those in the T<sub>1</sub> were primed and boosted with NDV, LaSota strain (Merial) on day 5 and Muktaswar virus strain (Veterinary Research Institute, Lahore) on day 21 of their age, respectively. On day 9 of age, chickens in different groups, except those in T<sub>1</sub>, were inoculated with an inactivated AIV (H9) vaccine (M/S Avicenna Laboratory). All AIV vaccinated chickens were boosted with an oil-based AIV (H9) vaccine (M/S Avicenna Laboratory) on day 24 day of age. Routine vaccination against infectious bursal disease and hydropericardium syndrome was also administered to chickens except in T<sub>1</sub>.

### *Collection, processing, and analysis of serum samples*

Blood samples from each group were collected on days 1, 7, 14, 21, 28, 35, and 42. The sera were separated and used to determine hemagglutination inhibition (HI) titers. Before HI testing, the sera were heat-inactivated in a water bath at 56°C for 30 minutes. Phosphate buffered saline (PBS), pH of 7.2, was used as a diluent in hemagglutination (HA) and HI tests. The chicken red blood cells (RBCs) were washed with PBS and 1% RBCs suspension was used in HA and HI tests. According to the procedure described earlier, the HI test was conducted with already standardized NDV and AIV antigens using 96-well, U-bottom microtiter plates (Alexander and Chettle, 1977). The microtiter plates were agitated gently and incubated at ambient temperature (22-25 °C) for 20-30 minutes after which the results were recorded as previously described. The HA and HI titers were expressed as log<sub>2</sub> of the reciprocal of the highest dilution exhibiting HA or HI activity, respectively.

### *Intestinal microflora*

Fresh fecal samples from each treatment group were used to study intestinal microflora. The fecal samples from each treatment group were collected, before medication and at 4, 8, 12, 24, 48, 72 and 120 h post-medication.

One gram of the pooled fecal material was used for viable bacterial count using the pour plate method (Collins *et al.*, 2004). The bacterial colonies were counted using a colony counter. Colony-forming unit per gram (cfu/g) of feces was calculated by multiplying the average number of colonies per countable plate by the reciprocal of the dilution.

#### Feed conversion ratio

The FCR of chickens in each group was estimated at the end of the experiment. FCR was calculated as per the formula of Morgan and Lewis (1962).

#### Impact of antibiotic treatment on the economics of flock production

The economics of flock production for each treatment group was determined as per Oyekole (1984).

#### Statistical analysis

The data obtained was statistically analyzed using ANOVA and differences among the treatments were determined by Fisher's least significant difference (LSD) test using SPSS version 20 (IBM, Armonk, NY, USA).

## RESULTS

#### Body weight gain

This study indicated that the non-medicated, non-vaccinated chickens gained higher mean body weight (MBW) than the non-medicated, vaccinated or GPA-medicated, vaccinated chickens. On day 42 of their age, the highest MBW (1604.62±85.20 g) was recorded in chickens from group T<sub>1</sub> (non-medicated, non-vaccinated), and the lowest MBW (1375.75±44.58 g) was recorded in chickens of group T<sub>2</sub> (non-medicated, vaccinated). The MBW of chickens in group T<sub>1</sub> was significantly higher (P<0.05) than the MBW of chickens in group T<sub>2</sub> and T<sub>4</sub> (Table I).

#### Morphometric analysis of lymphoid organs

Dohms and Saif (1984) parameters were followed to study the effect of FN, LN, and ZB on the immune system of broiler chickens. Table I shows comparison of mean bursal, splenic, thymic, and liver weights of the chickens in various treatment groups.

#### Bursal body weight ratio (BBR)

The chickens from group T<sub>5</sub> (ZB-medicated, vaccinated) had the highest BBR (Mean BBR: 1.63±0.21 g), and those from group T<sub>2</sub> (non-medicated, vaccinated) had the lowest BBR (mean BBR 0.89±0.11 g). The mean BBR of chickens from group T<sub>5</sub> was significantly higher (P<0.05) than the mean BBR of chickens in T<sub>2</sub> and T<sub>4</sub>.

#### Thymus body weight ratio (TBR)

The chickens from group T<sub>1</sub> (non-medicated, non-vaccinated) had the highest mean TBR (mean TBR 4.00±0.50 g), and those from group T<sub>2</sub> (non-medicated, vaccinated) had the lowest mean TBR (mean TBR 2.83±0.38 g).

#### Spleen body weight ratio (SBR)

The chickens from T<sub>1</sub> (non-medicated, non-vaccinated group) had the highest mean SBR (mean SBR 1.57±0.15 g), and chickens from group T<sub>4</sub> (lincomycin-medicated, vaccinated) had the lowest mean SBR (mean SBR 1.21±0.23 g). However, there were no significant differences between the mean SBR of different treatment groups (P>0.05).

#### Liver body weight ratio (LBR)

The chickens from group T<sub>1</sub> (Non-medicated, non-vaccinated) had the highest mean LBR (mean LBR 24.54±1.03 g), and those from group T<sub>3</sub> (flavomycin-medicated, vaccinated) had the lowest mean LBR (mean LBR 21.78±0.83 g).

**Table I. Effect of antibiotics on the mean (Mean±SEM) body, bursa, thymus, spleen, and liver weights of chickens.**

Group	Body weight (g)		Bursal body weight ratio (g)	Thymic body weight ratio (g)	Splenic body weight ratio (g)	Liver body weight ratio (g)
	1 <sup>st</sup> Day	42 <sup>nd</sup> Day				
T <sub>1</sub>	39.50±0.94 <sup>AB</sup>	1604.62±85.20 <sup>AB</sup>	1.61 ± 0.08 <sup>B</sup>	4.00 ± 0.50 <sup>AB</sup>	1.57 ± 0.15 <sup>AB</sup>	24.54 ± 1.03 <sup>AB</sup>
T <sub>2</sub>	39.00 ± 0.70 <sup>B</sup>	1375.75 ± 44.58 <sup>C</sup>	0.89 ± 0.11 <sup>C</sup>	2.83 ± 0.38 <sup>C</sup>	1.26 ± 0.07 <sup>B</sup>	24.00 ± 1.71 <sup>B</sup>
T <sub>3</sub>	39.62 ± 0.77 <sup>B</sup>	1448.00 ± 54.07 <sup>BC</sup>	1.55 ± 0.79 <sup>B</sup>	3.42 ± 0.26 <sup>BC</sup>	1.27 ± 0.20 <sup>B</sup>	21.78 ± 0.83 <sup>B</sup>
T <sub>4</sub>	39.12 ± 0.66 <sup>B</sup>	1376.12 ± 33.26 <sup>C</sup>	0.91 ± 0.12 <sup>C</sup>	3.52 ± 0.36 <sup>BC</sup>	1.21 ± 0.23 <sup>B</sup>	23.72 ± 1.78 <sup>B</sup>
T <sub>5</sub>	39.25 ± 0.72 <sup>B</sup>	1460.62 ± 67.57 <sup>BC</sup>	1.63±0.21 <sup>AB</sup>	3.78 ± 0.48 <sup>BC</sup>	1.21 ± 0.15 <sup>B</sup>	23.15 ± 0.37 <sup>B</sup>

\*NDV and AIV vaccines, † Mean ± standard error, <sup>A,B,C</sup>: Any two means carrying the same superscript are not significantly different from each other (P>0.05).

### HI antibody analyses

#### Newcastle disease virus

The geometric mean (G) HI titers of chickens from Day 1 through Day 42 are depicted in Figure 1A. On Day 1, chicks in each treatment group had a G HI titer of 6.5 against NDV. On day 7, the G HI titers of chickens in treatment groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, and T<sub>5</sub> were 50.63, 45.01, 63.97, 58.68, and 72.77, respectively. On day 14, the highest G HI titers were observed in chickens from group T<sub>5</sub> and the lowest in chickens from group T<sub>1</sub>. On day 21, the highest G HI titer (24.67) was observed in group T<sub>4</sub>, and the lowest titer was observed in chickens from group T<sub>1</sub>. On day 28, the highest G HI titer (39.00) was noted in group T<sub>5</sub>, and the lowest titer was observed in chickens from group T<sub>1</sub>. On day 35, the highest G HI titer (42.65) was observed in group T<sub>5</sub>, and the lowest titer was observed in chickens from group T<sub>1</sub>. On day 42, the highest G HI titer (11.25) was observed in group T<sub>2</sub> while the lowest titer (1.41) was observed in chickens from T<sub>1</sub>.

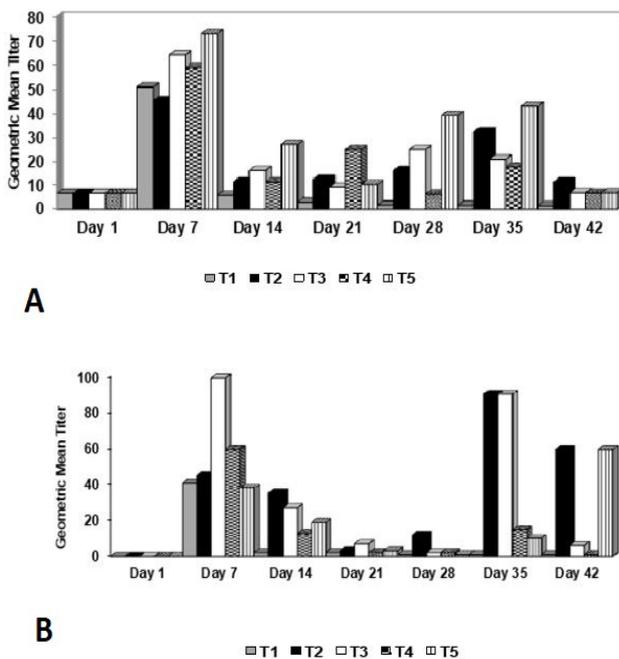


Fig. 1. Newcastle disease virus (A) and avian influenza virus (B) geometric mean HI titer in broilers.

T1, non medicated, NDV and AIV non vaccinated; T2, non medicated, NDV and AIV vaccinated; T3, flvomycin treated, NDV and AIV vaccinated; T4, lincomycin treated, NDV and AIV vaccinated; T5, zinc bacitracin treated, NDV and AIV vaccinated.

#### Avian influenza virus

The G HI titers for AIV are depicted in Figure 1B. The G HI AIV titers in one day old chickens in all

treatment groups were zero. On day 7, the highest G HI titer (99.00) was observed in the chickens from group T<sub>3</sub> (FN-medicated, vaccinated), while the lowest (38.00) in chickens from treatment group T<sub>5</sub>. On day 14, the highest G HI titer (35.00) was observed in the chickens from group T<sub>2</sub> (non-medicated, vaccinated), while the lowest (2.00) titers were observed in chickens from treatment group T<sub>1</sub>. On day 21, the highest G HI titer (7.00) was observed in the chickens from T<sub>3</sub> and lowest in the chickens from T<sub>1</sub> and T<sub>4</sub> groups. On day 28, the highest G HI titers (12.00) were observed in group T<sub>2</sub> while lowest in group T<sub>1</sub> and T<sub>5</sub>. On day 35, the highest G HI titer (90.00) was observed in group T<sub>2</sub> (non-medicated, vaccinated) and T<sub>3</sub> (FN-medicated, vaccinated) and the lowest (1.00) in group T<sub>1</sub> (non-medicated, non-vaccinated). On day 42, while the highest G HI titer (59.00) was observed in T<sub>2</sub>, and T<sub>5</sub>, the lowest titer was noted in T<sub>1</sub> and T<sub>4</sub>.

#### Total viable counts of intestinal microflora

Total viable counts (TVC) per gram of feces before and 4, 8, 12, 24, 48, 72, and 120 h after medication in various treatment groups are described in Table II. TVC per gram of feces in groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, and T<sub>5</sub> before medication were 14.88, 16.53, 15.32, 15.62, and 16.09 log<sub>10</sub>. The TVC 120 h after medication were 12.32, 6.69, 6.47, 6.47, and 6.73 log<sub>10</sub>, respectively. The statistical analysis revealed that overall effect of treatments is significant and the effect of time is highly significant (p<0.05). Pair-wise comparison of means of each treatment indicated the means of T<sub>1</sub> and T<sub>4</sub>, T<sub>2</sub> and T<sub>5</sub>, and T<sub>4</sub> and T<sub>5</sub> were significantly different (p<0.05). The effect of antibiotics on intestinal microflora was time-dependent. For example, the total microflora started to reduce 24 h after medication and this reduction was noted till the end of the experiment (p<0.05).

Table II. Bacteria (cfu Log<sub>10</sub>) in the fecal samples of broiler chickens before and after medication.

Group	Before medication	After commencing medication						
		4 h	8 h	12 h	24 h	48 h	72 h	120 h
T <sub>1</sub>	14.88	15.49	13.73	13.82	14.33	12.84	10.47	12.32
T <sub>2</sub>	16.53	12.29	13.73	11.36	13.74	13.68	9.30	6.69
T <sub>3</sub>	15.32	12.94	14.19	14.86	12.06	13.13	12.50	6.47
T <sub>4</sub>	15.62	12.43	12.93	13.23	12.71	10.48	10.74	6.47
T <sub>5</sub>	16.09	12.33	16.65	16.31	15.30	15.90	12.22	6.73

For details of treatment groups, see Figure 1.

#### Feed conversion ratio

The mean FCR for groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, and T<sub>5</sub> were as 2.25, 2.35, 2.24, 2.35, and 2.47, respectively (Table

III). The FCR study indicated that Flavomycin-medicated, vaccinated chickens had the best FCR (2.24). In contrast, ZB-medicated group had the poorest FCR (2.47) value.

**Table III. Feed Conversion ratio of chickens in various treatment groups.**

Group	Feed consumed (g)	Mean body weight (g)	Feed conversion ratio
T <sub>1</sub>	28248.30	12521.00	2.25
T <sub>2</sub>	25226.67	10694.00	2.35
T <sub>3</sub>	25283.92	11247.00	2.24
T <sub>4</sub>	25166.90	10696.00	2.35
T <sub>5</sub>	28100.00	11371.00	2.47

For details of treatment groups, see [Figure 1](#).

#### Economics of flock

Total profit and profit per bird in groups T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, and T<sub>5</sub> are shown in [Table IV](#). The highest profit Rs.132.85, was recorded in group T<sub>1</sub> (non-medicated and non-vaccinated). GPA-medicated, vaccinated groups demonstrated lower profit, with LN-medicated group at the lowest profit level.

## DISCUSSION

Antibiotics use for growth performance in animals has come under greater scrutiny in the recent years. Therefore, the present study was designed to evaluate the effect of antibiotics on the growth performance, immune response, and intestinal microflora of broilers. The study results revealed that none of the antibiotics adversely affected the development of antibodies to NDV or AIV. The total viable bacterial counts per gram of feces before and after medication were meaningfully different. Interestingly, the feed efficiency of non-medicated, non-vaccinated chickens was equal to the Flavomycin-medicated chickens but better than zinc bacitracin and lincomycin-medicated chickens. The economic analysis indicated that the non-medicated,

non-vaccinated group was the most cost-effective.

On day 42, the body weights analysis revealed that chickens fed on a non-medicated diet and without vaccination against NDV and AIV had significantly higher ( $P < 0.05$ ) body weights compared to vaccinated chickens with or without medication, suggestive of vaccination-related stress. Vaccination stress has already been well documented ([Samanta, 1992](#)). [Hentges et al. \(1984\)](#) have reported similar vaccination-related reactions in broilers: decreased protein synthesis rate, decreased final body weights, poor feed conversion ratios, and increased mortality rates. [Hentges et al. \(1984\)](#) has also reported maximum live weight gain by non-vaccinated chickens compared to vaccinated ones, consistent with our findings. One explanation for vaccination-related stress could be stimulation of the immune system in response to vaccination resulting in considerable metabolic changes that are antagonistic toward growth. For example, monokines released due to immune stimulation promote the utilization of dietary nutrients to support the immune response and disease resistance instead of skeletal muscle growth ([Klasing and Johnstone, 1991](#)). Characteristic metabolic alterations induced by monokines include decreased growth, impaired lipid utilization, decreased skeletal muscle protein synthesis, hepatic acute-phase protein synthesis, increased metabolic rate, and decreased feed intake ([Klasing et al., 1990](#)).

On day 42, bursal body weight ratio (BBR) of non-medicated, vaccinated and LN-medicated, vaccinated chickens were significantly lower than BBR of the chickens from non-medicated, non-vaccinated, FN-medicated, vaccinated and ZB-medicated, vaccinated groups. These data suggest LN-related decrease in BBR, consistent with [Al-Ankari and Homeida \(1996\)](#), who have reported a decrease in bursal weight due to antibiotic feeding. FN and ZB, however, did not adversely affect the morphometry of the chicken bursae, consistent with [Dafwang et al. \(1996\)](#), who reported that the bursal weights increased in GPA-fed chickens.

**Table IV. Economics of the medication studies.**

Group	No. of birds in the experiment		Total cost* (Pak. Rupees)	Total income (Pak. Rupees)	Total profit† (Pak. Rupees)	Profit per bird (Pak. Rupees)
	Beginning	End				
T <sub>1</sub>	9	8	443.15	576.00	132.85	16.60
T <sub>2</sub>	9	9	470.96	554.85	83.89	9.32
T <sub>3</sub>	9	9	471.86	583.20	111.34	12.37
T <sub>4</sub>	9	8	475.73	493.20	17.47	2.18
T <sub>5</sub>	9	8	474.24	525.60	51.36	6.42

\*, Total cost included cost of chicks, feed, vaccination, antibiotics etc. †, Total profit was calculated by multiplying the per kilogram rate of broiler meat on the day of sale. For details of treatment groups, see [Figure 1](#).

The mean thymus body weight ratio (TBR) of chickens from group T<sub>1</sub> was significantly higher ( $P < 0.05$ ) than the mean TBR of chickens in group T<sub>2</sub>. However, the differences in the mean TBR of chickens from T<sub>1</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> were not significant ( $P > 0.05$ ), suggesting that FN, LN, and ZB did not adversely affect the weight of the thymus. Our findings differ from those reported by [Al-Ankari and Homeida \(1996\)](#), who reported an antibiotic-related decrease in thymus size. This difference may be due to selective depression of thymus due to Oxytetracyclines used by these researchers.

This study indicated that FN, LN, and ZB did not have any adverse effects on the spleen body weight ratio (SBR) of chickens at 42 of their age, consistent with findings of [Al-Ankari and Homeida \(1996\)](#), who have reported that antibiotic-medication did not adversely affect spleen weight.

The differences amongst mean liver body weight ratio (LBR) of different treatment groups were non-significant ( $P > 0.05$ ), suggesting FN, LN or ZB did not interfere with the development of liver consistent with [Sarica \*et al.\* \(2005\)](#).

The mean HI titers of chickens for Newcastle disease virus from groups T<sub>3</sub>, T<sub>4</sub>, and T<sub>5</sub> were higher or comparable to the chickens in group T<sub>2</sub> throughout the experiment except for T<sub>4</sub> titers, which were lower than T<sub>2</sub> on day 28. Overall, these data indicate that the use of FN, LN, and ZB did not adversely affect the development of antibodies against NDV, consistent with [Landy \*et al.\* \(2011\)](#).

The mean HI titer of chickens for avian influenza virus from groups T<sub>3</sub>, T<sub>4</sub>, and T<sub>5</sub> were higher than or comparable to T<sub>2</sub> except on day 28 of their age when T<sub>2</sub> titer was higher. These data suggest that the use of FN, LN, and ZB did not adversely affect the development of antibodies against AIV, consistent with [Landy \*et al.\* \(2011\)](#).

The total viable counts per gram of feces before and after medication in treatment groups were different consistent with [Gunal \*et al.\* \(2006\)](#), who have reported a reduction in total bacterial counts due to antibiotic feeding. However, our findings differ with [Lattemann \*et al.\* \(1999\)](#), [Rakowska \*et al.\* \(1993\)](#), [Corpet \(1999\)](#), and [Kim \*et al.\* \(2000\)](#). These workers have observed little or no influence of feeding GPAs on the intestinal microflora of chickens. The decrease in TVC in the non-medicated, vaccinated group may be attributed to the stimulation of the innate immune system due to vaccination.

The present study's results indicating poorest feed efficiency by ZB-medicated chickens, differ from [Abdulrahim \*et al.\* \(1999\)](#), who have reported a drop in FCR or better feed efficiency of broilers with ZB compared to controls. On the other hand, [Erdogan \(1999\)](#) found no significant effect of ZB on feed efficiency. Interestingly

in our study, the FCR of non-medicated, non-vaccinated chickens was comparable to FN-medicated chickens, yet better than ZB and LN -medicated or non-medicated, vaccinated chickens. These findings are consistent with [Lin \*et al.\* \(1991\)](#), who reported that there was no significant difference in the feed conversion rates of groups fed on two different GPA (a mixture of LN and Spectinomycin) to that of the control group. However, our findings differ from those of [Rakowska \*et al.\* \(1993\)](#), who reported that the antibiotic treatment increased feed intake and body weight gain with improvements in feed utilization. This difference may be attributed to the use of a different antibiotic (Nisin) or rye-based diet by [Rakowska \*et al.\* \(1993\)](#).

The economic analysis indicated that GPAs could cause economic losses to poultry producers, consistent with [Graham \*et al.\* \(2007\)](#). The highest profit observed in non-medicated, non-vaccinated group can be attributed low cost of production: no costs incurred on medication and vaccination. Furthermore, this group had the highest mean live weight recorded on day 42, which added to this group's net profit. The profit per bird was also highest in this group. The lowest profit observed in the LN-medicated and vaccinated group was because of the higher cost of Lincomix<sup>®</sup> than other GPAs. The profit per bird in this group was the lowest.

Overall, this small-scale study has generated some interesting data on antibiotic use for broiler growth performance.

Ban on the use of GPAs in the European Union and growing consumer concern in North America on the use of GPAs in animal production warrants further investigation in search for and the use of alternatives to GPAs ([Selaleddi \*et al.\*, 2020](#)). These alternatives include but are not limited to exogenous enzymes, organic acids, herbs, and essential oils. Recent workers have claimed to obtain broiler performance similar to antibiotics from herbal extracts ([Petrolli \*et al.\*, 2012](#)). Further probiotics, prebiotics, and innate immune system stimulants aim at decreasing pathogen load in chickens ([Huyghebaert, \*et al.\*, 2011](#); [Nawab \*et al.\*, 2018](#)). A thorough understanding of the mode of action of GPA-alternatives capable of regulating chicken's immune system and intestinal microflora is likely to expedite their replacement for GPAs.

## CONCLUSIONS

In conclusion, this study suggested that dietary supplementation of FN, LN, and ZB at recommended dosage did not interfere with the development of the broiler's immune system and their effect on total intestinal microflora was time-dependent. Additionally, FN, LN, and ZB effect in improving feed efficiency was negligible,

suggesting GPAs contribute to poultry production's economic burden.

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### Statement of conflict of interest

The authors have declared no conflict of interest.

### Data availability

Data presented in this study will be available on a fair request to the corresponding author.

### Ethical approval

All experiments were conducted using standard ethical procedures in line with the institutional policies to minimize unnecessary pain and discomfort to birds.

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