

## Comparative Study of Probiotic, Acidifier, Antibiotic Growth Promoters and Prebiotic on Activity of Humoral Immune and Performance Parameters of Broiler Chickens

**Research Article**
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### ABSTRACT

The aims of this study were the comparative study of probiotic, acidifier, antibiotic growth promoters and prebiotic on activity of humoral immune and performance parameters of broiler chickens. 500 one day old male broilers (308 Ross strain) were divided into 5 groups: A, B, C, D and E. Each group with equal numbers of male included 4 replicates (25 chicks per replicate). Group A was as control and the other groups were administrated with distinct dose of drugs, comprised: Virginamycine 150 gr/ton, protexin 100 gr/ton, salkil 6 kg/ton, immunoval 1 kg/ton, respectively. 40 broilers were selected from each group randomly and performance parameters such as weight, mortality, amount of grain for consumption, feed conversion rate (FCR) were calculated by means of scale and reference formula. Moreover, at three time points (9<sup>th</sup>, 17<sup>th</sup> and 24<sup>th</sup> days) about 1 cc of blood was taken from broilers' brachial vein, within each of the five groups. Laboratory analysis conducted on blood samples featured Hemagglutination Inhibition (HI) test performed on sera and humoral immunity assessed as antibody production to Newcastle disease virus. The results showed that the group C and the group E were the best groups in terms of the performance parameters and the HI rate.

**KEY WORDS** acidifier, antibiotic growth promoters, broiler chickens, HI test, prebiotic, probiotic.

### INTRODUCTION

A great deal of research has been carried out to investigate the effect of antibiotic growth promoters in promoting performance parameters of broilers. Numerous studies have been conducted on antibiotic growth promoters such as Avilamycin, Virginiamycin, Lincomycin, Flavophospholipol, and Bacitracin (Bedford, 2000; Elwinger *et al.* 1998; Salminen *et al.* 1998). In general, the investigations have shown different reasons concerning the effect of antibiotic growth promoters. The reasons can be categorized briefly as follows:

1. Changes can occur in intestinal microflora, especially in gram positive bacteria because these bacteria absorb a lot of food energy. Some gram positive bacteria like Clostridiums have caused diseases such as necrotic enteritis (Bedford, 2000; Chen *et al.* 2005; George *et al.* 1982). These bacteria even destroy alimentary enzymes and reduce food digestion and absorption. Also, these bacteria have extended the length of intestine through producing volatile fatty acids and Polyamines.
2. The efficient microfloras of intestine may increase.
3. The membrane of intestine gets thinner and accordingly the food-intake increases.

4. Phagocytes are getting sensitive to bacteria.
5. The selection action occurred in a way that antibiotic growth promoters destroy deleterious and anaerobic bacteria (Milner and Roberford, 1999; Salminen *et al.* 1998; Savage *et al.* 1996). The findings of studies have revealed advantages and disadvantages of antibiotic growth promoters.

Controlling the replication of some microflora of intestinal pathogen, the growth of efficient microflora of intestine, the extensive activity against gram positive bacteria, the reduction of deleterious effects of metabolites of intestinal microflora by means of their destruction, the reduction in thickness of the mucous layer of intestine in order to increase food intake (the thickness of muscular layer of intestine membrane has increased in comparison with mucous layer), the reduction of turnover rate of enterocytes that results in reduction of body's energy, the reduction of immunology stress due to reduction of intestinal microflora, the competitive repression of microfloras of intestinal pathogen and promotion in absorption of nutritive foostuffs, the growth of efficient energy for production (by means of promoting AME in foodstuffs and reducing the essential energy for keeping and permanent endurance), the improvement of growth factors, the production of pathogen germs which have resistance against antibiotics whenever they are used in long-term periods, the prevention of colonizing efficient bacteria of intestine like lactobacillus and the reduction of non-specific immunity of mucous (Chen *et al.* 2005; Elwinger *et al.* 1998; Hofacre *et al.* 2003; Lemieux *et al.* 2003; Roberfoïd, 2000; Roberfroid, 1998; Salminen *et al.* 1998).

Probiotics have recently come into the market of poultry and are a compound of live microorganisms which promote natural intestinal microflora (for instance, Lactobacillus, Streptococcus, Bacillus, Bifidobacterium, Enterococcus, Aspergillus, Candida and Saccharomyces) and have a beneficial effect on broiler performance and immunomodulation (Lutful Kabir, 2009).

The findings of various studies have shown that the effect of probiotics can be mentioned as: turnover of efficient microflora in digestive system (Bello *et al.* 2001; Lemieux *et al.* 2003; Vegad, 2004); changes in bacteria metabolisms (Vegad, 2004; Zoppi, 1998); neutralization of enterotoxins (Vegad, 2004) and stimulation of immune system (Savage *et al.* 1996; Vegad, 2004).

Prebiotics belonging to the group of oligosaccharides are known as one of the most natural productions in promoting the immune level of body.

Mannan oligosaccharides (MOS) is one of the most important productions of this group. There are several studies on the effect of this substance on the immune system of poultry. In the case of prebiotics, Bailey *et al.* (1991) con-

ducted a study on the effect of Fructooligosaccharide on turnover of Salmonella in intestine mucous and mucous immune of intestine. Their findings indicated that these compounds were really effective in prohibiting turnover of deleterious bacteria like Salmonella (Bailey *et al.* 1991).

The mechanism of Mannan oligosaccharides can be summarized as follows: preventing from colonizing some microfloras of intestinal pathogen; reducing the deleterious effects of metabolites of intestinal microflora through changing the density of intestine microflora; extending the thickness of muscular layer of mucous and increasing the movements of intestine; decreasing the turnover rate of enterocytes and increasing the height of villi with respect to crypts; stimulating lymphoid tissue of intestine and immune system as a non-pathogenic antigen, promoting the health of brush boarder of intestine; the relative increase of goblet cells, the increase of mucous secretion, and colonizing the efficient bacteria which results in promoting non-specific immune of mucous (Bello *et al.* 2001; Chen *et al.* 2005; Elwinger *et al.* 1998; Savage *et al.* 1996; Vegad, 2004).

Since Immunoval (MOS) is one of the natural products of growth stimulating from the group of prebiotics, it has no medical leftover on poultry meat.

Also, with the consumption of poultry's meat by consumers no resistance on Immunoval (MOS) of other antibiotics is produced in individuals. Since June 1999 in Europe the consumption of most antibiotic growth promoters in poultry has been forbidden (because of antibiotic leftover on meat and also producing medicinal resistance on poultry and humans).

It seems that using natural compounds such as Immunoval (MOS) that has a high efficiency can be used as one of the best alternatives for antibiotic growth promoters (Bedford, 2000; Roberfoïd, 2000; Vegad, 2004; Zoppi, 1998).

Synbiotic (probiotic and prebiotic) have been determined to be antimicrobial, anticarcinogenic, antiallergenic and a stimulating factor of immunity system. They also are reasons for absorption of minerals and prevention of diarrhea and optimization of nutrients' digestion, however, synbiotics mechanism of act is generally unknown (Salminen *et al.* 1998).

These compounds improve and increase immunity level and production factors of broiler chickens. Using these substances in poultries' diet provide consumers with healthy meat without drug residues (Bedford, 2000). In fact, acidifiers are composed of organic acids with anti-bacterium property and pH regulation in intestine that contain acetic acid, propionic acid, phosphoric acid, citric acid, lactic acid, formic acid, fusaric acid and salts of each acid. Indeed, acidifiers are synthetic compounds between organic acids and their salts.

The main advantages of acidifiers can be mentioned as: pH regulation of intestine and microflora balance; the increase of digestive enzymes of intestine for extending food digestion; the increase in absorption of minerals in optimum pH; the increase of palatability of food, and the increase of minerals consumption for poultry.

The aims of this study were the comparative study of probiotic, acidifier, antibiotic growth promoters and Prebiotic on humoral immunity and performance parameters of broiler chickens.

## MATERIALS AND METHODS

500 one day old male broilers (308 Ross strain) were divided into 5 groups: A, B, C, D and E. Each group with equal numbers of male included 4 replicates (25 chicks per replicate).

The group A was taken as the control birds and the groups B, C, D, and E were considered as the experimental birds. From the beginning of experimental period till its end, virginiamycin (150 gr/ton) was added to the ration of group B as antibiotic growth promoters, protexin was added to the ration of group C as probiotics with the average dose of 100 gr/ton, salkil (6 kg/ton) was added to the ration of group D as an acidifier, and immunoval (1 kg/ton) was added to the ration of group E as prebiotics, whereas the group A was deprived of any growth stimulating substance (Bailey *et al.* 1991; Chen *et al.* 2005; Elwinger *et al.* 1998; Roberfroid, 1998; Salminen *et al.* 1998).

Vaccination programs were the same for all groups. B1 Newcastle vaccine was used in all groups of broilers by intramuscular injection (IM) and drinking water route at 10 days of age. Indeed, this vaccine was used in all groups of broilers by drinking water route at 25 and 35 days of age. In order to investigate the performance parameters such as weight, mortality, amount of grain for consumption, Feed Conversion Rate (FCR) per group, and 40 broilers were randomly chosen weekly and then intended performance factors were calculated by means of scale and reference formula for measuring feed conversion rate (FCR). The data gathering tools as regards performance factors were scale which is used to measure the weight of groups, the lowest weight of groups, the highest weight of groups, and the amount of consumption of grains. Later, the factors were calculated based on the mathematical formula and the mortality rate was determined by counting dead birds (Macfarlane and Cummings, 1999; Roberfroid, 1998; Salminen *et al.* 1998). For doing Hemagglutination Inhibition test (HI) for investigation of hummural immune (that is, the antibody rate of serum), the vaccine of antigen strains B1 Newcastle was used in distinctive number of broilers in 5 groups of broilers.

In each of the five groups, about 1 cc of blood was taken from broilers' brachial vein. In the first time, blood was taken on the 9<sup>th</sup> day of experimental period, in the second time on the 17<sup>th</sup> day of rearing period (one week after B1 Newcastle vaccine) and the third time on the 24<sup>th</sup> day of rearing period (two weeks after B1 Newcastle vaccine).

Blood samples were taken into laboratory for analysis on each of the three times. HI test was performed on sera and humoral immunity was assessed as antibody production to Newcastle disease virus.

The current research was based on an experimental study and its population was comprised of male broiler chicks (Ross 308 strain). It was calculated with the square test of 0.90 ( $\beta=0.10$ ), the confidence level of 0.95 ( $\alpha=0.05$ ), the least coefficient of 0.2 (these criteria are in line with similar studies).

This study consisted of 5 groups and 100 chicks per group (in total 500 chicks of one day). The broiler chick was considered as the unit of sampling and it was based on random sampling. The obtained results of performance parameters were calculated with one way analysis of variance (one way Anova) and SPSS version 12 was used to statistically compare findings.

In order to explore the performance parameters, 40 broilers were chosen from each group per week and then intended performance parameters were calculated in the way that carcasses were calculated weekly.

The weight of chicks and the amount of their consumption of grains were measured by scale and were recorded at the end of every week. The live broilers were calculated in percentage and were determined by subtracting the dead broilers from the live ones (Milner and Roberforid, 1999; Macfarlane and Cummings, 1999; Patterson and Burkholder, 2003; Roberfroid, 1998; Salminen, 1998).

## RESULTS AND DISCUSSION

The average cumulative weight of different groups was compared weekly from the first week to the sixth one. The Tukey and Duncan test was employed in order to find out whether the differences among groups were significant or not.

The similar letters written for groups mean that there were no significant differences among them, while different letters at the above of columns indicated that there were significant differences among them ( $P<0.05$ ). According to our findings in this research, the groups C and E had the highest average cumulative weight in all weeks. Also, these groups had significant differences in comparison with the control group (A) and other groups; in addition, at the end of sixth week of rearing period, these groups had the highest weight as compared to other groups.

Although broilers of the group C had lower weight at the end of first week as compared to the group E, they had higher weight at the end of six weeks as compared to broilers of the group E. Of course, statistically speaking, the differences between two groups in most weeks and at the end of sixth week were not significant.

The feed conversion rate of different groups was compared weekly from the first week to the sixth one. In these comparisons, the Tukey and Duncan test was employed in order to find out whether the differences among groups were significant or not.

According to our findings in this research, the groups C and E had the lowest conversion rate. Also, they had significant differences in comparison with the control group (A) and other groups, that is, the groups C and E were the best experimental groups due to low conversion rate. It is important to note that feed conversion rate increases when the age of broilers increases and the rate of increase in the control group (A) was higher than that of other groups. However, the lowest trend of increase was observed in the groups of C and E. In general, the group C and the group E were the best groups in terms of the Feed Conversion Rate (FCR) and final weight (Table 1).

**Table 1** Effects of different supplements on performance parameters of broiler chickens

TRT	ADG	FI	FCR
A	49.56 <sup>d</sup>	103.57 <sup>a</sup>	2.18 <sup>a</sup>
B	54.34 <sup>b</sup>	100.24 <sup>bc</sup>	1.89 <sup>bc</sup>
C	56.96 <sup>a</sup>	101.90 <sup>b</sup>	1.79 <sup>bc</sup>
D	52.10 <sup>c</sup>	102.02 <sup>ab</sup>	1.93 <sup>b</sup>
E	55.70 <sup>ab</sup>	99.02 <sup>c</sup>	1.75 <sup>c</sup>
SEM	0.51	0.85	0.05

The means within the same column with at least one common letter, do not have significant difference ( $P>0.01$ ).

TRT: treatment; ADG: average daily gain; FI: feed intake and FCR: feed conversion rate.

SEM: standard error of mean.

These results are in agreement with the findings of [Lutful Kabir \(2009\)](#) who reported that the use of probiotics in broilers diet have a beneficial effect on broiler performance ([Lutful Kabir, 2009](#)). The lowest mortality rate and the highest mortality rate were observed in the group B and the control group, respectively. Statistically speaking, there were no significant differences between the group C and the group B in terms of mortality rate ( $P<0.05$ ). The findings of this research indicated that the groups C and E, in exception of the first time, had the highest amount in other times of measurement antibody production to Newcastle disease virus and were the best groups in term of the HI rate (Table 2).

These results are in agreement with the findings of [Panda et al. \(2000\)](#) and [Cotter et al. \(2000\)](#) who reported that the use of probiotic and prebiotic in broiler chick's diet improved the immune response significantly ([Cotter et al.](#)

[2000; Panda et al. 2000](#)). It has been proved that Immunoval (MOS) and Fructooligosaccharides increase immunity level of broiler chickens and increase activity induction of macrophages (as antigen presenting cells) ([Hofacre et al. 2003](#)).

**Table 2** Effects of different supplements on humeral immunity and mortality ratio in broiler chicks

Groups	HI (1)	HI (2)	HI (3)	Mortality
A	2.02	3.62 <sup>d</sup>	4.04 <sup>b</sup>	0.16 <sup>a</sup>
B	2.03	3.98 <sup>bc</sup>	4.2 <sup>b</sup>	0.01 <sup>c</sup>
C	2.08	4.23 <sup>ab</sup>	4.58 <sup>a</sup>	0.05 <sup>bc</sup>
D	2.05	3.89 <sup>cd</sup>	4.14 <sup>b</sup>	0.12 <sup>ab</sup>
E	1.98	4.45 <sup>a</sup>	4.86 <sup>a</sup>	0.09 <sup>ab</sup>
SEM	0.04	0.09	0.11	0.025

The means within the same column with at least one common letter, do not have significant difference ( $P>0.01$ ).

HI (1), HI (2) and HI (3) are hemagglutination inhibition test in three times (9<sup>th</sup>, 17<sup>th</sup> and 24<sup>th</sup> days) of experimental period respectively.

SEM: standard error of mean.

Also prebiotics inhibit joining pathogenic bacteria to intestinal mucosa and creating acidic environment in intestine ([Chen et al. 2005](#)). Other important mechanisms which can be used in order to improve the immune level and the intestinal microfloras are changing acidity of intestine through increasing concentration of lactic acid in intestine and reducing activity of deleterious bacteria of intestine (*E. Coli*, Salmonella, and Clostridium) and increasing activity of lactobacillus. According to [Savage et al. \(1996\)](#) studies, it was found that the rate of both Bile IgA that comes into intestine from bile duct and also the rate of plasma IgA have increased ([Savage et al. 1996](#)).

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