Effect of Prebiotics, Probiotics, Acidfire, Growth Promoter Antibiotics and Synbiotic on Humoral Immunity of Broiler Chickens

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Abstract: 240 one-day Ross 308 chickens divided into 6 groups A, B, C, D, E, F. Group B received 150 gr/ton Virginamycin as growth-promoter antibiotic, group C received Protexin with average dose of 100 gr/ton as probiotic, group D received 6 kg/ton Salkyn as Acidifier, group E received 3 Kg/ton A-Max as Pre-biotic and group F received 1 kg/ton Synbiotic while group A received no growth-promoter. Each group included 40 chickens and the experiment conducted with four replications for each 10 chicken in each group. On 9th, 24th and 34th day of aviculture 20 chicken selected randomly from each group and their blood sample were taken to laboratory to conduct HI test (Prevention from Hemangontinasion) and obtained humoral immunity level recorded and studied: Obtained results analyzed by statistical tests and observed that using growth-promoters not only increase humoral immunity but they improve production factors (P>0.05).

Keywords: Prebiotic • Acidifier • Probiotic • Growth-Promoter Antibiotics • Synbiotic • Broiler Chicken • HI Test

INTRODUCTION

The most important natural growth promoter which have been just entered into market are prebiotics, probiotics, acidifiers and synbiotics. Prebiotics have been reported to improve gastrointestinal tract balance through bacterial antagonisms, competitive exclusion and immune stimulation [1]. Synbiotic (probiotic plus prebiotic) may improve the survival rate of probiotics during their passage through the digestive tract, thus contributing to the stabilization and/or potentiation of the probiotic effects [2]. These compounds improve and increase immunity level and production factors of broiler chickens. Using these substances in poultries’ diet provide consumers with health meat without drug residues [3-5]. The currently used feed additives such as antibiotics, probiotics and prebiotics in broiler diets to enhance nutrient utilization by mean of diverse mechanisms [6]. Effect of growth-promoter antibiotics has been studied and surveyed in different articles considering improvement of immunity level and production factors in broiler chickens [7-9]. Several studies have been also conducted on growth-promoter antibiotics such as Avilamycin, Virginamycin, Lincomycin, Flavophospholipol and Bacitracin [4, 10-14]. A huge amount of antibiotics have been used in small doses to control diseases and improve performances in livestock [15]. Prebiotics which are included in category of Oligosaccharides, are one of the most important natural production which improve body immunity level. The most important production from this category is Mannan Oligosaccharides which many studies have been conducted about the influence of this substance on immunity level of poultry. studied about influence of fructo-oligosaccharides (Prebiotics) on locating Salmonella in intestinal mucosa and on immunity of intestinal mucosa and obtained results represent efficiency of these components to inhibit locating harmful bacteria such as Salmonella [7]. Also, studies conducted by on new born piglets represent positive effect of Mannan Oligosaccharides on their growth [16]. Due to growing concerns about antibiotic resistance and the potential for a ban for antibiotic growth promoters in many countries, there is an increasing interest in finding alternatives to antibiotics in poultry production [17]. conducted studies about positive effect of Mannan Oligosaccharides on Turkey’s immunoglobin [18]. Mechanism of act for growth-promoter antibiotics could
be briefly represented as follow: To inhibit increasing some microflora of intestinal pathogens, increasing useful intestinal microflora, wide activity against positive gram bacteria, decreasing harmful effect of metabolites of intestinal microflora through removing them, decreasing thickness of intestinal mucosa layer to increase food absorption (thickness of muscular layer of intestine’s wall increases in compare with intestinal mucosa layer) [7]. Increasing turnover of Enterocytes and as a result decreasing body’s energy, decreasing immunologic stress through decreasing intestinal microflora’s load, competitive suppression of microflora of intestinal pathogen and developing food absorption, increasing efficiency of energy toward production (through improving foods’ AME and decreasing require energy for maintenance and durability), developing growth factors in different environmental condition, creating pathogen germs resistant against antibiotics if consumes in long-term and preventing colonization of useful intestinal bacteria such as Lactobacillus and decreasing non-specialized protection of mucosal surface [5, 7, 19].

However, using antibiotics in food 1) develops resistant bacteria against drugs 2) remains drug in birds’ body and 3) leads to normal bacteria imbalance [8]. Prebiotics mechanism of act is briefly as follow: They are one of immunity stimulus, increasing resistance against infectious diseases, sedation of lactose intolerance, reduction in cholesterol and blood pressure, producing vitamins of group B, increasing calcium and absorption of magnesium, prevention from connecting and colonizing some of intestinal bacteria and increasing nutrients absorption, developing growth-factors specially when they are polluted by intestinal pathogens and relatively increase of goblet cells and increasing mucus secretion [20, 9]. Prebiotic is a microbial production consumed lively and directly and creates equilibrium in population of intestinal bacteria and preventing from digestive infection, developing animal’s performance and increasing growth of livestock and birds. Determining consuming dosage and their system of performance is difficult and unlike antibiotics, probiotics remains no residues in livestock and birds and they do not create microbial resistance. Microorganisms added as probiotic to birds’ diet, are usually microbes of digestive system. Some of these microorganisms are Lactobacillus, Streptococcus, Probiobacteria, bifidobacteria and some Bacillus (such as Lactobacillus) [7]. Many scientific researches have been conducted to find the most effective complementary of probiotics and prebiotics so that they become applied in compounds to obtain symbiotic activity. Symbiotics are determined through anti-microbes, Anti Carcinogenic, anti-allergy and stimulating factor of immunity system. They also are reasons for absorption of minerals and prevent from diarrhea and optimize process of nutrients’ digestion, however, synbiotics’ mechanism of act is generally unknown [21-24, 9].

Acidifiers are, in fact, compounds of organic acids with antimicrobial nature and they are pH control agents in intestines including acid-lactic, acid-acetic, acid-propionic, acid-citric, acid-formic, acid-fumaric and salt of any acids. In fact, it is synthetic compound between organic acids and their salts. Advantages of using acidifiers are: 1) Regulating intestinal pH and microflora’s balance, 2) Increasing activity of intestinal digestive enzymes in order to increase nutrients’ digestion, 3) Increasing minerals absorption in delight PH condition, 4) Increasing palatability 5) Increasing and improving usage of minerals for bird [25].

MATERIALS AND METHODS

In this study, 240 broiler Ross308 chickens which were negative in Mycoplasma Septicom, Cynovieh and Salmonellapullorum and: Salmonella gallinarum (Negativity was certified by technical manager of the incubation factory) divided into 6 similar groups each containing 40 chickens. The experiment conducted with 4 replications per each 10 chicken in each group. The 6 groups were introduced by Latin alphabets A, B, C, D, E and F. Experimental group B received 150 gr/ton Virginiamycin (as growth-promoter antibiotic), group C received an average dose of Protexin amounted 100 gr/ton (as probiotic), group D received 6 kg/ton Salkyn (as acidifier), group E received 3 Kg/ton Symbiotic (as probiotic) and group F received 1 Kg/ton symbiotic while group A had no growth-promoter and fed just by basic diet. All condition for aviculture (a salon with tube ventilation in complete similar pans and simple bed of chaff with 7 cm depth disinfected previously by Formaldehyde gas and bell-type drinkers and cylindrical dankhori with thermal system of heater), environmental condition (a temperature of 30°C when entering chickens and 70% humidity) and vaccination plan (H120 bronchitis spray form vaccination on 1st day of birth, inactivated double oil influenza vaccination-N2 and H- and Newcastle B1 vaccination injecting in breast’s muscle and Newcastle B1vaccination as eye-drop on 10th day and D78 Gambro as drinking vaccination on 16th day) were completely similar and diet given to all six groups in starter period had 2900 kcal/kg energy and 21.26% protein, in grower period

Table 1: First Material and Chemical Compounds of Basic Diet in Each Six Groups (2)

<table>
<thead>
<tr>
<th>Type of diet</th>
<th>Type of Nutrient</th>
<th>Starter</th>
<th>Grower</th>
<th>Finisher</th>
<th>Type of diet</th>
<th>Type of Nutrient</th>
<th>Starter</th>
<th>Grower</th>
<th>Finisher</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Grain 0-14</td>
<td>Grain 15-30</td>
<td>Grain 31-42</td>
<td></td>
<td></td>
<td>Grain 0-14</td>
<td>Grain 15-30</td>
<td>Grain 31-42</td>
</tr>
<tr>
<td>First Materials%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>Compound %</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Corn</td>
<td>48.9</td>
<td>52.9</td>
<td>55.45</td>
<td>Metabolizable Energy Kcal/kg</td>
<td>2900</td>
<td>2985</td>
<td>3095</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat</td>
<td>9.4</td>
<td>10.2</td>
<td>11.1</td>
<td>Raw Protein</td>
<td>21.26</td>
<td>20.2</td>
<td>19.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soya</td>
<td>32.2</td>
<td>28.2</td>
<td>24.2</td>
<td>Digestible Protein</td>
<td>16.9</td>
<td>16.1</td>
<td>15.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish Meal</td>
<td>2.8</td>
<td>1.8</td>
<td>1.5</td>
<td>Raw Fiber</td>
<td>3.6</td>
<td>4.9</td>
<td>5.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat Powder</td>
<td>2.4</td>
<td>3.2</td>
<td>4.1</td>
<td>Raw Fat</td>
<td>4.5</td>
<td>5.4</td>
<td>6.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>0.2</td>
<td>0.15</td>
<td>0.22</td>
<td>Methionine</td>
<td>0.5</td>
<td>0.42</td>
<td>0.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>0.07</td>
<td>0.06</td>
<td>0.07</td>
<td>Methionine + Sitsein</td>
<td>0.91</td>
<td>0.82</td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salt</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>Lysine</td>
<td>1.4</td>
<td>1.2</td>
<td>0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Di Calcium Phosphate</td>
<td>1.53</td>
<td>1.3</td>
<td>1.2</td>
<td>Available Phosphor</td>
<td>0.6</td>
<td>0.6</td>
<td>0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shell Powder</td>
<td>1.28</td>
<td>1.4</td>
<td>1.32</td>
<td>Calcium</td>
<td>1.1</td>
<td>0.9</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complementary</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salinomycin</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

had 2985 kcal/kg energy and 20.2% protein and in finisher period had 3095 kcal/kg and 19.2% protein. Basic ration with all particulars could be seen in Table 1.

To survey humoral immunity and describing them in 3 times, (each time 20 birds were selected randomly from each group) blood samples were taken. The first stage of taking blood sample was on 9th day of aviculture. The second stage of taking blood sample was on 24th day of aviculture and the third stage of taking blood sample was on 34th day of aviculture. All blood samples were send to laboratory to conduct test HI (Prevention from Hemaglutination) and obtained level of humoral immunity were recorded and surveyed. Results obtained from all six groups evaluate through software SPSS by Variance Analysis tests ANOVA, multi-rang Duncan test and LSD [8, 14, 26-30].

RESULTS

Results obtained (Table 2) from the first HI test on 9th day of aviculture did not show any statistical differences between groups (P>0.05) and it represented that maternal titers of all six groups provided from one mother flock are likely the same. 2.08 ± 0.23 in group A, 2.03±0.23 in group B (Virgiamycin), 2.03±0.23 in group C (Protexin), 2.03 ± 0.23 in group D, (Salkyn), 2.03±0.23 in group E (A-max), 2.03± 0.23 in group F (Symbiotic) showed that there are not any statistical differences among groups (P>0.05) but results obtained from the second HI test in 24th day (5.01±0.34 in group A, 5.27±0.34 in group B, 5.13 ± 0.34 in group C, 5.28 ± 0.34 in group D, 5.31 ± 0.34 in group E and 5.23±0.34 in group F) showed that there is not any statistical differences among groups (P<0.05) but there was a relative increase in antibody in each five experimental groups in compare with control group. This increase in antibody was more in group E. Results obtained from the third HI test in 34th day (5.04±0.44 in group A, 5.87±0.44 in group B, 5.61±0.44 in group C, 5.9 ± 0.44 in group D, 5.98 ± 0.44 in group E and 5.8 ± 0.44 in group F) represented a significant difference (P<0.05) between five experimental groups with Control groups but there was not any statistical differences among five experimental groups, although antibody in group E showed 0.11 Log increase in compare with group B, 0.37 Log increase in compare with group C, 0.08 Log increase in compare with group D and 0.18 Log increase in compare with group F. Mean, standard deviation and P value for each 4 groups obtained by Variance Analysis Test ANOVA, multi-rang Duncan test and LSD, are represented in Table 2.

Table 2: Mean, Standard Deviation and Comparison of statistical differences of obtained standards from 6 groups (Control, Virgiamycin, Protexin, Salkyn, A-Max and Symbiotic)

<table>
<thead>
<tr>
<th>Age by day</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>2.08±0.23</td>
<td>2.03±0.23</td>
<td>2.03±0.23</td>
<td>2.03±0.23</td>
<td>2.03±0.23</td>
<td>2.03±0.23</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>24</td>
<td>5.01±0.34</td>
<td>5.27±0.34</td>
<td>5.13±0.34</td>
<td>5.28±0.34</td>
<td>5.31±0.34</td>
<td>5.23±0.34</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>34</td>
<td>5.04±0.44</td>
<td>5.87±0.44</td>
<td>5.61±0.44</td>
<td>5.9±0.44</td>
<td>5.98±0.44</td>
<td>5.8±0.44</td>
<td>P&gt;0.05</td>
</tr>
</tbody>
</table>

☐ and ☐: Means with different alphabetic exponent have difference with each other (P<0.05)
DISCUSSION AND CONCLUSION

Applying each of these compounds, it means Virginiamycin, Protexin, Salkyn, A-Max and Symbiotic could improve level of immunity response in broiler chickens [27]. These compounds which are in markets in commercial forms, could be likely used to develop humoral immunity system and increasing chickens’ resistance against infectious and diseases. On the other hand high and active immunity system could make vaccination plan successful which finally lead to developing production factors that is final goal of aviculture industry. Research conducted by Mcfarlance and Cummings [10-28] about performance method of prebiotics on intestinal microflora’s performance showed that these compounds prevent from intestinal cells turnover and occupation of intestinal cells by pathogenic bacteria [27]. Mannan Oligosaccharides are kinds of prebiotics which are formed a sugar unit called Mannose. To cause diseases in digestive system, most bacteria should be first joint by surface of intestinal epithelial cells. They do this through lectins. These lectins exist on fimbriae of bacteria type I. Mannan Oligosaccharides existed in useful intestinal bacteria which are increased due to consumption of prebiotic (For example A-Max), occupy junctions of lectin on fimbriae type I and prevent from connecting pathogenic bacteria to intestinal wall. it was showed that prebiotics are effective to inhibit and control bacteria such as Clostridium perfringens which do not have any dependency on lectins sensitive to Mannose for intestinal joint. Other mechanism to improve immunity and intestinal microflora is changing intestine acidity through increasing density of lactic acid in intestine and decreasing activity of intestinal harmful bacteria (Escherichia coli, Salmonella, Clostridium) and increasing activity of lactobacillus. Some studies conducted on direct influence of Mannan Oligosaccharides (Prebiotic) on immunity level [11, 14-29]. It has approved that immunowall (Mos) and Fructo-Oligosaccharides increase immunity of chickens and increase activity induction of macrophages (as the most important cell supplying antigen in poultries) weather directly or indirectly [28, 14, 18-30]. Immunowall induce activities of macrophages through occupying Mannose specific receptors. When 1/3 or more of these receptors occupied, macrophages become more active and more ready to remove antigenic bacteria and create more proper immunity reaction. In addition presenting antigens by macrophage to antibody-producer cells increases. Immunowall (Mos) not only develop immunity response but increase the similarity in immunity response [9]. In fact results obtained by this study and other studies about increasing immunity levels show that prebiotics could improve immunity system of broiler chickens through direct influence on macrophage and inhibiting from joining pathogenic bacteria to intestinal mucosa and creating an acidic environment in intestine. Also arranging intestinal microflora, increasing nutrient absorption, improving production factors such as feed conversion coefficient and improvement of responses to vaccination plans are other advantages of these compounds based on conducted researches [7-12]. on piglets, develop in growth were observed. A-Max has a proper price and more important that it is a natural growth promoter product and is categorized on probiotics group. It does not remain in poultries’ meat, therefore no resistance against antibiotics create in consumers. As using most of growth-promoter antibiotics were forbidden (due to remaining on consuming meat and creation medicine resistance in human and poultries), it considers that natural compounds such as A-Max have either more efficiency or they are one of the best alternative compounds for growth-promoter antibiotics. This is also important to say that compounds such as A-Max are completely health or Green biologically because it is natural [3, 11, 114, 18, 21, 28, 30]. using Mannan Oligosaccharides in turkeys, it was observed that amount of biliary LgA which entered to intestine from bilious ducts and also amount of plasma LgA increased [13, 31]. Many scientific researches conducted to find the most effective complementary of prebiotic and probiotics and symbiotic activities obtain due to their application in compounds. There are many discussion about symbiotic but their mechanism of act is not completely known and there are a few articles about the effectiveness of this product in the world. Conclusions obtained by this study represent that symbiotic is a stimulating factor for immunity system. It should briefly stated that growth-promoters improve immunity system but as acidifiers, prebiotics, probiotics and symbiotic are natural growth-promoter products, remain no drug residue in birds’ meat and no resistance against A-Max, Protexin, Symbiotic and other antibiotics create due to birds meat consumption. Regarding that from January 1999, using most of growth-promoter antibiotics were forbidden for birds (due to remaining antibiotic in consuming birds ‘meat and also creating drug-resistance in birds and human). It considers that natural compounds such as A-Max and symbiotic which have higher efficiency in compare with other natural growth-promoters, could be one of the best replacing compounds for growth-promoter antibiotics.
This is also necessary that compounds such as A-max, Protexin, Symbiotic and Salkyn are completely healthy or green biologically as they are natural [4, 12, 18, 21, 25, 29].

REFERENCES


