Effects of probiotics on some acute phase proteins in broilers exposed to Salmonella typhimurium lipopolysaccharides

Einfluss von Probiotika auf einige leberspezifische Blutproteine beim Broiler nach Belastung mit Salmonella typhimurium Lipopolysacchariden

Serpil Kefal and N. Y. Toker


Introduction

Probiotics are defined as selected and concentrated amounts of live lactic acid bacteria (i.e. Lactobacillus spp., Streptococcus spp.) by VanBelle et al. (1990). Also the definition “direct fed microbials” (DFM) was proposed by American Food and Drug Association (FDA) instead of the definition “probiotic”. DFM are defined as live originated natural formed microorganisms including bacteria, fungi and yeasts. Some microbial species which are containing Bacillus spp., Bifidobacterium spp., Enterococcus faecium (Ent. faecium), Enterococcus faecalis (Ent. faecalis), Escherichia coli (E. coli), Lactobacillus bulgaricus (L. bulgari-cus), Lactobacillus acidophilus (L. acidophilus), Lactoba-cillus lactis (L. lactis), Lactobacillus salivarius (L. salivar-ius), Lactobacillus plantarum (L. plantarum), Lactobacillusspp., Streptococcus thermophilus (Str. thermophilus), several yeasts and undefined culture mixtures are used as probiotics (Fuller, 1989; Patterson and Burkholder, 2003). Jin et al. (1997) suggested that probiotics can be an useful alternative to antibiotics if the correct bacteria is given with the optimal concentration to chicks that are under stress conditions. There are a number of reports investigating the effects of using probiotics in broilers for control of Salmonella (Nurmi and Rantala, 1973; Palmu and Camelin, 1997; Schnitz et al., 1998). However, to our knowledge, there is no published data evaluating the effects of using probiotics on acute phase proteins (APPs) in chickens exposed to Salmonella or Salmonella lipopolysaccharides (LPS). Acute phase response is characterized by changes in concentrations of some liver-synthesized plasma proteins, and it is a nonplastic reaction to maintain homeostasis in case of immune deficiency, infection, inflammation, tissue damage and tumor formations (Kent, 1992). Despite an increase in recent years, the numbers of studies on changes of acute phase proteins induced by inflammation in poultry are more limited compared to those conducted in mammalian species (Xie et al., 2001). The relationship between avian APP levels and inflammatory (pathogens, inflammation) and non-inflammatory (gender, age, nutritional status) factors is of importance. Korver and Klaasing (1997) diagnosed some avian diseases (which are difficult to diagnose postmortem) by using APP levels that were increased during acute phase response. Saini and Weber (1991) used APPs for determining early-inflammatory changes which occur prior to antibody synthesis and cannot be detected by visual inspection of poultry meat. APPs are used for selection of poultry lines on high immune response (Chamanza et al., 1999).

The most significant positive APPs in avian species are ceruloplasmin, α1-acid glycoprotein (AGP), amyloid A, transferrin, mannan-binding protein, haptoglobin, hemopexin, fibrinogen and fibronecin (Chamanza et al., 1999). The acute phase proteins which were investigated in this study are: fibrinogen, ceruloplasmin, total protein and albumin. Fibrinogen has an active role with tromboplastin by blood clotting (Kaneko, 1989). Ceruloplasmin is the most important plasma protein in the copper metabolism (Harris et al., 1997). The changes in the plasma total protein levels are used to diagnose several diseases (Bell, 1977). Transferrin has an important role in the iron metabolism (Chamanza et al., 1999). On the other hand, albumin was reported as a negative APP in poultry (Takahashi et al., 1995). Acute phase response in various animal species under different pathological conditions requires further studies and classifications of APPs (Chamanza et al., 1999). The objective of the present study was to investigate the effects of commercial probiotics (Broilact, Bioplus 2B) on immune response in Salmonella LPS-sensitized-broilers using APPs.

Materials and methods

The study was conducted in the Poultry Research House of the Biochemistry Department in the Veterinary Faculty of the Istanbul University by using 300 Ross 308 mixed sex broilers and lasted for 42 days. There were 150 broilers in the “Control (C) Group” and also 150 broilers in the “Trial (T) Group”. The animals were housed in 25-broiler capacity cages with glass windows. Each of these cages with glass windows was 1.2 m². It is indicated in Ross Breeders Broiler Management Manual (Ross Breeders, 1999) as recommended in the Codes of Recommendations for the Welfare of Livestock of the United Kingdom Ministry and Departments of Agriculture that stocking density in broilers for 1.4 kg body weight can be 24.4 animals per m² and also that the average body weights for male Ross 308 broilers

1This project was supported by Scientific Research Fund of Istanbul University. Project No: T-1266/0112001
2This article was summarized from the Ph.D. thesis by the first author.
are 1522 g and for female Ross 308 broilers 1339 g on 30th day. All animals were kept under the same physical and environmental conditions. Light was provided for 24 h/day between days 1 and 9, for 23 h and 30 min/day between days 10 and 15, for 23 h/day between days 16 and 42. Relative humidity was 60-70% during the study. Temperature was 27-31 °C within the first week, 25-28 °C for the 2nd week, 22-24 °C for the 3rd week, 21-22 °C for the 4th week, 20-22 °C for the 5th week and 20 °C for the 6th week.

Arranging of the groups

Salmonella typhimurium (S. typhimurium) LPS was not applied to the control group broilers. Control group was divided into 3 subgroups and each subgroup included 50 broilers. These subgroups were arranged as below:

- **C-Check**: No S. typhimurium LPS injection, no probiotic
- **C-Broilact (C-Bro)**: No S. typhimurium LPS injection, Broilact
- **C-Bioplus 2B (C-Bio)**: No S. typhimurium LPS injection, Bioplus 2B

To each broiler in the trial group S. typhimurium LPS was applied. Trial group was divided into 3 subgroups and each subgroup included 50 broilers. These subgroups were arranged as below:

- **T-Check**: S. typhimurium LPS injection, no probiotic
- **T-Broilact (T-Bro)**: S. typhimurium LPS injection, Broilact
- **T-Bioplus 2B (T-Bio)**: S. typhimurium LPS injection, Bioplus 2B

(Table 1)

Feeding of the broilers

The diet used for feeding of broilers was prepared according to National Research Council's (NRC, 1994) recommendations on an isocaloric and isonitrogenic basis. The animals were fed with broiler starter mash diet ad libitum between days 1 and 10, with broiler pelleted grower diet between days 11 and 28 and with broiler pre-slaughter pelleted diet between days 29 and 42. Bioplus 2B was given to C-Bio and T-Bio subgroups mixed in the diet (Table 2 and 3).

### Table 1. Applied treatments

<table>
<thead>
<tr>
<th>Applications</th>
<th>C-Check</th>
<th>C-Bro</th>
<th>C-Bio</th>
<th>T-Check</th>
<th>T-Bro</th>
<th>T-Bio</th>
</tr>
</thead>
<tbody>
<tr>
<td>No LPS</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No probiotic</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S. typhimurium LPS injection</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Broilact</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Bioplus 2B</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

### Table 2. Composition and calculated contents of nutrients in experimental diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Starter diet 0-10. days</th>
<th>Grower diet 11-28. days</th>
<th>Pre-slaughter diet 29-42. days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>527.03</td>
<td>545.91</td>
<td>592.32</td>
</tr>
<tr>
<td>Soy meal (48%)</td>
<td>328.04</td>
<td>315.04</td>
<td>263.73</td>
</tr>
<tr>
<td>Bone meal</td>
<td>43.23</td>
<td>40.00</td>
<td>30.00</td>
</tr>
<tr>
<td>Fat (Sunflower oil 45%)**</td>
<td>30.00</td>
<td>38.74</td>
<td>43.25</td>
</tr>
<tr>
<td>Marble Powder</td>
<td>8.12</td>
<td>7.02</td>
<td>7.72</td>
</tr>
<tr>
<td>Vitamin-Mineral mix (VM221)***</td>
<td>3.00</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>Salt</td>
<td>2.69</td>
<td>2.75</td>
<td>2.80</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.21</td>
<td>-</td>
<td>1.07</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.17</td>
<td>0.91</td>
<td>1.34</td>
</tr>
<tr>
<td>Clinacox (Anticoccidial)****</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>Corn meal</td>
<td>40.00</td>
<td>40.00</td>
<td>40.00</td>
</tr>
<tr>
<td>Fish meal (70%)</td>
<td>13.51</td>
<td>-</td>
<td>4.91</td>
</tr>
<tr>
<td>Lignobon (Pellet binder)*****</td>
<td>-</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>DCP (18%) (Dicalcium phosphate)****</td>
<td>-</td>
<td>3.14</td>
<td>6.36</td>
</tr>
<tr>
<td>Vit-E</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* The diets (starter, grower and pre-slaughter) given to broilers in C-Bio and T-Bio subgroups contained Bioplus 2B at 500 g/ton diet
** Fat: Sunflower oil 45%
*** Content of VM221 (2.5 kg VM221/ton): Vitamin A 12000000 IU, Vitamin D3 2500000 IU; Vitamin E 40000 mg; Vitamin K3 5000 mg; Vitamin B1 3000 mg; Vitamin B2 6000 mg; Vitamin B6 5000 mg; Vitamin B12 20 mg; Pantothenic acid 12000 mg; Niacin 25000 mg; Folic acid 1000 mg; Biotin 50 mg; Butylated hydroxytoluen 10000 mg; Mangan 80000 mg; Iron 60000 mg; Zinc 60000 mg; Copper 5000 mg; Iodine 1000 mg; Cobalt 200 mg; Selenium 150 mg
**** Clinacox is: Anticoccidial
***** Lignobon: Pellet binder
******* DCP is: 18%
Probiotics and their applications

Broilact. 1 g Broilact contains:

- Gram (-) anaerobs: $2.0 \times 10^8$ CFU
- Gram (+) anaerobs: $3.0 \times 10^8$ CFU
- Enterococci: $1 \times 10^9$ CFU
- Lactobacilli: $2.3 \times 10^9$ CFU
- Coliform Lactobacilli: $4.4 \times 10^9$ CFU

Preparation and application of concentrated Broilact solution.
Commercial probiotic Broilact was given to broilers in C-Bro and T-Bro via drinking water on their first day.

Regeneration agent was added to water to protect the freeze-dried bacteria from impurities such as chlorine, and enhance to their recovery.

In total, 20 g regeneration agent was dissolved in lukewarm water. When it was completely dissolved, 4 g Broilact was added to the solution with gentle mixing to avoid bubble formation. The concentrated solution was kept for approximately half an hour until all material has dissolved. The concentrated solution was added into 1.5 l of water and the end solution was divided into 2 parts (0.75 l each) in order to apply them to C-Bro animals.

All procedures described under the title “Preparation and application of concentrated Broilact solution” were repeated also for T-Bro animals.

Bioplus 2B. Bioplus 2B contains:

- $1.6 \times 10^6$ spores / g of strains of *Bacillus licheniformis* – 0.5%
- $1.6 \times 10^6$ spores / g of strains of *Bacillus subtilis* – 0.5%
- Sodium silicon silicated – 1.0%
- Whey powder as carrier – 98%

Commercial probiotic Bioplus 2B was given to broilers in C-Bio and T-Bio via diet (0.5 kg / ton diet).

Preparation and application of the *S. typhimurium* LPS solution:

To each broiler in the trial group 3 ml *S. typhimurium* LPS (Sigma. L-6511. 100 mg. Batch number: 12K4090) was applied IP with an automatic syringe on the 20th day of the study. As Roufia et al. (1992) described sterile *S. typhimurium* LPS solution (100 mg/l) was prepared by dissolving in 8.2 g/l NaCl. Total amount of injected LPS was approximately 450 ml.

It has been reported that orally administered *S. typhimurium* organisms are very infective for the day-old chick, with 100% infection arising from $10^2$ and 50% mortality from $10^{3.5}$ viable organisms/ chick. Furthermore, it has been found that intravenous (IV) inoculation of 1x10$^6$ *S. typhimurium* organisms into adult chickens established an infection in the liver, spleen, and intestinal tract of all birds (Williams and Stanley, 1989).

**Blood sampling**

Blood samples were collected from wing veins (V. subcutanea ulnaris) of each broiler beginning at 9:00 h in the morning on days 19, 21, 22 and 42 of the study i.e. at the same time on each blood sampling day of the study. All collected blood samples were centrifuged at 3000 rpm for 10 minutes to obtain serum and plasma. All serum and plasma samples were numbered and kept frozen ($-20^\circ$C) till the day of analysis.

**Biochemical analysis**

Serum albumin levels (n=1200) were determined by Bromcreosol Green Method using a commercial test kit (Spain-Spinreact-1001020) as 100 tests per day, plasma fibrinogen levels (n=200) by Richterich’s (1960) Biuret Method as 100 tests per day, serum ceruloplasmin levels (n=200) by Ravin’s Method (1961) as 100 tests per day, plasma total protein levels (n=200) by modified Lowry Protein Method (Tokr, 2000) as 100 tests per day. Serum iron levels (n=200) were determined by colorimetric meth-
od without deproteinization using a commercial test kit (Spain-Spinreact-1001247) as 50 tests per day and serum total iron binding capacity levels (n=200) were determined by using a commercial test kit (Spain-Spinreact-1001241) as 50 tests per day. Serum transferrin saturation levels (n=200) were determined by calculating from serum iron levels and serum total iron binding capacity levels with the following formulation (Vovoda et al., 1992): Transferrin saturation (%) = (Fe / TIBC) x 100.

Statistical analysis

Data were subjected to One-Way ANOVA statistical analysis. Duncan’s test was employed to determine significant differences between groups and sampling days. The results were presented as mean values with standard errors (x ± SE). The significance levels were accepted as p < 0.05.

Results

No clinical symptoms were observed in animals of control group during the study. However, in animals of trial group sleepiness, depression, decreases in diet and water consumption, dizziness and dishevelled feathers were observed after S. typhimurium LPS exposure.

Serum albumin levels: Mean albumin levels in T-Check group were significantly (p < 0.05) higher on days 19, 21 and 22 than in all of the other groups on the same days. Mean albumin levels in control group on day 42 were significantly (p < 0.05) higher on days 21, 22 and 42 than in control group animals as there are (Fuller, 1989; Jin et al., 1997):

- Maintaining beneficial microflora in the alimentary tract

Discussion

For comparing modes of action of Broilact and Bioplus 2B, modes of action of probiotics have to be known, in general, as there are (Fuller, 1989; Jin et al., 1997):

<table>
<thead>
<tr>
<th>Days</th>
<th>Groups</th>
<th>C-Check</th>
<th>C-Bro</th>
<th>C-Bio</th>
<th>T-Check</th>
<th>T-Bro</th>
<th>T-Bio</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.</td>
<td>Control</td>
<td>1.06 ± 0.07^B</td>
<td>1.12 ± 0.07^B</td>
<td>0.98 ± 0.05^B</td>
<td>1.59 ± 0.05^A</td>
<td>1.24 ± 0.06^A</td>
<td>1.27 ± 0.06^A</td>
</tr>
<tr>
<td>21.</td>
<td>Control</td>
<td>1.19 ± 0.05^B</td>
<td>1.24 ± 0.05^B</td>
<td>1.15 ± 0.06^B</td>
<td>1.64 ± 0.07^A</td>
<td>1.29 ± 0.07^A</td>
<td>1.22 ± 0.06^B</td>
</tr>
<tr>
<td>22.</td>
<td>Control</td>
<td>1.11 ± 0.06^B</td>
<td>0.99 ± 0.06^C</td>
<td>1.07 ± 0.07^B</td>
<td>1.59 ± 0.05^A</td>
<td>1.21 ± 0.08^B</td>
<td>1.34 ± 0.06^B</td>
</tr>
<tr>
<td>42.</td>
<td>Control</td>
<td>1.69 ± 0.05^A</td>
<td>1.48 ± 0.06^A</td>
<td>1.59 ± 0.05^A</td>
<td>1.80 ± 0.07^A</td>
<td>1.43 ± 0.09^A</td>
<td>1.46 ± 0.08^A</td>
</tr>
</tbody>
</table>

abc : numbers with different superscripts with the same row are significantly different (p < 0.05)

ABC : numbers with different superscripts with the same column are significantly different (p < 0.05)

<table>
<thead>
<tr>
<th>Days</th>
<th>Groups</th>
<th>C-Check</th>
<th>C-Bro</th>
<th>C-Bio</th>
<th>T-Check</th>
<th>T-Bro</th>
<th>T-Bio</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.</td>
<td>Trial</td>
<td>656.2 ± 28.09^A</td>
<td>408.0 ± 23.48^A</td>
<td>445.3 ± 21.37^B</td>
<td>402.8 ± 25.07^A</td>
<td>465.3 ± 15.78^C</td>
<td>484.1 ± 23.46^B</td>
</tr>
<tr>
<td>21.</td>
<td>Trial</td>
<td>634.1 ± 29.34^A</td>
<td>407.9 ± 23.10^A</td>
<td>489.8 ± 22.00^G</td>
<td>1026.2 ± 40.65^A</td>
<td>900.0 ± 38.85^G</td>
<td>1025.0 ± 43.14^A</td>
</tr>
<tr>
<td>22.</td>
<td>Trial</td>
<td>626.1 ± 27.16^B</td>
<td>430.2 ± 23.49^A</td>
<td>468.5 ± 20.06^G</td>
<td>916.6 ± 36.23^A</td>
<td>1002.5 ± 45.25^A</td>
<td>995.2 ± 32.73^A</td>
</tr>
<tr>
<td>42.</td>
<td>Trial</td>
<td>607.2 ± 27.43^A</td>
<td>478.9 ± 25.69^A</td>
<td>512.7 ± 18.87^A</td>
<td>970.1 ± 36.65^A</td>
<td>1153.3 ± 41.25^A</td>
<td>1001.3 ± 42.16^A</td>
</tr>
</tbody>
</table>

abc : numbers with different superscripts with the same row are significantly different (p < 0.05)

ABC : numbers with different superscripts with the same column are significantly different (p < 0.05)
Kefali and Toker: Effects of probiotics on broilers exposed to Salmonella

- Antagonistic activity (production of antibacterial compounds)
- Competitive exclusion (competition for nutrients and for adhesion sites)
- Increasing feed intake and digestion
- Alteration of bacterial metabolism
- Digestive enzyme activity
- Bacterial enzyme activity
- Ammonia production
- Enterotoxin neutralization
- Stimulation of immune system

The first probiotic which was used in this study is Broilact and it is a competitive exclusion product (Cameron and Carter, 1992; Palmu and Camelin, 1997; Salvat et al., 1992; Schneitz et al., 1998). Salvat et al. (1992) reported that Broilact exhibits inhibitory effects on S. typhimurium.

The second probiotic which was used in this study is Bioplus 2B and it contains 2 Bacillus strains: Bacillus licheniformis and Bacillus subtilis. It acts through maintaining beneficial microflora in the alimentary tract, and through increasing feed intake and digestion (Alexopoulos et al., 2004).

Comparing trial group broilers, no significant differences for mean serum albumin levels could be observed before and after S. typhimurium LPS application. This situation in our study might depend on the applied immunogen to broilers, the application time and the dosage of immunogen. Our results on the mean serum albumin levels are consistent with those reported by Inoue et al. (1997). In

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Table 6. Changes in the mean serum ceruloplasmin levels (mg/dl) (x±SE) (n=50)  
Veränderungen des Serum-Ceruloplasminspiegels (mg/dl; x±SE; n=50)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days</th>
<th>Control</th>
<th>Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>19</td>
<td>C-Check</td>
<td>C-Bro</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.89 ± 0.42&lt;sub&gt;AB&lt;/sub&gt;</td>
<td>4.12 ± 0.34&lt;sub&gt;B&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>4.72 ± 0.41&lt;sub&gt;B&lt;/sub&gt;</td>
<td>4.19 ± 0.35&lt;sub&gt;C&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>4.73 ± 0.45&lt;sub&gt;B&lt;/sub&gt;</td>
<td>4.12 ± 0.34&lt;sub&gt;A&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>6.18 ± 0.59&lt;sub&gt;A&lt;/sub&gt;</td>
<td>5.16 ± 0.40&lt;sub&gt;B&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

<sub>abc</sub>: numbers with different superscripts with the same row are significantly different (p < 0.05)  
<sub>ABC</sub>: numbers with different superscripts with the same column are significantly different (p < 0.05)

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Table 7. Changes in the mean plasma total protein levels (mg/dl) (x±SE) (n=50)  
Veränderungen des Plasma-Gesamtproteinspiegels (mg/dl; x±SE; n=50)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days</th>
<th>Control</th>
<th>Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>19</td>
<td>C-Check</td>
<td>C-Bro</td>
</tr>
<tr>
<td></td>
<td></td>
<td>49.9 ± 1.31&lt;sub&gt;B&lt;/sub&gt;</td>
<td>61.7 ± 1.93&lt;sub&gt;AB&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>40.1 ± 1.30&lt;sub&gt;C&lt;/sub&gt;</td>
<td>53.2 ± 1.42&lt;sub&gt;B&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>55.1 ± 1.54&lt;sub&gt;A&lt;/sub&gt;</td>
<td>62.3 ± 1.49&lt;sub&gt;A&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>57.1 ± 1.71&lt;sub&gt;B&lt;/sub&gt;</td>
<td>56.3 ± 1.36&lt;sub&gt;D&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

<sub>abc</sub>: numbers with different superscripts with the same row are significantly different (p < 0.05)  
<sub>ABC</sub>: numbers with different superscripts with the same column are significantly different (p < 0.05)

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Table 8. Changes in the mean serum transferrin saturation levels (%) (x±SE) (n=50)  
Veränderungen des Serum-Transferrin-Sättigungspiegels (%; x±SE; n=50)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days</th>
<th>Control</th>
<th>Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>19</td>
<td>C-Check</td>
<td>C-Bro</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32.7 ± 0.90&lt;sub&gt;1A&lt;/sub&gt;</td>
<td>34.3 ± 0.62&lt;sub&gt;A&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>33.9 ± 0.80&lt;sub&gt;A&lt;/sub&gt;</td>
<td>34.3 ± 0.71&lt;sub&gt;A&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>33.8 ± 0.85&lt;sub&gt;A&lt;/sub&gt;</td>
<td>34.6 ± 0.68&lt;sub&gt;A&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>33.8 ± 0.91&lt;sub&gt;A&lt;/sub&gt;</td>
<td>34.6 ± 0.75&lt;sub&gt;A&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

<sub>abc</sub>: numbers with different superscripts with the same row are significantly different (p < 0.05)  
<sub>ABC</sub>: numbers with different superscripts with the same column are significantly different (p < 0.05)
contrast to our results on the serum albumin levels, some investigators determined that injections of immunogens to avian species cause decreases in plasma albumin concentrations (Takahashi et al., 1995; Xie et al., 2000).

When mean plasma fibrinogen levels of control and trial group broilers were compared, significant (p < 0.05) differences were found on days 21, 22, and 42. The increase in plasma fibrinogen levels in trial group on days 21, 22, and 42 might have been caused by S. typhimurium LPS application on day 20. Mean plasma fibrinogen levels on days 19, 21, 22 and 42 in C-Check broilers were similar to those reported by Kaya (2003). The difference in mean plasma fibrinogen levels between C-Check and T-Check animals on day 19 might be due to stress. There were no significant differences between groups C-Check and T-Check for serum albumin, serum ceruloplasmin, plasma total protein and serum transferrin levels on day 19. Our results on the mean plasma fibrinogen levels are consistent with those reported by Pindyck et al. (1977) and Ambani et al. (1986). Immunogenic injections to avian species might stimulate acute phase response by cytokin-induced AFP and fibrinogen synthesis in the liver (Pindyck et al., 1983).

When mean ceruloplasmin levels of control and trial group broilers were compared, significant (p < 0.05) differences were found on days 21, 22 and 42. The increase in serum ceruloplasmin levels in trial group on days 21, 22, and 42 might have been caused by S. typhimurium LPS application on day 20. Immunogenic injections applied to broilers are claimed to be responsible for occurrence of acute phase response and for increase of serum ceruloplasmin levels (Curtis and Butler, 1980; Koh et al., 1996; Starcher and Hill, 1965). Our results on the mean serum ceruloplasmin levels are consistent with those reported by Koh et al. (1966), Curtis and Butler (1980) and Starcher and Hill (1965).

Mean plasma total protein concentrations of control group animals were significantly (p < 0.05) lower on day 21 than on day 19, while they were significantly (p < 0.05) higher on day 22 than on days 19 or 21. Mean plasma total protein concentrations in trial group animals were significantly (p < 0.05) higher on days 21 and 22 than on day 19. The increase in plasma total protein levels in trial groups on days 21, 22, and 42 might have been caused by S. typhimurium LPS application on day 20. In general, repeated blood sampling did not have any effect on blood data of albumin, fibrinogen and ceruloplasmin. Repeated blood sampling may affect serum transferrin saturation levels of trial group animals. Significant (p < 0.05) differences were observed between control and trial groups animals for plasma fibrinogen, serum ceruloplasmin, plasma total protein and serum transferrin levels due to the S. typhimurium LPS application.

Mean plasma total protein levels of control group animals were significantly (p < 0.05) lower on day 21 than on day 19, while they were significantly (p < 0.05) higher on day 22 than on days 19 or 21. Mean plasma total protein concentrations in trial group animals were significantly (p < 0.05) higher on days 21 and 22 than on day 19. The increase in plasma total protein levels in trial groups on days 21, 22, and 42 might have been caused by S. typhimurium LPS application on day 20. In general, repeated blood sampling did not have any effect on blood data of albumin, fibrinogen and ceruloplasmin. Repeated blood sampling may affect serum total protein levels by the following ways: Mean plasma total protein levels of control group animals were significantly (p < 0.05) higher on days 22 and 42 than on day 21. Comparing mean plasma total protein levels of C-Bro and C-Bio for all the sampling days, it can be seen that mean plasma total protein level of C-Bro broilers is significantly (p < 0.05) higher than for C-Bio broilers on day 21, and mean plasma total protein level of C-Bio broilers is significantly (p < 0.05) higher than for C-Bro broilers on day 22. It is well known that serum and plasma total protein levels can be affected by physiological condition of the animal, environmental conditions of the poultry house and presence of an antigen (Bell, 1971). In the study of Xie et al. (2000), it was reported that plasma total protein levels of 3-weeks old broilers increased 24 h and 48 h after S. typhimurium LPS application significantly. Our results on mean plasma total protein levels are consistent with those reported by Xie et al. (2000) and Chamanzia et al. (1999).

When mean serum transferrin saturation levels of control and trial group broilers were compared, significant (p < 0.05) differences were found on days 21 and 22. The decrease in serum transferrin saturation levels in trial group on days 21 and 22 indicates an increase in serum transferrin level. This situation depends on the fact that serum transferrin saturation is inversely proportional to total iron binding capacity (TIBC) which is the direct indicator of serum transferrin concentration. The increase in serum transferrin levels in trial group on days 21 and 22 might have been caused by S. typhimurium LPS application on day 20. Immunogenic injections to avian species might be responsible for transferrin synthesis in the liver (Hallquist and Klasing, 1994). Our results on mean serum transferrin levels are consistent with those reported by Hallquist and Klasing (1994), Töhö et al. (1995) and Chamanzia et al. (1999). Repeated blood sampling may affect serum transferrin saturation levels by the following way: Mean serum transferrin saturation levels of trial group animals were significantly (p < 0.05) higher on the 42nd day than on days 21 and 22 and they were similar to the serum transferrin saturation levels on day 19.

No significant differences were found for serum albumin, plasma fibrinogen, serum ceruloplasmin and serum transferrin levels between groups C-Bro and C-Bio in all sampling days.

Conclusions

The study revealed that probiotics alone have no effect on serum albumin, plasma fibrinogen, serum ceruloplasmin, plasma total protein concentrations and serum transferrin saturation levels of trial groups animals. Significant (p < 0.05) differences were observed between control and trial group animals for plasma fibrinogen, serum ceruloplasmin, plasma total protein and serum transferrin levels due to the S. typhimurium LPS application.

In conclusion, no beneficial effects of probiotics were seen on serum albumin, plasma fibrinogen, serum ceruloplasmin, plasma total protein and serum transferrin levels in broilers. Furthermore, no significant difference was observed between the two commercial probiotics (Broilact and Bioplus 2B).

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Summary

In this study the effects of two commercial probiotic preparations - Broilact used via drinking water and Bioplus 2B used via diet - on immunological response of the broilers that were sensitized with IP S. typhimurium LPS (lipopolysaccharide) application were investigated. The effects were verified by determination of changes in some acute phase proteins including albumin, fibrinogen, ceruloplasmin, total protein and transferrin. The study was conducted on 300 “Ross 308” broilers. Three ml of S. typhimurium LPS was applied to each broiler in trial group intraperitoneal on day 20 of the study. S. typhimurium LPS was not applied to control group broilers. The experimental design included 2 groups, control (no LPS exposure) and trial group (LPS exposure). Each group was divided into 3 subgroups as Check, Bro and Bio and each of the subgroup contained 50 broilers.
These subgroups were arranged as below:

C-Check: No S. typhimurium LPS injection, no probiotic;  
C-Broilact (C-Bro): No S. typhimurium LPS injection, Broilact;  
C-Bioplus 2B (C-Bio): No S. typhimurium LPS injection, Bioplus 2B;  
T-Check: S. typhimurium LPS injection, no probiotic;  
T-Broilact (T-Bro): S. typhimurium LPS injection, Broilact;  
T-Bioplus 2B (T-Bio): S. typhimurium LPS injection, Bioplus 2B.

Blood samples were collected on days 19, 21, 22 and 42 of the study and analysed for acute phase proteins.

Results revealed that blood levels of transferrin, fibrinogen, ceruloplasmin and total protein in trial group were significantly (p < 0.05) higher than in control group birds, but no significant change in albumin concentrations of broilers could be observed. The results indicate that probiotics alone do not affect levels of serum albumin, plasma fibrinogen, serum ceruloplasmin, plasma total protein and serum transferring in broilers, and that they do not show any effects on S. typhimurium LPS.

Zusammenfassung

Einfluss von Probiotika auf einige leberspezifische Blutproteine beim Broiler nach Belastung mit Salmonella typhimurium Lipopolysacchariden

In der vorliegenden Studie wurde der Einfluss von zwei kommerziellen Probiotika-Produkten (Broilact über das Trinkwasser, Bioplus 2 B über das Futter) auf die Immunreaktion von Broilern nach Belastung mit Salmonella typhimurium Lipopolysacchariden (LPS) untersucht. Als Indikatoren wurden die leberspezifischen Blutproteine Albumin, Fibrinogen, Zeruloplasmin, Gesamtproteine und Transferrin verwendet. Die Untersuchung wurde an 300 Broilern durchgeführt, die aus je 50 Broilern bestanden:

- C-Check: Keine S. typhimurium LPS Injektion, kein Probiotikum
- C-Broilact (C-Bro): Keine S. typhimurium LPS Injektion, Broilact
- C-Bioplus 2B (C-Bio): Keine S. typhimurium LPS Injektion, Bioplus 2B
- T-Check: S. typhimurium LPS Injektion, kein Probiotikum
- T-Broilact (T-Bro): S. typhimurium LPS Injektion, Broilact
- T-Bioplus 2B (T-Bio): S. typhimurium LPS Injektion, Bioplus 2B

Blutproben wurden am 19., 21., 22. und am 42. Tag der Untersuchung entnommen und auf einige leberspezifische Proteine analysiert.

Die Transferrin-, Fibrinogen-, Ceruloplasmin- und Gesamtproteinspiegel im Blut waren in den Behandlungsgruppen signifikant höher (p < 0.05) als in den Kontrollgruppen. Dagegen konnten keine signifikanten Unter-


Stichworte

Broiler, Salmonella typhimurium, Probiotika, leberspezifische Blutproteine

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