Salmonella typhimurium Lipopolisakkariti Verilen Broylerlerde Probiyotiklerin Etkileri[1, 2]

Effects of Probiotics on Broilers Exposed to *Salmonella typhimurium* Lipopolysaccharide [1, 2]

* Serpil Kefalı, ** Nezir Yaşar Toker

* Ecoform Yem ve Katkıları Tarım ve Hayvancılık Ürünleri San. ve Dış Tic. Ltd. Şti., 34704 Üsküdar-İstanbul-TÜRKİYE.

** Istanbul University Faculty of Veterinary Department of Biochemistry, 34320 Avcılar-Istanbul-TURKEY.

Özet

Çalışmada broylerlerde, mikroorganizmalar tarafından üretilen, büyüme artırıcı faktör ve antibiyotiklere alternatif olarak sunulan probiyotiklerin (Broilact, Bioplus 2B), *Salmonella typhimurium (S. typhimurium)* lipopolisakkariti (LPS) uygulamasına etkileri araştırıldı. Bu amaçla, 150 adeti kontrol ve 150 adeti deneme grubu olarak ayrılan, 300 adet Ross hattı broyler civciv kullanıldı. Deneme (D) ve Kontrol (K) grupları kendi içlerinde üçer alt gruba ayrıldı. Deneme alt gruplarına (D-Kör, D-Bro ve D-Bio) intraperitonal (IP) yolla 3'er ml *S. typhimurium* LPS verildi. D-Kör alt grubuna sadece *S. typhimurium* LPS verildi. D-Bro alt grubuna sadece ilk gün su ile probiyotik olarak Broilact, D-Bio alt grubuna da yem ile deney süresince probiyotik olarak Bioplus 2B verildi.

Kontrol alt gruplarına (K-Kör, K-Bro ve K-Bio) ise *S. typhimurium* LPS verilmedi, fakat K-Bro ve K-Bio alt gruplarına deneme alt gruplarıyla aynı şartlarda Broilact ve Bioplus 2B verildi. Araştırma 42 gün sürdürüldü. Probiyotiklerin, broylerlerde *S. typhimurium* LPS'ye karşı oluşan immun yanıtta ne derece etkin oldukları Salmonella O antijeniyle yapılan lam aglütinasyon testi ile takip edildi.

Sonuç olarak; Salmonella (+) yüzde oranının, çalışmanın deneme gruplarında,

S. typhimurium LPS uygulamasından sonra yükseldiği tespit edildi. Broylerlere destek sağlama amacıyla ve Salmonella'ları inhibe etme özellikleri açısından, suyla uygulanan Broilact ve yemle uygulanan Bioplus 2B probiyotikleri arasında herhangi bir farklılık gözlenmemiştir.

Anahtar kelimeler :Broyler, *Salmonella typhimurium*, lipopolisakkarit, probiyotik.

Summary

Effects of probiotics (Broilact, Bioplus 2B), which are used as an alternative to antibiotics and chemical growth promoters, on broilers that were exposed to *Salmonella typhimurium (S. typhimurium)* lipopolysaccharide (LPS) were studied. For this purpose, 150 chicks for control group and 150 chicks for test group were used resulting in a total of 300 Ross line broiler chicks. Each of control (C) and test (T) group animals are further divided in to 3 subgroups as Check, Bro and Bio. Test subgroups (T-Check, T-Bro, T-Bio) received 3 ml of *S. typhimurium* LPS intraperitonally (IP). T-Check animals received LPS only while T-Bro received Broilact, a commercial probiotic, via drinking water on day 1 after hatching only, and T-Bio animals received Bioplus 2B, another commercial probiotic, via feed during the 42-day study period. Control subgroups were not exposed to LPS but C-Bro and C-Bio received probiotics same as the test subgroups (T-Check, T-Bro and T-Bio). Effects of probiotics on the immune response of chickens against *S. typhimurium* LPS were followed by slide agglutination test using Salmonella "O" antigen.

Results indicated that percentage of *Salmonella* (+) animals increased in test subgroups after LPS exposure. No difference was found between Broilact and Bioplus 2B in regard to inhibiting Salmonella and supporting broiler's health.

Key words: Broiler, *Salmonella typhimurium*, lipopoysaccharide, probiotic.

Introduction

Stress factors affecting avian species may cause illnesses by impairing the balance in the intestinal flora of the chickens and by weakening the body defense systems (Weinack et al. 1985, Padron M. 1990, Arda et al. 2002). Up to date, antibiotics are widely used to eliminate harmful microorganisms from the intestines of chickens. Increasing resistance against antibiotics used for treating infections resulted in increased incidence of infectious diseases. Probiotics are, however, growth promoters produced by microorganisms and are presented as an alternative to antibiotics (Yavuz 2001). Vanbella et al. (1990) defined probiotics as selected and concentrated amounts of lactic acid bacteria (*Lactobacillus spp., Streptococcus spp.*) and they established that the use of probiotics provides increased live weight gain and increased feed conversion rate.

Salmonellas in broilers have a high incidence rate affecting a variety of organ systems. Compared to adults, young chickens are relatively more susceptible to *S. typhimurium* which is the causative agent of paratyphi in chickens with a mortality rate of up to 20 % within 5-8 days (Arda et al. 2002).

Use of probiotics as an alternative to antibiotics is becoming more popular in today's poultry industry in the world (Yavuz 2001). Jin et al. (1997) reported that probiotics are presented as an alternative to antibiotics and meaningful results can be obtained when appropriate strains of probiotics are given at optimum doses to the chickens. Tortuero (1973) reported that feed conversion ratio and live weight gain obtained with antibiotic use were achieved by implementation of Lactobacilli. Results of the researcher further indicated that probiotic addition to the feedstuff resulted in an increased egg weight in laying hens.

This study was undertaken to investigate the effects of some commercial probiotics that are given via feed or drinking water on immune response of broilers that are exposed to *S. typhimurium* LPS by IP.

Materials and Methods

The study was completed in 42 days. Animal material used was composed of a total of 300 Ross line newly hatched broiler chicks. 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 as of day 16. Relative humidity was 60-70 % during the study. Temperature was 27-31 °C within the first week, 25-28°C for the 2nd week, 22-23 °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. The chicks were divided in to 2 groups of 150 each as control (C) and test (T) groups. Each of these groups was further divided in to 3 subgroups of 50 chickens each as Check, Bro and Bio (C-Check, C-Bro, C-Bio, T-Check, T-Bro, T-Bio). Control subgroups did not receive any LPS injections. Subgroups were C-Check (no probiotic); C-Bro (received Broilact; i.e., Enterecocci, Lactobacilli and Coliforms via drinking water on day 1), C-Bio (received Bioplus 2B; i.e., *Bacillus licheniformis* and *Bacillus subtilis* via feed during the entire study period).

Test groups of animals received *S. typhimurium* LPS (Sigma. L- 6511. 100 mg. Lot 12K4090) as 3 ml/ per bird on day 20 of the study. Sterile *S. typhimurium* LPS (100 mg/l) was prepared by dissolving in 8.2 g/l NaCl (Roura et al., 1992). Subgroups were T-Check (no probiotics); T-Bro (Broilact) and T-Bio (Bioplus 2B) which received commercial probiotics as described for control subgroups. All chickens are provided with ad libitum water and broiler's starter powder feed between days 1 and 10, with broiler's pelleted growing feed between days 11 and 29, and with pre-slaughter pelleted feed between days 30 and 42. C-Bio and T-Bio subgroups received Bioplus 2B as 0,5 kg/ton. The rations were prepared according to National Research Council's recommendations as isocaloric and isonitrogenic. All chickens are provided with drinking water ad libitum during the study period.

Table 1. Composition of feedstuff given to broilers in control and test subgroups during the study

Blood samples were collected from the wing veins (V. subcutanea ulnaris) of control and test subgroups of animals for the serum extraction on days 19, 21, 22, and 42.

S. typhimurium Slide Agglutination Test: Serum samples of control and test subgroups taken on days 19 and 42 were subjected to slide agglutination test to determine presence of antibody against *Salmonella* "O" antigen (Omega Diagnostics). A drop of *Salmonella* "O" antigen was placed on a dry, clean, and greaseless slide and mixed gently with a drop of serum sample using a glass rod for 1 min. Visual agglutination occurred within 1 min. was accepts as a positive result.

Results and Discussion

Table 2. Results of *S. typhimurium* slide agglutination tests conducted on control and test subgroups on days 19 and 42 (% + % -) (n=150)

Some clinical symptoms including decrease in feed and water intake, massy feathers, and depression were observed in the chickens of control groups. In regard to these symptoms, it was found that LPS exposure caused significant clinical changes in test group of chickens. These results are consistent with the findings of Xie et al. (2000) and Koh et al. (1996) who reported depression, decreases in water and feed consumption after LPS injection. Results of slide agglutination test are presented in Table 2. Seroposistivity all test animals (61.65 %) on day 42 was found significant positive than those of control chickens (50.28 %). This result can be explained probably due to potential antigen properties of Salmonella LPS (Schimizu et al. 1998). Similarly, Sunwoo et al. (1996) reported that level of anti-salmonella antibodies increased within 1 week after *Salmonella typhimurium* LPS injections in 40-weeks old laying hens. Sunwoo et al. (1996) reported also that time period and rate of level of increase in antibody in poultry are related to whether LPS applied is detoxified or not.

As seen in Table 2, percentage of seropositivity of control subgroups were relatively maintained between days 19 and 42 whereas those in test subgroups increased appreciably. In conclusion, percentage of Salmonella seroposistivity increased in test subgroups after LPS injections (Table 2). In comparison of control and test subgroups, no difference was found between Broilact and Bioplus 2B in terms of inhibiting Salmonella and supporting health of broilers. Intake of probiotics via feed or drinking water did not differ. Instead, economical site of probioticts should be focused.

Table 1. Composition of feedstuff given to broilers in control and test subgroups during the study.

Ingredients	Amounth (kg)		
	Starter Feed*	Growing Feed*	Pre-slaughter Feed*
Corn	527.03	545.91	592.32
Soy meal (48%)	328.04	315.04	263.73
Bone meal	43.23	40.00	30.00
Fat (Sunflower oil 45 %)	30.00	38.74	43.25
Marble Powder	8.12	7.02	7.72
Vitamin-Mineral mix (VM221) **	3.00	2.50	2.50
Salt	2.69	2.75	2.80
Lysine	1.21	-	1.07
Methionine	2.17	0.91	1.34
Clinacox (Anticoccidial)	1.00	1.00	-
Corn meal	40.00	40.00	40.00
Fish meal (70 %)	13.51	-	4.91
Lignobon (Pellet binder)	-	3.00	3.00
Dicalcium phosphate (18 %)	-	3.14	6.36
Vit-E	-	-	1.00

* The rations (starter, growing and pre-slaughter) given to broilers in C-Bio and T-Bio subgroups contained Bioplus 2B at 500 g/ton ration.

** Content of VM221 (2.5 kg VM221/ton): Vitamin A 12000000 IU, Vitamin D_3 2500000 IU; Vitamin E 40000 mg; Vitamin K_3 5000 mg; Vitamin B_1 3000 mg; Vitamin B_2 6000 mg; Vitamin B_6 5000 mg; Vitamin B_{12} 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

Table 2. Results of *S. typhimurium* slide agglutination tests conducted on control and test *sub*groups on days 19 and 42 (% + % -) (n=150)

	Day 19		Day 42	
Subgroups	Salmonella+ (%)	Salmonella- (%)	Salmonella+ (%)	Salmonella- (%)
C-Check	53.33	46.67	51.67	48.33
C-Bro	58.33	41.67	46.67	53.33
C-Bio	55.00	45.00	50.85	49.15
T-Check	37.78	62.22	81.40	18.60
T-Bro	52.08	47.92	63.41	36.59
T-Bio	33.33	66.67	47.73	52.27

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Corresponding author:

Serpil Kefalı; Ecoform Yem ve Katkıları Tarım ve Hayvancılık Ürünleri San. ve Dış Tic. Ltd. Şti., 34704 Üsküdar-İstanbul-TÜRKİYE.