

Original Research Article

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Effect of Supplementation of Various Levels of Inulin on Immunity and Lymphoid Organs Weight in Raja II Broilers

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ABSTRACT

A biological trial was conducted to study the effect of supplementing various levels of inulin on immunity and lymphoid organs weight in Raja II broilers from 1 to 42 days of age. 225 day old straight run Raja II broiler chicks were selected randomly and out of this 45 chicks were assigned randomly to each treatment: control (without inulin), control + 0.25 percent inulin (T₂), control + 0.5 percent inulin (T₃), control + 0.75 percent inulin (T₄) and control + 1 percent inulin (T₅). The supplementation of various levels of inulin in broiler diets found effective in improving significantly antibody titre against IBDV, but not in NDV and also change in lymphoid organs weight. The results indicated that inulin have a better effect on antibody titre against IBD in broilers.

Keywords

Broiler, Antibody, Inulin and Immunity.

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Introduction

Antibiotics have been widely used in animal production for many years. Although, some antibiotics are used therapeutically to improve the health and well-being of animal a large portion was employed for prophylactic purposes and to improve growth performance.

The long-term presence of antibiotics in the microbial population has made antibiotic-resistant strains by gene mutation more efficient in survival compared to normal microbes (Murray and Moellering, 1978). However due to the emergence of microbes resistant to antibiotics which are used to treat human and animal infection, antibiotics are being taken out of poultry diets around the

world. The immune system highly depends on an adequate supply of nutrients to function properly. Besides the essential nutrients, non-essential food constituents such as non-digestible carbohydrates may also have an impact on the immune system (Seifert and Watzl, 2007).

Prebiotics cause potential possibilities of their practical using in non-specific immune prophylaxis lots of animal diseases. The ban placed on using antibiotic growth promoters raised an interest in prebiotics as alternative solution in poultry farms. Commercially available prebiotics are mostly fructo-oligosaccharides, isomalto-oligosaccharides, inulin, raffinose, lactulose, malto-

oligosaccharides, guar gum, sucrose thermal oligosaccharides, galacto-oligosaccharides, transgalacto-oligosaccharides, xylo-oligosaccharide.

Inulin contains oligosaccharides components and polysaccharides. Because of the β (2-1) glycosidic bond, it is resistant to host – derived digestive enzymes and is believed to enhance the growth of health-promoting bacteria and to suppress the growth of potential pathogenic bacteria. By promoting the growth of lactic acid producing bacteria, prebiotics have an indirect beneficial effect on the immune system of the host. These bacterial populations produce immune stimulating substances that react with the immune system at different levels, including the production of cytokines, mononuclear cells and macrophage phagocytosis as well as the induction of synthesis of large amounts of Ig, particularly IgA (Yasui and Ohwaki, 1991; Macfarlane and Cummings, 1999). Moreover, consumption of inulin- type fructans could be one way to modulate mucosa-associated biofilms so that members of the biofilm community, generally considered as health- promoting, are stimulated (Kleessen and Blaut, 2005). It is believed that inulin enhanced with oligofructose (OF) causes beneficial changes in the immune function of GALT (Roller *et al.*, 2004). Hence, this study was designed to study the effect of various levels of inulin with the immuno competence and lymphoid organs weight in Raja II broilers.

Materials and Methods

Experimental design and diets

A total of 225 day old straight run Raja II broiler chicks were wing banded, weighed and randomly assigned to five treatment groups with three replicates in each treatment group and with 15 chicks in each replicate consisting of five dietary treatments: control

(without inulin), control + 0.25 percent inulin (T₂), control + 0.5 percent inulin (T₃), control + 0.75 percent inulin (T₄) and control +1 percent inulin (T₅). The chicks were reared under deep litter system with all standard managemental practices till 6 weeks of age. Standard vaccination schedule were followed for immunizing the chicks. Birds were fed with broiler pre starter diet (0-7 days), starter diet (8-21 days) and finisher diet (22-42 days). The basal diet was formulated as per the specifications of BIS-2007. Inulin sample required for the trial was procured from Quadragen Vet health Private Limited, Bangalore.

Immuno competence

HI titer against new castle disease virus

The micro-test method described by Allan and Gouch (1974) was used for detection of HI titers from serum samples collected on 42nd day of post immunization of birds to assess the antibody titers. The HI test was done manually by β - procedure in ‘U’ bottom micro-plates using diluters, droppers and 4 HA units of ND viral antigen. Serial two fold dilution of serum in normal saline was taken and 25 μ l/well 4 HA unit of antigen was added. Plates were incubated for 45 minutes at room temperature. Fifty μ l of 0.8 per cent chicken erythrocytes were added to each well and the plates were incubated for one hour at room temperature before reading the results. The titers were expressed as the reciprocal of highest dilution of serum showing the haemagglutination inhibition or button formation.

Antibody titer against infectious bursal disease virus

The serum antibodies against IBDV were titered by using Poultry Diagnostic and Research Centre (PDRC) indirect ELISA Kit. Each of the steps was followed as per the

manufacturer’s instructions. Each of the wells of antigen pre-coated plate provided in the kit was used for the test. 100 µL each of the positive control serum and the negative control serum were added in duplicates to the respective control wells. Then, 100 µL of each test serum sample diluted in the sample buffer were added in duplicates to corresponding wells of the plate (apart from the control wells) and incubated at 37 °C for one hour. The plate was washed using the wash buffer provided in the kit. One hundred µL of mouse anti-chicken IgG conjugated with Horse Raddish Peroxidase (HRP) in wash buffer was added to each of the wells and incubated at 37°C for one hr. The plate was washed as before mentioned. One hundred µL of freshly prepared chromogen-substrate solution containing OPD and 3% H₂O₂ as substrate (4 L / ml of chromogen) were added to each of the wells and the plate was kept at room temperature for 15 min. Finally, 50 µL of 2.5 N HCL was added to each of the wells to stop enzyme-substrate reaction. Absorbance values were read using the ELISA reader (Bio Rad) with an interference filter at 492 nm. Readings were taken after the wells with only substrate-chromogen and HCL were blanked to ‘zero’ at 492 nm.

Lymphoid organs weight

The weight of lymphoid organs such as

spleen, bursa of fabricius and thymus was taken on 42nd day of the experiment during the slaughter of the birds and expressed as per cent of live body weight.

Results and Discussion

The results of the current study evaluating the effect of various levels of inulin in Raja II broilers on immune response for ND and IBD vaccination showed that there was no significant (P>0.05) difference in titer value against NDV and significant (P≤0.05) difference in titer value against IBDV among different treatment groups presented in table 1. This is in agreement with the findings of Elrayehand Yildiz (2012) conducted an experiment with Ross broilers fed 0.7% inulin for 6 week in which the antibody titer against ND viruses was investigated on 26 day and at the end of the experimental period showed non-significant difference. Sadeghi *et al.*, (2013) reported that dietary inclusion of prebiotics had no significant differences in antibody titers against ND viruses at 21 and 42 days of age.

But results of the present experiment are in contrary with the finding of Saeed *et al.*, (2015) revealed that addition of different levels of chicory leaf extract (5, 10 and 15 ml/L) in drinking water exhibited significant effect (P<0.05) on ND titer and unaffected (P>0.05) against infectious bursal disease (IBD) in broilers.

Table.1 Effect of supplementation of inulin on antibody titer against New Castles Disease (NDV) and Infectious Bursal Disease Virus (IBDV) of Raja II broilers

Experimental group	Description of the treatment	NDV log HI titer	IBDV titer (ELISA)
T ₁	Control (without Inulin)	0.90± 0. 30	1359.0± 288.3 ^a
T ₂	Control + 0.25% Inulin	0.93± 0. 16	1762.0± 163.8 ^{ab}
T ₃	Control + 0.5% Inulin	0.74±0. 10	2084.6± 359.7 ^{ab}
T ₄	Control+ 0.75% Inulin	0.94± 0. 21	1851.8± 231.0 ^{ab}
T ₅	Control + 1% Inulin	0.78± 0. 12	2413.0± 291.4 ^b

Means bearing different superscript column wise differ significantly (P≤0.05)

Table.2 Effect of supplementation of inulin on lymphoid organs weight (g/100g body weight) of Raja II broilers

Experimental group	Description of the treatment	Lymphoid organs weight (g/100g body weight)		
		Spleen	Bursa of Fabricius	Thymus
T ₁	Control (without Inulin)	0.15± 0.01	0.10± 0.01	0.35± 0.02
T ₂	Control + 0.25% Inulin	0.19±0.01	0.09±0.01	0.42±0.04
T ₃	Control + 0.5% Inulin	0.16± 0.01	0.13±0.01	0.43± 0.01
T ₄	Control+ 0.75% Inulin	0.14±0.01	0.12± 0.01	0.44± 0.05
T ₅	Control + 1% Inulin	0.18±0.02	0.04±0.01	0.39±0.01

The higher level of antibody titer against IBDV in birds using chicory leaf extract-inulin may be due to its antimicrobial effect. Another possible reason might be the increased activity of neutrophils in blood after vaccination, which could play a major role to boost up the immunity (Guo *et al.*, 2003).

The results of this study on lymphoid organs weight revealed that there was no significant difference in relative weights of lymphoid organs viz., spleen, bursa of fabricius and thymus presented in table 2 and this result is in agreement with the findings of Nabizadeh *et al.*, (2012) reported that 0.5% and 1% inulin inclusion had no significant effect on relative weight of thymus and spleen at the end of the grower and finisher period and bursa of fabricius weight at grower period. The lack of effect on immunological organs weights might be due to the production of Ig and lymphocytes was not increased with dietary inulin supplementation. This may be due to a lack of challenge, as the birds were used in this trail and thus more isolated from pathogen presence than in a production houses.

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