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Influence of Honey on Immune Response Against Newcastle Disease Vaccine

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Abstract: This study aims to investigate the effect of supplement of honey in drinking water on immune response of broiler chicks. A solution of 10% of honey in drinking water was used for 28 days. We evaluated the mortality rate, body weight, lymphoid organ weight and immune response of broiler chicks previously vaccinated with Newcastle Disease Virus (NDV) vaccine. The use of honey as a natural feed additive in drinking water through the experiment reduced mortality rates. The results revealed an increase in body weight (73-92.2%), lymphoid organ weight (22-29%) as well as an increase in antibody titer (6-9%) of chicks previously vaccinated with Newcastle Disease Virus (NDV) vaccine.

Key words: Newcastle Disease Virus Vaccines · Honey · Immune Response

INTRODUCTION

Appropriate nutrition may aid in minimizing the incidence of diseases by enhancing immunity [1-3]. Certain nutrients are capable of modulating the function of the immune system through a variety of mechanisms [4]. The immune response of chickens has been shown to be influenced by number of nutrients which regulate animal immune response [5,6]. The medical properties of some natural products have some consideration such as herbal extracts [7, 8], probiotics [9], prebiotics [10] and enzymes [11]. There is an increase demand for using natural biological compounds as feed additives. The information concerning characteristics of honey as feed additives for poultry is still quite limited and deficient. The present study aimed to determine the effect of using honey as a feed additive in drinking water on immune response of broiler chicks vaccinated with Newcastle disease virus (NDV) vaccine from one day old up to 28 days of age.

MATRIALS AND METHODS

Honey: All reagents are of analytical purity grade. Distilled water was used for all dilution steps. Coriander honey was collected as market sample of Egyptian origin.

Chicks: One hundred and twenty, day-old, commercial broiler chicks (100 gm) were housed in wire cages (60 cm×100 cm) in rooms lighted for 24 h at the beginning of pretrial period. The temperature was gradually declined to the room temperature. Broiler chicks fed commercial ration and water ad libitum and reared under strict hygienic measures.

Newcastle Disease Virus Vaccines:

- HB1 vaccine: Hebra, batch no.40M/2, 1000 dose.
- La Sota vaccine-Intervet-batch no. 12836KJ01, 1000 doses and titer of $10^{7.5}$ EID50 /dose.

Blood Samples: Blood samples were drawn by heart puncture into centrifuge tubes and left to clot, for 30 minutes, at room temperature for collection of chicken sera. Sera were kept at-20°C in the deep freezer for analysis.

Haemagglutinating Antigen: It was prepared according to methods of Allan *et al.* [12].

Serum Samples for HI Test: Serum from blood samples was collected from wing vein, labeled and kept at-20 °C till used.

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Chicken Red Blood Cells: Red blood cells (RBCs) from susceptible adult birds were collected on 4% sodium citrate as anticoagulant. The RBCs were washed three times with phosphate buffered saline (PBS) at PH 7.0-7.2.

Physiological Saline: Prepared and autoclaved according to Cruickshank *et al.* [13] then stored at 4°C till used.

Haemagglutination Inhibition (HI) Test: The test was carried out according to the standard procedure described by Majiyagbe and Hitchner [14]. The end point was estimated according to scheme described by Kaleta and Siegmann [15].

Determination of Body and Lymphoid Organ Weights: The body weight and lymphoid organ weight of each broiler chick was determined using digital balance at the second and fourth weeks of the experiment, the mean body weight of each group was measured.

Experimental Design: One hundred and twenty, day-old, broiler chicks (100gm) were randomly distributed. Chicks were divided into four groups. The first group served as normal control. The second group received a solution of 10% Coriander honey in drinking water day after day till the end of the experiment (28 days) as honey control group. The third group was served as control vaccinated group while the fourth group received 10% Coriander honey in drinking water day after day till the end of the experiment. The third and fourth groups were vaccinated with Hitchener B1 by ocular route at 5 days of age followed by boaster dose using La Sota live vaccine at 14 days of age in drinking water. The mortality rate was monitored throughout the experiment and the body weight was evaluated at 7, 14, 21 and 28 days. Five chicks from each group were sacrificed after 7, 14, 21 and 28 days where serum samples were taken for analysis using HI test for ND antibody titers as described by Hegazi *et al*. [16] and lymphoid organs (bursa of Fabricius, thymus and spleen were removed, weighed and weight was expressed as percentage of live body weight or relative weight (mg/100 gm) [17].

Statistical Analysis: The results obtained in the present study were represented as means ± standard error and were analyzed using analysis of variance (ANOVA). The significance of difference between means at $P<0.05$ was calculated using the Duncan Multiple Range Test [18].

RESULTS

Data displayed in (Table 1) summarized the differences in live body weights from 7 days to 28 days of age between four broiler chicks groups. The supplemented honey at level 10% showed more increment in the live body weight of broiler chicks than the control group and vaccinated group only during the 2nd week up to the end of the experiment

Table 1: Average body weight gain / grams (BW) of treated and control broiler chicks groups

	body weight /grams							
	1st week		2nd week		3rd week		4th week	
Treatment	BW	%	BW	%	BW	%	BW	%
Control negative	159	4.6	401	23.5	710	47.5	1012	71.0
10% Coriander honey	160	4.7	417	24.7	742	50.0	1039	73.0
Vaccinated with NDV vaccine	162	4.8	425	25.3	743	50.0	1054	74.3
10% Coriander honey + vaccinated	161	4.7	442	26.6	793	54.0	1283*	92.2

* This weight (the highest at the end of the experiment) was used as reference to calculate the percentage increase in body weight.

Table 2: Means of mortality rate (%) for broiler chicks treated and control broiler chicks groups

	Mortality rate			
Treatment	1st week	2nd week	3rd week	4th week
Control negative	0.00 %	5.10 %	3.10 %	2.10 %
10% Coriander honey	0.00 %	2.20 %	2.10 %	0.00 %
Vaccinated with NDV vaccine	0.00 %	1.20 %	0.00 %	0.00 %
10% Coriander honey + vaccinated	0.00 %	0.00 %	0.00 %	0.00 %

Table 3: Means of relative lymphoid organs weights (spleen, thymus, bursa) (gm/100 gm L.B.W.) for broiler chicks treated and control negative broiler chicks groups

	Experimental periods					
	2nd week			4th week		
Treatments	spleen	thymus	bursa	spleen	thymus	bursa
Control negative	0.07	0.11	0.24	0.10	0.13	0.19
10% Coriander honey	0.08	0.14	0.26	0.12	0.16	0.26
Vaccinated with NDV vaccine	0.08	0.16	0.27	0.16	0.18	0.29
10% Coriander honey + vaccinated	0.09	0.20	0.35	0.20	0.26	0.37

Table 4: Determination of logarithmic (log 2) mean antibody titers among sera of chicks vaccinated with NDV vaccine and subsequently treated with honey

	Mean HI titer / days		
Treatment	2nd week	3rd week	4th week
Control negative	2.7	2.3	1.6
10% Coriander honey	2.8	2.5	1.9
Vaccinated with NDV vaccine	3.8	6.1	7.2
10% Coriander honey + vaccinated	4.6	6.7	7.8

The mortality rates between broiler chicks groups were illustrated in Table (2). The results indicated that there were significant differences in the mortality rates through all the experimental period. It's worthy to note that the control group and 10% of supplemented honey showed increased mortality rate throughout the experiment. Irrespective of honey supplemented levels, mortality rate was still under normal percentages. The supplementation of honey to vaccinated group showed no deaths throughout the whole period of the experiment.

The differences in lymphoid organs relative weights between broiler chicks groups were illustrated in Table (3). It was found that the supplemented honey at all periods showed an increase in the lymphoid organ relative weight of broiler chicks when compared with control group during experiment. The supplemented honey at all levels showed increase in lymphoid organ relative weight when compared with control group.

The antibodies titers of broiler chicks groups were summarized in Table (4). Irrespective of honey supplemented in water, it was found that there were no significant differences in the (NDV) antibodies titer between the treatments through all the experimental period when compared with control group. The 10% honey supplementation and vaccinated with (NDV) vaccine showed higher antibody titer than the control vaccinated NDV vaccine group. 10% of honey showed the highest (NDV) antibodies titer among other groups.

DISCUSSION

The present study aimed to determine the effect of using honey as a feed additive in drinking water for broiler chicks from 7-28 days of age. The effect of supplement of 10% honey in drinking water on immune response of broiler chicks was evaluated. The supplemented honey at level 10% caused an increase in the live body weight of broiler chicks compared to the control and vaccinated only group during the 2nd week up to the end of the experiment. This means that formulation diet matches a desired nutrient complete growth. Results obtained here indicated that honey at the last week of the experiment may be beneficial in increasing feed intake and improving broiler chicks' performance [16]. Our results were in disagreement with some earlier studies. It's well known that the main components of honey are sugars. Addition of honey in drinking water increased the metabolic energy which in turn decreases the feed intake. Monsuru *et al*., [19] found that honey had no significant effect on feed intake, weight gain, feed conversion ratio, water intake, survival, dressed percentage, breast meat, gizzard, drumstick, shank, thigh, tibia volume and magnesium. Moreover, Miraei-Ashtiani *et al.* [20] had earlier given a report similar to this. The authors found that there was no difference in weight gain, feed intake, feed conversion ratio and final live weight offered feed supplemented with or without 200 ppm vitamin C. Regardless of honey, the mortality rates were lower than the control group.

This result may be due to the chemical composition of honey which includes huge mixture of flavonoids and phenolic acids which act as antimicrobial agent [21]. It is clear that the similarity in performance that is associated with an immune response is due to the diversion of nutrients away from growth or egg production to be used by the immune system. Improvements in immunity or functions that support immunity are associated with Zn, Mn, Cu and Se. Dietary Se interacts with vitamin E in antioxidant protection of cells because it is a component of glutathione peroxidase [21]. In addition to antioxidant activity, Selenium (Se) has been shown to impact disease resistance. For example, broilers infected with *Emeria tenella* had improved resistance (i.e., reduced mortality and cecal lesions) when supplemented with Se [22]. Because Se may be included in chicken's diet only at a level of 0.3 mg/kg, highly available organic sources may benefit poultry productivity.

The weights of the lymphoid organs (spleen, thymus and bursa) were measured as a percentage of BW. The splenic, thymic and bursal weights were not significantly different for all experimental supplemented honey when measured at 4 weeks of age. But, there was an improvement in lymphoid organs weight due to the natural feed additives. The role of nutrition on the developmental events of the immune system must consider the diet of the hen as well as the diet of the chick [16, 23]. These findings are clear and agree with our results in all three Trails at the early age. Defense against an infectious challenge requires a highly orchestrated response by the immune system. From a nutritional viewpoint, substrates (amino acids, energy, enzyme co-factors) are needed to support the clonal proliferation of antigen-driven lymphocytes, the recruitment of new monocytes and heterophils from bone marrow, the synthesis of effector molecules (e.g., immunoglobulins, nitric oxide, lysozyme, complement) and communication molecules (e.g., eicosanoids, cytokines) [24, 25].

The total antibody response of the 10% honey additives as supplement to chicks vaccinated with (NDV) vaccine was evaluated. Broiler chicks fed with honey had significantly higher antibody response as compared with control group. It is concluded that honey in drinking water may be considered as a new natural additive due to their components of micronutrients which enhanced and developed the immune system. The use of honey as a broiler immunostimulant for poultry farming purposes needs validation with further studies, using different levels and other poultry species [18]. Serum antibody titer is the indicator of humoral immunity. Results in this study also showed that the antibody titers in most of treatment groups at each time point were higher than that from control group, suggesting that they could promote humoral immunity.

Previous studies have shown that flavonoids have an immunossupressor effect on the lymphoproliferative response [26-28]. According to this study the micronutrient components of natural additive of honey which added to drinking water of broiler chicks may improve the development of the immune system more than macronutrients such as carbohydrates and protein. Clearly the immune system is sensitive to frank deficiencies as well as excesses of nutrients. Nutrient deficiencies that are especially damaging to development of the immune system include linoleic acid, vitamin A, iron, selenium and several of the B vitamins [29, 30].

From over mentioned results, it could be concluded that the honey supplementation increases body weight, lymphoid organ weight and antibody titer. On the other hand it reduces mortality rates. This may be due to the antimicrobial and immunostimulant activity of honey. It opens perspective uses of honey as food additive to improve chicken performance.

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