



Original Research Article

Blood Serum Interferon - Alpha and - Gamma Concentrations in Broiler Chickens Treated with the Immunomodulator Helpankar

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ABSTRACT

Keywords

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The experiment was conducted with two broiler chicken flocks; the one was orally treated with the immunomodulator Helpankar – a natural product containing in concentrated form the lipopolysaccharide components isolated from Gram-negative bacteria of the *Enterobacteriaceae* family, in the drinking water. Blood serum α -IFN and γ -IFN concentrations were assayed immunoenzymatically (ELISA). At the age of 36 days, the average α -IFN concentration in control group was 8.05 ± 0.43 pg/ml, whereas in the experimental group treated with the immunomodulator - 12.44 ± 0.87 pg/ml. Average control γ -IFN level was 6.45 ± 0.55 pg/ml vs 18.62 ± 0.64 pg/ml in treated birds. The data showed that despite the short life of broiler chickens, the blood serum concentrations of both interferons were substantially increased even following a single administration of the immunomodulator Helpankar.

Introduction

In birds, type I interferons (IFN- α and IFN- β) are produced by leukocytes and fibroblasts, whereas type II (IFN- γ) – by T-lymphocytes and natural killer cells. IFN α/β are mainly involved in antiviral immunity, while IFN γ is a pleiotropic molecule that influences all stages of the immune response at a specific extent (Rosenberger *et al.*, 2000; Seo *et al.*, 2002; Schroder *et al.*, 2004).

Emadi *et al.* (2010a) showed that Arg supplementation in the chickens' basal diets significantly increased the serum levels of

interferon- α , interferon- γ in chickens challenged by orally administration of intermediate plus strain of IBD virus. The same authors in another article (Emadi *et al.*, 2010b) showed that tryptophan also significantly increased the serum levels of interferon- α and interferon- γ in broiler chickens. It is acknowledged that bacterial endotoxins are able to trigger the production of interferons in the body (Bailey *et al.*, 2007; Petrunov *et al.*, 2007; Sato and Iwasaki, 2005). According Kaiser *et al.* (2012) LPS induced immune response was regulated by both pro- and anti-

inflammatory cytokines. They found significantly higher splenic levels of IL6, IFN- γ , iNOS, and IL10 RNA expression in chickens receiving LPS. The present experiments aimed to assay blood serum concentrations of IFN- α and IFN- γ in broiler chickens, after single administration of the polybacterial immunomodulator Helpankar.

Materials and Methods

Experimental animals

In this trial, Ross 308 broiler chickens were used, divided into 2 flocks of 36,000 birds each – control and experimental. Throughout the experiment, the birds were reared under identical conditions conforming to the current immunoprophylactic schedule at the farm.

Immunomodulator **Helpankar** (registration certificate No 1734)

It is a natural product, containing a concentrate of lipopolysaccharide components isolated from gram-negative bacteria belonging to the *Enterobacteriaceae* family.

Route of treatment

During the experimental period Helpankar was given orally in drinking water for 10 days. One dose of Helpankar (0.4 ml) was dissolved in 700 ml water (this water is for 10 days for each bird in the group).

Samples

Blood samples were collected from v. ulnaris profunda 26 days after the last administration of the immunomodulator. The samples were left to clot for 30 min, and sera were separated by centrifugation at 2000 rpm.

Interferon assay

Interferon concentrations were determined by immunoenzymatic assay. Chicken interferon alpha (IFN- α) ELISA kit and Chicken interferon gamma (IFN- γ) ELISA kit (NovaTeinBio, Massachusetts, USA) were used for that purpose. In the wells of the ELISA plate, 7 standards were added with concentrations 0, 15.6, 31.2, 62.5, 125, 250, 500 and 1000 pg/ml (for IFN- α) and 0, 6.25, 12.5, 25, 50, 100 and 200 pg/ml (for IFN- γ). Absorptions were measured at a wavelength of 450 nm. Interferon concentrations were calculated from the standard curve by means of a software product.

Statistical analysis

The results are presented as means \pm SE. All given parameters were compared between the control group and the Helpankar treated group using the one way ANOVA with fixed effects of the factor using Statistica 6.0 (StatSoft Inc.). Differences were considered significant at $P < 0.05$.

Results and Discussion

Table 1 presents the average blood IFN- α and IFN- γ concentrations in experimental and control broiler chickens. In the experimental group, IFN- α concentration was 12.44 ± 0.87 pg/ml, whereas in controls – 8.05 ± 0.43 pg/ml. The data demonstrated that blood serum IFN- α of birds supplemented with Helpankar was by 35% higher than that of untreated birds ($P < 0.001$). Average IFN- γ level in the blood of experimental broiler chickens was 18.62 ± 0.64 pg/ml vs. 6.45 ± 0.55 in controls; i.e. the concentrations in treated birds were by 65% higher ($P < 0.001$).

Table.1 Average values of IFN- α and IFN- γ concentrations in blood sera of 35 days old chicken broilers (n = 60)

Tested birds	IFN- α (pg/ml)	IFN- γ (pg/ml)
Experimental group	12.44 \pm 0.87***	18.62 \pm 0.64***
Control group	8.05 \pm 0.43	6.45 \pm 0.55

*** - P<0.001

The obtained results confirmed the opinion of a number of researchers (Bailey *et al.*, 2007; Murtaugh and Foss, 2002; Seo *et al.*, 2002), that bacterial endotoxins induce interferon synthesis after subcutaneous or intramuscular application. Our experiments demonstrated that the *per os* route also results in a similar immune response. Most probably, lipopolysaccharide is presented to dendritic cells after challenging the mucous coatings of the intestinal tract and through the M-cell transepithelial transport (Neutra, 1998; Sato and Iwasaki, 2005).

This mechanism for interaction between mucous and systemic immunity has been elucidated only after the discovery of dendritic cells in birds (Kaiser *et al.*, 2012). TLRs (Toll-like receptors) are also involved in the recognition, by binding to bacterial lipopolysaccharides and immune response activation (Moresco *et al.*, 2011; Neutra, 1998; Newberry and Lorenz, 2005; Ogra *et al.*, 2001; Rosenberger *et al.*, 2000). According to Sekellick *et al.* (1998), avian type I and II interferons act synergically and their ability to activate macrophages develops simultaneously with their antiviral activity.

In conclusion, the results from the experiment allowed to conclude that the immunomodulator Helpankar, applied orally in drinking water, increased blood serum IFN- α and IFN- γ concentrations in broiler chickens.

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