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Effect of artemisinin on immune system in chickens: preliminary results

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We investigated the effect of artemisinin on immune system in broiler chickens following chronic oral intake by lymphocyte proliferation assay (mitogens: concavalin A, lipopolysaccharide, artemisinin), phagocytosis assay, evaluation of serum gamma-globulin (SGG) levels, and development of lymphoid organs (bursa of Fabricius, spleen, and thymus). The chickens received artemisinin (5, 50, and 500 ppm) in their diet from 18 to 46 day-old. The level of serum gamma-globulin was significantly higher in chickens in-feed treated with artemisinin than in untreated chickens. However, only in chickens treated with 5 and 50 ppm the level of SGG increased over time, while in chickens treated with 500 ppm decreased as in control group. The in vitro phagocytic activity was supressed by artemisinin only at 28 days after in-feed treatment with artemisinin, but without statistical significance comparing with control group. Interestingly, in variants without stimulation, and stimulation with lipopolysaccharide, in-feed treatment with 50, and 500 ppm artemisinin stimulated the in vitro phagocytic activity. As regarding the thymus weight we did not register statistical significant difference among the groups, even chickens in-feed treated with 50, and 500 ppm artemisinin had a lower weight. Generally, the stimulation index of lymphocytes was higher in chickens treated with 50, and 500 ppm artemisinin and in vitro stimulated with concavalin A and lipopolysaccharide, but lower in case of in vitro stimulation with artemisinin. Over time, stimulation index decreased in all treated chickens, but greater in chickens treated with 50 ppm artemisinin, and in vitro stimulated with concavalin A and artemisinin, while in case of lipopolysaccharide increased. Also, the weight of bursa was significant lower in chickens treated with 50 ppm artemisinin, followed by chickens treated with 500 ppm artemisinin, comparing with untreated chickens. The spleen had a lower weight in chickens treated with 50, and 500 ppm artemisinin, but without any statistical significant difference. Artemisinin is relatively immunotolerant in broiler chickens, and further investigations are needed to make a final conclusion.

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INTRODUCTION

Artemisinin is a sesquiterpene lactone isolated from the Chinese herb Artemisia annua, and has been used for centuries to treat malaria. This compound has antimalarial activity as well as other potential applications such as antitumor effects (Efferth, 2006) and antibacterial properties (Dhingra et al., 2017). Artemisinin was found to be effective in controlling experimental visceral leishmaniasis (Utzinger et al., 2003; Keiser et al., 2006), and trypanosomiasis (Romero et al., 2006; Efferth et al., 2008), and has been shown to have anti-inflammatory and immunomodulatory effects (Galasso et al., 2010). Although artemisinin has been proven to be safe in therapeutic doses, it is important to consider the potential effects of chronic oral intake.

MATERIAL AND METHODS

We used four experimental groups of 10 chickens (ROSS 308), divided in two replicates of five chickens (one females, and one males). One group received no treatment (control group) and three groups received artemisinin in their diet at 5, 50 and 500 ppm concentrations. The treatment started when chickens were 18 days and lasted for 28 days (46 days old). Blood and serum samples were harvested from each chicken before treatment and two times after treatment at 14 and 28 days. Finally, chickens were euthanized by cervical dislocation and lymphoid organs (bursa of Fabricius, spleen, and thymus) collected. Feed and water were provided ad libitum, and the lighting was continuous. The chickens were reared in cages. Artemisinin of 98% purity was purchased from INTTRADE Chemicals GmbH, Germany.

RESULTS

Serum gamma-globulin levels and weight of lymphoid organs

The level of serum gamma-globulin was significantly higher in chickens in-feed treated with 50 and 500 ppm artemisinin than in untreated chickens. Generally, the weight of lymphoid organs was lower in experimental groups comparing with control group, but only in case of bursa the difference was statistically significant. Interestingly, the weight of bursa and spleen was higher in chickens treated with 5 ppm artemisinin than in untreated chickens.

Lymphocyte proliferation assay

The stimulation index of lymphocytes was higher in chickens treated with 50, and 500 ppm artemisinin and in vitro stimulated with concavalin A and lipopolysaccharide, but lower in case of in vitro stimulation with artemisinin. Over time, stimulation index decreased in all treated chickens, but greater in chickens treated with 50 ppm artemisinin, and in vitro stimulated with concavalin A and artemisinin, while in case of lipopolysaccharide increased.

Phagocytosis assay

The in vitro phagocytic activity was suppressed by artemisinin only at 28 days after in-feed treatment with artemisinin, but without statistical significance comparing with control group. Interestingly, in variants without stimulation, and stimulation with lipopolysaccharide, in-feed treatment with 50, and 500 ppm artemisinin stimulated the in vitro phagocytic activity.

CONCLUSIONS

Artemisinin is relatively immunotolerant in broiler chickens, and further investigations are needed to make a final conclusion.

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EFFECT OF ARTEMISININ ON IMMUNE SYSTEM IN CHICKENS: PRELIMINARY RESULTS

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INTRODUCTION

Artemisinin is a sesquiterpene lactone isolated from the Chinese herb Artemisia annua, and has been used for centuries to treat malaria. This compound has antimalarial activity against Plasmodium species, and also exhibits anti-cancer, antioxidant, immunomodulatory, and anti-inflammatory properties (Yang et al., 2008). Artemisinin is relatively immunotolerant in broiler chickens, and further investigations are needed to make a final conclusion.

MATERIAL AND METHODS

We used four experimental groups of 10 chickens (ROSS 308), divided in two replicates of five chickens (one males, and one males). One group received no treatment (control group) and three groups received artemisinin in their diet at 5, 50, and 500 ppm concentrations. The treatment started when chickens were 15 days and lasted for 28 days (46 days old). Blood and serum samples were harvested from each chicken before treatment and two times after treatment at 14 and 28 days. Finally, chickens were euthanized by cervical dislocation and lymphoid organs (bursa of Fabricius, spleen, and thymus) collected. Fed and water were provided ad libitum, and the lighting was continuous. The chickens were reared in cages. Artemisinin of 98% purity was purchased from INTARADE Chemicals GmbH, Germany.

The effect of artemisinin on immune system in broiler chickens was assessed by lymphocyte proliferation assay using whole blood in-vitro (concavalin A, lipopolysaccharide, artemisinin), phagocytosis assay, evaluation of serum gamma-globulin levels, and development of lymphoid organs (weight).

RESULTS

Serum gamma-globulin levels and weight of lymphoid organs

The level of serum gamma-globulin was significantly higher in chickens in-feed treated with 50 and 500 ppm artemisinin than in untreated chickens. Generally, the weight of lymphoid organs was lower in experimental groups comparing with control group, but only in case of bursa the difference was statistically significant. Interestingly, the weight of bursa and spleen was higher in chickens treated with 5 ppm artemisinin than in untreated chickens.

Lymphocyte proliferation assay

The stimulation index of lymphocytes was higher in chickens treated with 50, and 500 ppm artemisinin and in vitro stimulated with concavalin A and lipopolysaccharide, but lower in case of in-vitro stimulation with artemisinin. Over time, stimulation index decreased in all treated chickens, but greater chickens in treated chickens with 50 ppm artemisinin, and in vitro stimulated with concavalin A and artemisinin, while in case of lipopolysaccharide increased.

Phagocytosis assay

The in vitro phagocytic activity was suppressed by artemisinin only at 28 days after in-feed treatment with artemisinin, but without statistical significance comparing with control group. Interestingly, in variants without stimulation, and stimulation with lipopolysaccharide, in-feed treatment with 50, and 500 ppm artemisinin stimulated the in vitro phagocytic activity.

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