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Effects of feed supplementation with glycine chelate and iron sulfate on selected parameters of cell-mediated immune response in broiler chickens

Łukasz Jarosz [a,*], Małgorzata Kwiecień [b], Agnieszka Marek [c], Zbigniew Grądzki [a], Anna Winiarska-Mieczan [d], Marcin Kalinowski [a], Ewa Laskowska [a]

[a] Department of Epizootiology and Clinic of Infectious Diseases, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Głęboka 30, 20-612 Lublin, Poland
[b] Faculty of Biology and Animal Breeding, Institute of Animal Nutrition and Bromatology, Department of Animal Nutrition, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland
[c] Department of Veterinary Prevention and Avian Diseases, Institute of Biological Bases of Animal Diseases, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Głęboka 30, 20-612 Lublin, Poland
[d] Faculty of Biology and Animal Breeding, Institute of Animal Nutrition and Bromatology, Department of Bromatology and Nutrition Physiology, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland

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ABSTRACT

Because little is known about the impact of chelated (Fe-Gly, Fe-Gly + F) and inorganic ($FeSO_4$, $FeSO_4$ + F) iron products on immune response parameters in broiler chickens, the objective of the study was to determine the effects of inorganic and organic forms of iron on selected parameters of the cell-mediated immune response in broiler chickens by assessing the percentage of $CD3^+CD4^+$, $CD3^+CD8^+$, $CD25^+$, and MHC Class II lymphocytes, as well as the $CD4^+/CD8^+$ ratio and IL-2 concentration in the peripheral blood. The experiments were conducted using 50 day-old Ross 308 roosters. The test material was peripheral blood. Flow cytometry was used to determine selected cell-mediated immune response parameters. The results obtained indicate that the use of iron chelates in the diet of broiler chickens may stimulate cellular defense mechanisms. As a result of the experiment an increase was observed in the percentage of Th1, mainly T $CD4^+$ and T $CD8^+$. It was also noted that application of chelated iron can increase production of T $CD8^+$ cytotoxic cells and IL-2, which promotes the body's natural response to developing inflammation. There were no changes in T $CD4^+$, T $CD8^+$, T $CD25^+$ or MHC II lymphocyte subpopulations in the chickens following application of the inorganic form of iron.

1. Introduction

Dietary characteristics are known to modulate the body's susceptibility to infectious challenge, and thus the level of certain nutrients may at times be of critical importance. One of these nutrients, which affects immune system development and participates in cellular energy metabolism and neurotransmitter synthesis, is iron (Spear and Sherman, 1992; Pineda and Ashmead, 2001; Rincker et al., 2004; Shelton and Southern, 2006, Lu et al., 2006). Iron is an important factor in the development and proliferation of rapidly dividing immune cells. All subpopulations of B cells, T cells and NK cells have been shown to be dependent on transferrin receptor (TfR), which mediates iron import (Davies and Nightingale, 1975; Langini et al., 1988).

Iron deficiency results in impaired production of IL-2 by activated T cells. This process is known to play a fundamental role in the regulation of immune responses and is responsible for transition of the T lymphocyte G1 phase to the S phase of the cell cycle. The decrease in IL-2 synthesis and secretion by cells in a state of iron deficiency reduces the response of Th cells to antigens and thus disturbs the balance of cooperation between Th1 and Th2 cells, thereby weakening Th1 activity. This is accompanied by increased synthesis of cytokines such as IL-4 by Th2 cells. These phenomena negatively impact defense mechanisms in the development of many diseases, especially bacterial infections and tumor processes (Spear and Sherman, 1992; Chen et al., 2009).

Both iron deficiency and surplus in the body can be detrimental to immune function. For example, an excessive supply of iron has been found to change the proportion of T-helper (CD4) and T cytotoxic (CD8) cells in rats (Golding and Young, 1995; Kuvibidila et al., 1999). Oversupply of iron can also affect neutrophil function, manifested as impaired intracellular phagocytosis and killing of bacteria (Spear and Sherman, 1992; Kulkarni et al., 2011).

As a feed additive, iron is usually used in the form of an inorganic salt, such as sulfates, oxides and carbonates. Current nutrition standards for poultry assume 80 mg Fe/kg of dry matter in broiler feed, while a dose above 2000 ppm is considered toxic (Spears, 1999; Theil, 2004; Vieira, 2008). It has been demonstrated that the bioavailability of this element can be increased as compared to its inorganic forms by applying it in the form of chelates, amino acid compounds or protein compounds

* Corresponding author.
E-mail address: lukasz.jarosz@up.lublin.pl (Ł Jarosz).

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(Langini et al., 1988; Henry and Miller, 1995; Spears, 1999; Yu et al., 2000). According to some authors, the use of such forms of iron can significantly raise production rates in dairy cattle and pigs (Vieira, 2008; Feng et al., 2007; Feng et al., 2009). Similarly, the use of compound poultry feed enriched with iron chelates, alone or in combination with methionine, affects the content of this component in hen eggs and poultry meat (Hallberg et al., 1979; Hill et al., 1983; Park et al., 2004; Bao et al., 2007; Seo et al., 2008). Studies on humans and rats have shown that combinations of iron chelates with glycine were well absorbed in the digestive tract and increased hematological indicators and growth rates (Olivares et al., 1997; Shelton and Southern, 2006). A positive effect of chelates on certain immunological indicators has been noted as well, for example as increased activity of antioxidation enzymes (Spear and Sherman, 1992; Pineda and Ashmead, 2001).

As little is known about the impact of chelated and inorganic iron products on immune response parameters in broiler chickens, the objective of the study was to determine the effects of inorganic and organic forms of iron on selected parameters of the cell-mediated immune response in broiler chickens by assessing the percentage of $CD3^+CD4^+$, $CD3^+CD8^+$, $CD25^+$, and MHC Class II lymphocytes, the $CD4^+/CD8^+$ ratio, and IL-2 concentration in the peripheral blood.

2. Material and methods

2.1. Experimental animals

All procedures used in the research were approved by the Local Ethics Committee for Animal Testing at the University of Life Sciences in Lublin, Poland. The experiments were conducted at the Didactic and Research Station for Small Animals of the University of Life Sciences in Lublin, using a total of 50 day-old Ross 308 roosters divided into 5 groups of 10 chickens each, i.e. 4 experimental groups and a control group. On the first day of the experiment, the birds were individually weighed and marked with wing tags.

The experimental birds were kept in cages in a room with controlled temperature and humidity. During the study, electric lighting was used for 24 h a day during the first 10 days of the experiment and for 16 h a day thereafter. In the first week the chickens were kept at 33 °C, which was reduced by 2 °C each week to a final temperature of 24 °C. The total bird rearing period was 42 days.

The birds were fed *ad libidum* mixtures appropriate for each period of rearing, i.e. starter - S (days 1–21), grower - G (days 22–35) and finisher - F (days 36–42), with unlimited access to water. The starter mixture was provided to the chickens in crushed form, and the grower and finisher as granulate. Iron was introduced into the mineral and vitamin premix, which contained no iron, at a level meeting 25% of the requirement for an adult chicken.

The Fe requirement in the feed mixtures, based on the dietary recommendations for Ross 308 broiler chickens, was 40 mg kg^{-1} Fe, which did not include the content of the element in the mixture components (Table 1).

In accordance with current recommendations, the birds received the same Fe content throughout all rearing periods. The birds in the control group received a balanced feed mixture in accordance with requirements for Ross 308 chickens, as well as a mineral and vitamin prefix without iron. In experimental group 2, Fe was added in inorganic form ($FeSO_4$), and in group 3 in the form of a phytase enzyme ($FeSO_4$ + F) supplement. Groups 4 and 5 had Fe provided in organic form, in combination with glycine (Fe-Gly). Group 5 additionally received a phytase supplement (Fe-Gly + F). See Table 2.

For all experimental groups 40 mg kg^{-1} of iron was added to the feed. 500 phytase activity units (FTU)/kg (RONOZYME® HiPhos) were added to the mixtures. The GLYSTAR FORTE chelate by ARKOP Sp. z o.o., containing 15% Fe, was used in the experiment.

Table 1
Raw material composition (%) and nutrition value of experimental mixtures.

Components (%)	Starter (day 1–21)	Grower (day 22–35)	Finisher (day 36–42)
Maize	24.44	40.00	40.00
Wheat	42.99	27.84	28.84
Soybean extraction meal*	25.0	24.97	22.87
Soy oil	2.50	3.69	3.98
1-Ca phosphate	0.90	0.90	0.81
Feed lime	1.40	1.13	1.09
Acidic sodium carbonate	0.08	0.08	0.08
NaCl	0.29	0.25	0.26
Vit.-min. prefix (no Fe)	0.50[a]	0.50[b]	0.50[c]
Protein and fat concentrate**	1.00	–	1.00
DL-methionine 99%	0.30	0.23	0.23
L-lysine HCl	0.42	0.28	0.27
L-threonine 99%	0.18	0.13	0.07
Nutrient value of 1 kg of mixture			
ME, MJ kg^{-1}	12.7	13.1	13.2
[d]BO, %	21.7	20.2	19.6
[d]WS, %	2.41	2.32	2.31
[d]TS, %	4.52	5.28	5.64
[d]Lysine, %	1.28	1.14	1.10
[d]Meth + Cys, %	0.94	0.84	0.83
[d]Ca total, %	0.87	0.79	0.76
[d]P total, %	0.67	0.66	0.64
[e]P assimilable, %	0.43	0.40	0.41
[e]Ca total/P assimilable	2.11	1.91	1.90

[a] Content of vitamins and minerals in 1 kg of starter mixture: Mn 100 mg, J 1 mg, Se 0.15 mg, vit. A 15000 UI, vit. D_3 5000 UI, vit. E 75 mg, vit. K_3 4 mg, vit. B_1 3 mg, vit. B_2 8 mg, vit. B_6 5 mg, vit. B_{12} 0.016 mg, biotin 0.2 mg, folic acid 2 mg, nicotinic acid 60 mg, pantothenic acid 18 mg, choline 1800 mg.
[b] Content of vitamins and minerals in 1 kg of grower mixture: Mn 100 mg, J 1 mg, Se 0.15 mg, vit. A 12000 UI, vit. D_3 5000 UI, vit. E 50 mg, vit. K_3 3 mg, vit. B_1 2 mg, vit. B_2 6 mg, vit. B_6 4 mg, vit. B_{12} 0.016 μg, biotin 0.2 mg, folic acid 1.75 mg, nicotinic acid 60 mg, pantothenic acid 18 mg, choline 1600 mg.
[c] Content of vitamins and minerals in 1 kg of finisher mixture: Mn 100 mg, J 1 mg, Se 0.15 mg, vit. A 12000 UI, vit. D_3 5000 UI, vit. E 50 mg, vit. K_3 2 mg, vit. B_1 2 mg, vit. B_2 5 mg, vit. B_6 3 mg, vit. B_{12} 0.011 μg, biotin 0.05 mg, folic acid 1.5 mg, nicotinic acid 35 mg, pantothenic acid 18 mg, choline 1600 mg.
[d] Analyzed values;
[e] Calculated values.
* 46% general protein in dry matter.
** 1 kg protein and fat concentrate contains: 2% raw fat, 39% raw protein, 10.8 MJ EM.

2.2. Blood samples

The test material was 2 ml samples of peripheral blood taken from the wing vein and by decapitation of day-old chicks, into sterile, heparinized test tubes (Equimed, Warsaw, Poland). Samples were taken before the start of the study (day 0), on day 20 of rearing, and on day 42, i.e. after rearing was completed.

Table 2
Experiment design.

Group	n=	Form of mineral component in mixtures			
		Inorganic	Inorganic + F^1	Organic	Organic + F^1
I (Control)	10	–	–	–	–
II	10	$FeSO_4$a	–	–	–
III	10	–	$FeSO_4$ + F (25%)a	–	–
IV	10	–	–	Fe-Gli (25%)a	–
V	10	–	–	–	Fe-Gli + F (25%)a

F - phytase
[a] Iron in chelate or sulfate form, in amounts equal to 25% of the requirement for chickens.