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Short communication

The effect of dietary supplementation with chromium-enriched soya meal on lymphatic cells in caecal tonsil of laying hens

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ABSTRACT

The study aimed to determine the effects of dietary supplementation with high dose of inorganic chromium from a new additive (soya meal enriched with Cr (III) by biosorption) on lymphatic cells in caecal tonsil of laying hens. The feeding experiment was conducted over 4 weeks on 60 laying hens from Hy-Line Brown line, aged 18 weeks. All birds were kept in a furnished Battery Cage System, in a room with a controlled climate conditions and light regimen of 13–15L:11–9D and were fed either a basal diet – control group (n = 30) or the basal diet supplemented with chromium – Cr-treated group (n = 30). The dietary treatments for Cr-treated group consisted of the basal diet supplemented with 87.7 mg kg^{-1} chromium from a enriched soya meal (20.588 ± 0.212 mg of Cr(III) g^{-1}). The samples of caecal tonsils were stained against chicken Bu-1, CD4, and CD8α antigens and the positive area fractions were counted. It was revealed that the population of $CD4^+$ cells was significantly higher ($p < 0.001$) by unchanged number of the Bu-1^+ and CD8α^+ cells in caecal tonsils of chromium-supplemented hens. The obtained results showed that the supplementation with high dose of chromium from a enriched soya meal stimulates the adaptive immune system in the caecal tonsil what may enhance the response against potentially dangerous antigens.

Abbreviations: Cr, chromium; CT, caecal tonsil; GALT, gut-associated lymphoid tissue; H/L, heterophil/lymphocyte ratio; Th, T helper; Tc, T cytotoxic; IEL, intraepithelial lymphocytes.

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1. Introduction

Many minerals are essential for metabolic pathways and their deficiency can be the source of several diseases (Underwood and Suttle, 1999). The beneficial effect of chromium was first documented for its role as an integral component of the glucose tolerance factor (Schwartz and Mertz, 1959). As a component of the enzyme, trivalent chromium is crucial in carbohydrate metabolism and play important role in cholesterol and amino acid metabolism (Vincent, 2000). A lot of studies have demonstrated that chromium supplementation supports the immune functions, reduces the negative effects of stress and significantly increase the performance (Bahrami et al., 2012; Ghazi et al., 2012; Shrivastava et al., 2002). Hence, the trivalent chromium in inorganic or organic form is widely used as mineral additive for mammals and birds (Burton et al., 1993; Khan et al., 2014). However chromium from organic complex such as chromium picolinate, nicotinate and high chromium yeast is absorbed more efficiently (about 25–30%) than inorganic compounds like chromium chloride (1–3%) (Bahrami et al., 2012; Naghieh et al., 2010). A new additive – a soya meal enriched with trivalent chromium by biosorption may become an alternative in chromium supplementation (Witkowska et al., 2014). With the ion exchange occurring in this process, it is possible to enrich feed with minerals important for health (Chojnacka, 2007).

Caecal tonsil (CT) is one of the most important and readily accessible aggregations of lymphoid tissue within the GALT (Lillehoj and Trout, 1996; Kitagawa et al., 1998). This is the first study that aims to determine the effects of supplementation with high dose (87.7 mg kg^{-1}) of trivalent chromium from a new additive (soya meal enriched with inorganic chromium by biosorptionon) on lymphatic cells in caecal tonsil of laying hens.

2. Materials and methods

2.1. Experimental design – biosorption

Soya meal (Vetos, Zebowice, Poland) was enriched with inorganic chromium ($Cr(NO_3)_3 \cdot 9H_2O$; POCh S.A. Gliwice, Poland; allowed by law as feed additive) by biosorption. The salt was dissolved in distilled water to obtain initial concentration of the salt at 300 mg/L. The process was conducted in a column reactor with a capacity of 4 L at a temperature of 20 °C until complete bed saturation. The received biomass was air-dried at 25 °C for 48 h. The level of chromium was determined using an ICP-OES spectrometer (Varian Vista-MPX; Varian, Palo-ALto, USA) in the Chemical Laboratory of Multi-elemental Analysis at Wroclaw University of Technology, accredited by ILAC-MRA and Polish Accreditation Centre (PCA) (No AB 696).

2.2. Birds, managements and diets

All procedures used in this experiment were approved by the Local Bioethics Committee (Permission No. 129/2010). This study is part of research carried out to evaluate the properties of soya meal enriched with different minerals including high dose of chromium. The feeding experiment was conducted over 4 weeks on 60 laying hens from Hy-Line Brown line, aged 18 weeks, kept in a furnished Battery Cage System, in a room with a controlled climate conditions (temperature: 19–20 °C; humidity: 55–60%) and light regimen of 13–15L:11–9D. Birds were randomly divided into 2 groups and were fed either a basal diet – control group (n = 30) or the basal diet supplemented with 87.7 mg kg^{-1} inorganic chromium – Cr-treated group (n = 30). The composition of the basal diet (Tasomix Universal, Tasomix, Poland) was formulated according to the nutrient recommendations for laying hens (Smulikowska and Rutkowski, 2005), no additional inorganic chromium salt was included in this composition. Additionally, the basal diet was completed with 4.259 g kg^{-1} enriched soya meal (20.588 ± 0.212 mg of Cr (III) g^{-1}) in Cr-treated group. Feed and water were available *ad libitum* in both groups. All birds had the same standard vaccination program which was completed in 16 weeks of age. The design of the experiment was presented in Table 1.

2.3. Sample preservation and staining

After 4 weeks of supplementation the samples of caecal tonsils were collected from randomly selected hens (aged 22 weeks): 20 from control group and 20 from Cr-treated group and preserved immediately after birds killing. The haematoxylin and eosin (H&E) staining was done according to standard protocol. The samples for immunohistochemistry were fixed in 4% buffered paraformaldehyde for 1 h then rinsed with 0.1 M phosphate buffer, infiltrated with buffered 30% saccharose, frozen in cryostat (Leica CM1850, Leica Microsystems GmbH, Wetzlar, Germany) and cut into 10 μm serial sections. All slides were stained with monoclonal mouse anti-chicken antibodies (Southern Biotech, Birmingham, AL, USA) directed against chicken Bu-1, CD4 and CD8α antigens. Non-specific binding was blocked by pre-incubation with Antibody Diluent with Background Reducing Component (Dako, Glostrup, Denmark) for 15 min. Sections were incubated with primary antibodies for 1 h at room temperature in humid chamber. Then slides were washed with 0.01 M PBS and visualised using LSAB+ System HRP (Dako) with DAB+ (Dako). B cells were visualised by means of monoclonal antibodies against chicken Bu-1 antigen (Clone AV 20, chB6, 1:500) (Southern Biotech, Birmingham, AL, 100 USA). T cell subpopulations were detected using anti-CD4 (clone CT-4, 1:200, Southern Biotech) and anti-CD8α (clone CT-8, 1:200, SouthernBiotech) antibodies. The CD4 antigen is primarily found on T helper (Th) cells, and CD8α is typical marker of cytotoxic T cells and NK cells.