"META-ANALYSIS OF THE EFFECT OF DIETARY SELENIUM SUPPLEMENTATION ON GLUTATHIONE PEROXIDASE ACTIVITY IN POULTRY"

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Abstract - The term Glutathione Peroxidase (GPx) defines a family of enzymes in the antioxidant system of aerobic organisms. Among them we find the seleno-enzymes, which contain Selenium (Se) in their active site as a fundamental factor in the efficiency of the enzyme's activity. Because of its pivotal role, supplementing animal diets with Se has become a common practice in farming. In the present work a meta-analysis (MA) of means was performed on the results of a literature search of electronic databases, complemented with a one way analysis of variance (ANOVA). The objective was to evaluate and compare the tendency of the effects produced by supplementation with different doses and sources of Se on the activity of Se-dependant GPx in the blood of poultry, which were initially fed either with a basal Se or Se deficient diet. The results showed a significant increase in enzymatic activity only when the initial diet was deficient in Se. Only one group showed a significant difference between organic and inorganic Se. From these observations it can be concluded that when the initial diet has a normal Se content, supplementation with Se would not produce a significant effect on GPx activity.

I. INTRODUCTION

Glutathione Peroxidase (GPx) is a family of enzymes in the antioxidant system of aerobic organisms. It catalyzes the reaction in which reduced Glutathione reacts with peroxides to transform them to water and alcohol. GPx has the capacity to protect the integrity of unsaturated bonds of membrane phospholipids by extinguishing free radical attacks capable of initiating and propagating lipid oxidation (1).

The main form of GPx is Selenium (Se) dependant and uses it as a cofactor. The importance of Se in animal nutrition lies in the fact that several levels of antioxidant defence

rest on the Se-dependant GPx activity, which depends on an adequate Se status in the cell.

Lipid oxidation is accelerated when there is a deficiency in Se (2). There are different mechanisms through which the antioxidant system can be altered or regulated. The main one is the synthesis of antioxidant enzymes (e.g. SOD and GPx), as the animal responds to stress conditions. However, this response will only be effective if the cofactors needed for the enzymatic action are available. Therefore, the nutritional intake of Se is a crucial factor in the regulation of GPx activity and the efficiency of the antioxidant system (3).

Lipid oxidation is one of the main factors limiting the quality and acceptability of meat and meat products. It leads to discolouration, drip losses, off-odour and off-flavour development, and the production of potentially toxic compounds (4). There are several critical phases of lipid oxidation: the balance between production of reactive oxygen species and the antioxidant defence system in the living animal; oxidative damage in the immediate post-slaughter period and oxidation during handling, processing, storage and cooking.

There are indicators that show that dietary supplementation with selenium, particularly organic selenium, improves quality and shelf life of poultry meat. Studies on chicken and turkey have shown that organic selenium is deposited more effectively in muscles than inorganic selenium. The increased tissue concentrations of selenium not only decreases stress, including oxidative protecting unsaturated fatty acids from peroxidation damage, but can also reduce drip loss from meat and the incidence of pale soft, exudative meat. It also improves the shelf life during refrigeration. (5)

Meat colour and quality analysis in a study by Yang (6) showed that birds fed with diets containing 0.3 ppm of organic Se had a decrease in cooking loss and myoglobin oxidation, and an increase in the degree of red colour in the meat. These findings suggest that Se-enriched yeast improved the antioxidative status of broilers by increasing the activity of antioxidant enzymes, hence dietary Se supplementation, especially with organic Se, may have a beneficial effect on the oxidative stability and extended shelf life of fresh meat.

In the present study the effect of Se supplementation on GPx activity was analysed for different species and tissues in the following diverse situations: initial diet deficient in Se; initial basal diet with normal Se content and diets supplemented with organic or inorganic sources of Se.

The objective of the analysis was to study the tendency of the effect of dietary supplementation with Se on GPx activity in animals in different situations, performing the relevant comparisons between groups. Only the results where GPx activity was measured in poultry blood are shown.

II. MATERIALS AND METHODS

A literature electronic search was performed to identify the relevant studies. The EBSCO meta-search service was used to search in the databases: following Academic Search Complete, CAB Abstracts, Directory of Open Access Journals, MEDLINE, Ovid Journals, Science Direct and Scopus, using the key words: GPX (abstract) AND Selenium (abstract) AND muscle (all text) without restrictions of date, language or type of document. In addition, a search for "grey literature" was performed through the search engine Google to minimize publication bias, which can happen when searching exclusively for literature published in refereed journals.

Then the literature retrieved was filtered according to inclusion and exclusion criteria, which were previously set to ensure the articles were methodologically homogeneous. Subsequently the quality of the studies used in the meta-analysis was evaluated using as a guideline the table described by Berman (7). The parameters described there were adapted according to the conditions of the present study.

The programme NCSS 2007 was used for statistical processing of the data. A meta-

analysis of means was performed, with α = 0.05 and a One Way ANOVA for each group to complement the meta-analysis, studying GPx activity vs. Se dose and GPx activity vs. type of Se (organic or inorganic). For the latter the Tukey-Kramer Multiple Comparison Test was used.

Homogeneity was evaluated using the Cochran's Q Test and the Radial Plot (Galbraith Plot).

Publication bias was assessed with a Funnel Plot, using the graph Standard Error vs. mean of Se supplemented GPx Activity.

III. RESULTS AND DISCUSSION

The obtained measurements were grouped according to their species, tissue, initial diet and dose of Se supplementation. This paper shows the results of two of the four groups analysed, which measured GPx activity in blood of poultry (laying hens, broiler chickens and turkeys). The groups are as follows:

Group 1: Poultry, without stress, initial basal diet in Se supplemented with low-medium dose of Se (between 0.01 and 0.46 ppm).

Group 2: Poultry, without stress, initial diet deficient in Se supplemented with low-medium dose of Se.

Even though data was obtained for several different tissues in each group, it was only possible to perform the MA for those tissues which had a minimum of six measures.

The heterogeneity test was performed on the different groups and the result was significant for each of them, showing that the data was heterogeneous in all cases. This was confirmed by the Radial Plots; therefore the Model of Random Effects was used to perform each MA.

Figure 1 shows the Forest Plot for group 1. This indicates that there are non-significant results (where the confidence interval line intersects the vertical line 0.0), but the average measure is significant. The size of the symbol is correlated with the weight each study has in the MA.



Fig. 1. Forest Plot Group 1

Comparing the MA results for Group 1 and Group 2 verifies that for Group 1 the GPx activity does not increase by the same amount as Group 2, which correlates with the study design. Though in both cases the Se supplementation dose was low-medium, Group 1 started with a basal diet (normal Se intake) while Group 2 started with a diet deficient in Se. The MA forest plot for Group 2 is shown in figure 2.



Fig. 2. Forest Plot Group 2

To complement the meta-analysis, a One Way ANOVA (OWAV) was performed on each group to study the effect on GPx activity of the dose of Se supplementation and type of Se (organic or inorganic).

The increase in GPx activity based on Se dose for Group 1 was not significant (probability level > α), which correlates with the study design; animals in Group 1 started from a diet with a normal Se content. From the Tukey-Kramer Multiple Comparison Test we observe that there are no significant differences between the control group and group 1, which coincides with the results of the MA. There is no increase in the activity of GPx based on Se dose, neither for inorganic Se nor for organic Se.

For Group 2, the increase in GPx activity based on Se dose was significant (probability level $< \alpha$), which corresponds with the study design, because this group was fed initially with a diet which was deficient in Se. The results for the Tukey-Kramer test show that the group with a Se deficient diet differ significantly from the supplemented group. It also shows a significant difference between both extremes in the range of the evaluated supplementation; with higher supplementation having a greater effect.

The results also show that there is a significant difference between the control group (deficient in Se) and the supplemented groups (specifically with inorganic Se), which is in accordance with the result of the meta-analysis performed for this group. There is no significant difference between the group supplemented with organic Se and the one supplemented with inorganic Se.

IV. CONCLUSIONS

According to the results obtained, we observe that when we start from a basal diet with a normal Se content, there is no effect produced by supplementation, as we see in Group 1. This may be due to the better oxidative stability caused by sufficient intake of Se, which without stress in the study design, causes a lower need for the synthesis of the antioxidant enzyme (8) (9). However, when we start from a diet deficient in Se, we can see a clear effect caused by Se supplementation in the diet on GPx activity, as is evident in Group 2. In addition, in this group a significant difference was observed between the results of adding different doses of Se, increasing the enzyme's activity to a higher degree when supplementing with a higher dose. Several articles (10) (11) state that if the initial diet is deficient in Se, when the dose is increased, the GPx activity also increases until it reaches a well defined plateau, therefore any further

increase in the dose will not produce a greater effect.

Regarding the different sources of Se, in Group 1 there was no difference observed between the animals fed with a diet supplemented with organic Se and the ones fed with a diet supplemented with inorganic Se. However, in Group 2 the increase in enzymatic activity was only significant when inorganic Se was administered. With respect to this observation, there is much controversy among researchers. Some of them comment that inorganic Se is metabolised more effectively and as a result GPx activity increases to a higher degree than when organic Se is supplied (12) (9). Others obtained results that favour the supplementation of organic Se over inorganic, based on a more effective absorption of Se in the intestine when it is attached to an organic source (1)(13)(6).

There are many known factors that affect the GPx activity apart from dietary Se. These include age, sex and ambient variables that can produce stress. It is interesting to note that some studies in young chickens on growth response to nutritional Se deficiency showed that these had a hereditary component (14). As a result, the consequences of Se nutritional deficiencies in animals could differ among genotypes. Therefore, it is of paramount importance to take into account all factors involved in a particular study to thoroughly understand and evaluate the results.

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