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# THE INFLUENCE OF IRON AND COPPER OVER THE INTESTINAL ENZYMATHICAL ACTIVITY

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***Abstract***

*The purpose of this study is the analysis of copper and iron microelements influence over the activity of intestinal amylase and maltase unfolding research on rats that the physiologically resemble swine, making it possible that the data obtained can be extrapolated. In most cases, the addition of microelements influence has manifested by inhibition of the enzymatic activity of intestinal maltase and amylase, growing with the increasing of concentration. A slight activation of intestinal amylase was observed with the administration of 600 ferric sulphate mg/kg food, maximal activation being of 104.3%.*

***Keywords***: *copper, iron ,intestinal amylase, intestinal maltase.*

## INTRODUCTION

Enzymes are biocatalysts, representing a special class of protein molecules that alter the kinetics of reactions which they catalyze, participating in small amounts in these reactions and do not undergone transformations during their development.

Enzymatic reactions can be influenced by various substances that act directly on the enzyme molecules. For example, salts of heavy metals, such as silver, copper, mercury or lead, in high concentrations, inactivate most of the enzymes. Some enzymes are very sensitive to even low concentrations of metal, such as for fructofuranosidase, which is inhibited by the silver salts.

The mechanism of inhibition is relatively well known, some authors suggesting that metallic ions combine with thiolic groups, while others do with the carboxyl groups.

Although the economic importance of the use of trace elements in animal rations is known, a relatively low number of data regarding their influence on the activity of various digestive enzymes is noticed.

Amylase is an enzyme that catalyzes the reaction for conversion of starch to dextrins and then to maltose. Amylase occurs in the digestion of carbohydrates, being secreted by the salivary glands, pancreas and small intestine mucosa.

Maltase is an enzyme secreted by the lining of the small intestine mucosa that breaks down the disaccharide maltose into two molecules of glucose.

Therefore, the aim of this study is the analysis of copper and iron microelements influence over the activity of intestinal amylase and maltase, the biological material being represented by rats, knowing from the metabolically point of view the rat specie resembles the swine, which will make possible extrapolation of data obtained in swine.

## MATERIALS AND METHODS

In order to study the action of the microelements trace of copper ($Cu^{2+}$) and iron ($Fe^{2+}$ and $Fe^{3+}$) the metal sulphate (cupric sulphate, ferrous sulphate, ferric sulphate)was used in these, being added to the vitamin and mineral premixes.

The biological material was represented by Wistar rats. They were fed a compound food similar to the one given to the young swine (Table 1). The batches of animals received premixes with a variable content of iron and

copper (Table 2). In a first stage 50 rats were used, being assigned to 10 batches, that have received as a supplement in premix divalent iron as ferrous sulphate which provided a content of 150, 300, 450, 600, 750, 900, 1050, 1200, 1350 mg/kg feed. In the second phase another 50 rats received mixes in which the trivalent iron content in the form of addition of ferrous sulphate varied, providing 150, 300, 450, 600, 750, 900, 1050, 1200, 1350 mg/kg feed. In the third step another 10 batches of 5 rats were given copper in premixes under the form of copper sulphate, providing 10, 20, 30, 40, 50, 60, 70, 80, 90 mg/kg.

Table 1. Composition and nutrients of diet

| Ingredient | Composition (%) |
|---|---|
| Maize | 65.50 |
| Soya meal | 20 |
| Sunflower meal | 10 |
| L-lysine | 0,37 |
| DL-methionine | 0,08 |
| Threonine | 0,05 |
| Calcium carbonate | 1,21 |
| Dicalcium phosphate | 0,86 |
| Salt | 0,50 |
| Vitamin-mineral premix | 1,00 |
| Total | 100.00 |
| Nutrients level (calculated) | |
| Metabolizable energy (Mj/kg) | 3126 |
| Crude protein (%) | 17,95 |
| Lysine (%) | 1,19 |
| Methionine+cystine (%) | 0,61 |
| Calcium (%) | 0,85 |
| Total phosphorus (%) | 0,48 |
| Available phosphorus (%) | 0,29 |

Table 2. Content of iron and copper of the administrated ratio

| Experimental batch | Iron (mg/kg feed) | Copper (mg/kg feed) |
|---|---|---|
| E 1 | 0 | 0 |
| E2 | 150 | 10 |
| E3 | 300 | 20 |
| E4 | 450 | 30 |
| E5 | 600 | 40 |
| E6 | 750 | 50 |
| E7 | 900 | 60 |
| E8 | 1050 | 70 |
| E9 | 1200 | 80 |
| E10 | 1350 | 90 |

The action of the two microelements has been studied using samples collected from the intestinal mucosa of rats after slaughter. Portions of the mucosa were homogenized in distilled water (4 ml per 1 g of tissue), after which they were kept for 24 hours at 4$^{o}$C and then centrifuged for 15 minutes.

The determination of enzyme activity was performed by dosing of a substrate of enzymatic reaction (starch) in the presence of the enzyme (amylase and maltase) that catalyze these reactions. The enzymatic activity represents the difference between the transformed substrate concentration in reaction product by enzyme and unchanged substrate concentration.

The quantitative expression of the enzymatic activity is done by activity units (U). U is the amount of enzyme that catalyzes the conversion of 1 μM substrate/min/l under optimum conditions of temperature and pH (Diaconescu, 2004). In current mode, the enzymatic activity is expressed as mU/ml. One unit of amylase or maltase activity is defined as the amount of enzyme that catalyses the conversion of 1 μg of substrate, namely glucose, per 1 minute/l at a pH of 7.

## RESULTS AND DISCUSSIONS

The results concerning the influence of iron and copper microelements on the enzymatic activity of amylase and maltase are presented in Table 3.

The action of ferrous sulphate on intestinal amylase activity is materialized by a low reduction (3.1%) until the concentration of 300 mg/kg feed, while at the amount of 1050 mg/kg the decreased activity is of 24.7%, at 1200 mg/kg is of 38.5% and at 1350 mg/kg it reaches 56.3%.

Rat intestinal amylase is easily activated in the presence of small amounts of ferric sulphate, the maximal activation (104.3%) being found in a concentration of 600 mg/kg feed. At the following amount of 750 mg ferric sulphate / kg feed, there is a slight decrease from the maximum activity (with 15.7%). An almost total reduction of the enzyme (9.3 $Uml^{-1}$) occurs at a concentration of 1350 mg/kg feed.

From the analysis of the influence of copper sulphate over the action of intestinal amylase on rats, a slight decrease in activity up to the concentration of 30 mg/kg feed is observed. The noticeable reduction is of about 5.3%. At an increase in the amount of copper sulphate in feed above this value, a strong reduction of amylase activity about 57.3% in the amount of 70 mg/kg feed, 80.6% at 80 mg/kg and 94.2% at 90 mg/kg are observed.

Table 3. Influence of iron and copper of the intestinal enzymatic activity

| Content in microelements of feed (mg/kg feed) | Amylase $Uml^{-1}$ % | Maltase $Uml^{-1}$ % |
|---|---|---|
| Divalent Iron | | |
| 0 | 15.57±1.12 100 | 13.93±1.12 100 |
| 150 | 15.18±1.04 97.5 | 13.70±2.42 98.4 |
| 300 | 15.09±0.87 96.9 | 13.44±1.37 96.5 |
| 450 | 14.23±1.65 91.4 | 12.70±1.63 91.2 |
| 600 | 14.18±1.53 91.1 | 11.92±1.93 85.6 |
| 750 | 14.14±1.84 90.8 | 11.86±2.01 80.5 |
| 900 | 13.45±0.97 86.4 | 10.43±1.11 74.9 |
| 1050 | 11.72±1.35 75.3 | 9.79±0.86 70.3 |
| 1200 | 9.57±1.74 61.5 | 9.48±1.04 68.1 |
| 1350 | 6.80±0.53 43.7 | 8.98±0.64 64.5 |
| Trivalent Iron | | |
| 0 | 20.17±2.12 100 | 12.41±0.79 100 |
| 150 | 20.47±3.07 101.5 | 12.42±1.17 100.1 |
| 300 | 20.85±2.52 103.4 | 12.41±2.04 100.0 |
| 450 | 20.91±1.95 103.7 | 12.41±1.89 100.0 |
| 600 | 21.03±2.75 104.3 | 12.43±0.99 100.2 |
| 750 | 17.00±1.29 84.3 | 12.45±2.10 100.4 |
| 900 | 15.18±1.49 75.3 | 12.39±1.49 99.9 |
| 1050 | 10.51±1.11 52.1 | 12.41±1.75 100.0 |
| 1200 | 6.99±0.48 34.7 | 12.38±1.36 99.8 |
| 1350 | 1.87±0.07 9.3 | 12.39±1.92 99.9 |
| Copper | | |
| 0 | 17.34±1.93 100 | 14.35±1.37 100 |
| 10 | 17.25±1.14 99.5 | 14.69±1.59 102.4 |
| 20 | 16.52±1.47 95.3 | 14.91±2.00 103.9 |
| 30 | 16.42±1.57 94.7 | 15.11±1.26 105.3 |
| 40 | 13.94±1.04 80.4 | 16.14±1.42 112.5 |
| 50 | 13.14±0.85 75.8 | 14.29±0.95 99.6 |
| 60 | 7.40±0.34 42.7 | 10.80±0.54 75.3 |
| 70 | 3.36±0.08 19.4 | 6.63±0.25 46.2 |
| 80 | 1.85±0.02 10.7 | 4.33±0.08 30.2 |
| 90 | 1.00±0.01 5.8 | 1.35±0.01 9.4 |

L. Fang et al. (2012) observed that pancreatic amylase was more sensitive than salivary amylase to small amounts of copper chloride.

Li et al. (2007) noticed that the addition of copper to hybrid tilapia reduced the activity of intestinal protease and inhibited the activities of amylase in intestine and hepatopancreas. Addition of iron reduced the activities of amylase in the intestine by 47.9%, but had no effect on amylase activities in the hepatopancreas.

Intestinal maltase in rats shows a steady decrease in activity related to the $Fe^{2+}$ ion concentration, inactivation being more than 35.5% for an amount of 1350 mg ferric sulphate/kg feed.

Analyzing the influence of ferric sulphate ($Fe^{3+}$) over the activity of intestinal maltase it was found that $Fe^{3+}$ ions had no effect over the studied enzyme, its values remaining constant at the variation of the amount of administered ferric sulphate.

Intestinal maltase undergoes a further activation in the presence of copper sulphate up to the amount of 40 mg/kg feed, when the activation was of 12.5%. For 50 mg copper sulphate/kg feed, the intestinal maltase activity is slightly inhibited, and for the subsequent increase in the amount of copper sulphate, the maltase activity is inhibited by 53.8% at 70 mg/kg and by 69.8% at 80 mg/kg. For the maximum concentration analyzed (90 mg/kg) it was found to inhibit the enzyme activity, which reaches only 9.4% of the initial activity.

Studying the effects of heavy metals over the intestinal maltase activity for rats, it was observed that the average amount of added copper ions, the concentration of enzyme was moderately inhibited.

## CONCLUSIONS

From analysis of the presented results, it follows that the addition of divalent or trivalent iron and copper influence the activity of intestinal amylase and maltase in rats that resembles the physiologically with swine, which allows the data obtained to be extrapolated from them.

In most cases, the addition of microelements influence has manifested by inhibition of the enzymatic activity of intestinal maltase and amylase, growing with the increasing of concentration.

A slight activation of intestinal amylase was observed with the administration of 600 ferric sulphate mg/kg food, maximal activation being of 104.3%. Further, with the increase of the addition of divalent iron source a nearly complete inactivation of the enzyme (9.3 $Uml^{-1}$) at a concentration of 1350 mg/kg feed is achieved.

The addition of copper sulphate until the amount of 40 mg/kg of feed determines an activation of intestinal maltase, so that, afterwards, at increased amounts of copper sulphate to lower the enzyme activity, reaching the maximum concentration analyzed (90 mg/kg), the inhibition the enzymatic activity to be of 90.6%.

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