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THE INVESTIGATION OF ALFALFA EFFECT ON THE ACTIVITY OF SUPEROXIDE DISMUTASE IN CHICKEN MEAT in dependence on TIME STORAGE

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ABSTRACT

This study was conducted in order to monitor the effect of adding lucerne meal to chicken feed mixtures. The experiment was conducted at the Department Food Hygiene and Safety, Faculty of Biotechnology and Food Science, Slovak University of Agriculture in Nitra. Chickens for meat production - final type Cobb 500 were used in the experiment. Chickens were placed in boxes all together for one group at the beginning of the experiment and from 14 days of age chickens were divided individually into floor enriched cages. Feeding of chickens lasted 38 days. The experiment was carried out without sex segregation. For the production of a feed composition was used alfalfa (*Medicago sativa*) as lucerne meal, which was added to the feed at a rate of 4%, namely: starter (HYD-01), growth (HYD-02) and final (HYD-03). The control group did not include the addition of lucerne meal. Chickens were fed *ad libitum*. Chickens were slaughtered after completion of feeding and the meat samples were taken for analysis. The collected samples were stored at -18 °C. Collected samples of meat were analyzed after slaughter chickens at time intervals of 6, 12 and 18 months. In the experiment was monitored the content of supeoxid dismutase in the chicken meat depending on the length of storage time. Superoxide dismutase content was increasing by storage time, while there were some statistically significant differences between groups.

Keywords: oxidation, superoxid dismutase, lucerne meal, meat, chicken, time storage

INTRODUCTION

Oxidation processes are one of the primary mechanisms of quality deterioration in meat and meat products because they lead to the degradation of lipids and proteins (including haem pigments) and they cause the loss of flavour, colour and nutritive value and limit the shelf-life of meat and meat products **(Kanner, 1994; Karwowskaet al., 2007)**.

The mechanisms of oxidative degradation can be autoxidation in presence of atmospheric oxygen **(Angelovič et al., 2015)**.

Lipid peroxidation is a primary cause of quality deterioration in meat and meat products. Free radical chain reaction is the mechanism of lipid peroxidation and reactive oxygen species (ROS) such as hydroxyl radical and hydroperoxyl radical are the major initiators of the chain reaction. Lipid peroxyl radical and alkoxyl radical formed from the initial reactions are also capable of abstracting a hydrogen atom from lipid molecules to initiate the chain reaction and propagating the chain reaction **(Min and Ahn, 2005)**.

Enzymes such as superoxid dismutase, katalase and glutation peroxidase can prevent of meat oxidation because they have influence to oxidation of muscle fiber **(Daun and Akesson, 2004; Tkáčová and Angelovičová, 2013)**.

The superoxide dismutases (SODs) are the first and most important line of antioxidant enzyme defense systems against ROS and particularly superoxide anion radicals. At present, three distinct isoforms of SOD have been identified in mammals, and their genomic structure, cDNA, and proteins have been described **(Chang et al., 1988; Keller et al., 1991; Crapo et al., 1992; Liou et al., 1993; Zelko et al., 2002)**.

Superoxide dismutases (SODs) are metalloenzymes found widely distributed in prokaryotic and eukaryotic cells **(Fridovich, 1995; Johnson and Giulivi, 2005)**.

These SODs are historically designated, in higher eukaryotes, by their primary location as follows: SOD1 (cytoplasmic), SOD2 (mitochondrial) and SOD3 (extracellular) **(Marklund, 1984; Johnson and Giulivi, 2005)**.

The superoxide dismutases (SODs) are ubiquitous components of cellular antioxidant systems. As described by McCord and Fridovich over 47 years ago, these proteins protect redox sensitive cellular machinery from damage by catalyzing the disproportionation of superoxide anion to oxygen and hydrogen peroxide **(McCord and Fridovich, 1969; Culotta et al., 2006).**

Zhou and Prognon (2006) dates discovery of the protein superoxide dismutase (SOD, EC 1.15.1.1) in 1969.

Superoxide dismutase is pervasive metaloenzym in aerobic organisms **(Bannister, 1987)**. Belongs to the superoxide dismutase enzyme groups, which in SOD molecules content metal - copper, zinc, manganese **(Crapo et al, 1992)**. Copper/zinc superoxide dismutase is widely distributed in eukaryotes, whereas the iron-manganese superoxide dismutase is primarily found in the mitochondria or in prokaryotes **(Fridovich, 1986).** Copper / zinc are mainly presented in the cytosol **(Steinman, 1982)**. The main function of superoxide dismutase is dismutating. It provides protection of biological tissue before not controled reactive oxygen species **(Crapo et al., 1992),** especially before superoxide radicals **(Rotilio Bannister, 1987)**. It is believed that superoxide dismutase belongs to among the most important line of antioxidant enzyme defense systems **(Fridovich, 1995)**. Superoxide dismutase helps to use zinc, copper with manganese in the body **(Bannister Rotilio, 1987; Tkáčová and Angelovičová, 2013).**

Functions of superoxide dismutase have been widely studied by many authors. They have found that it have impact on the various human diseases, such as arteriosclerosis, diabetes mellitus, Down syndrome **(Kakko et al., 2003; Haskins et al., 2004; Engidawork and Luberc; 2001, Culotta et al., 2006)**.

The strongest connection between SODs and human disease is found for the copper and zinc dependent forms, mutations in which can cause the neurodegenerative disease amyotrophic lateral sclerosis (ALS) **(Rosen et al., 1993; Culotta et al., 2006).**

The role of superoxide dismutase is to accelerate the dismutation of the toxic superoxide radical ($O_2^{\bullet}$), produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen **(Randox, 2009)**.

Nowadays there is a strong tendency towards isolating organic antioxidants from natural sources as alternative methods to retard oxidative processes in meat and meat products **(Wenk, 2003; Karwowska et al., 2007).**

The addition of antioxidants to meat products is known to be effective in colour stability and lipid oxidation. Many authors report about the benefits of adding vitamin E **(Buckley et al., 1995; Faustman et al., 1998; Haak et al., 2006; Houben and Gerris, 1998)**, tea catechins rosemary extract in the compound feed **(O'Grady et al., 2006)** Thereby affecting meat quality **(Karwowska et al., 2007)**, but in the literature are little reports with information about the benefits alfalfa diets for the oxidation of meat**.**

Alfalfa is well balanced in amino acids and rich in vitamins, carotenoids **(Sen, 1998)** and saponins **(Whitehead, 1981)**. Carotenoids are polyenoic terpenoids having conjugated trans-double bonds. They include carotenes (β-carotene and lycopene), which are polyene hydrocarbons, and xanthophylls (lutein, zeaxanthin, capsanthin, canthaxanthin, astaxanthin, and violaxanthin) having oxygen in different form **(Bonnie, 2000; Tkáčová and Angelovičová, 2013).**

Moreover, the alfalfa has anti-carcinogenic and antioxidant effects **(Rao and Gurfinkel, 2000; Tkáčová and Angelovičová, 2013).**

The aim of the experiment was to determine an activity of superoxide dismutase in chicken meat in dependence on time storage. Meat samples were analyzed after time storage 6, 12, 18 months.

Scientific hypothesis

At the beginning of our experiment we supposed that the storage of chicken meat will increase the activity of superoxide dismutase. We also supposed that will be statistical confirmed differences of superoxide dismutase activity between groups of chickens and between storage periods (6, 12, 18 months).

MATERIAL AND METHODOLOGY

The experiment was performed at the experimental facility of the Department Food Hygiene and Safety, Faculty of Biotechnology and Food Science, Slovak University of Agriculture in Nitra. At the start of the experiment the chickens was housed in the box and they were divided individually into two-storey enriched cages from 14 days of age.In the experiment were used chickens for meat production - final type Cobb 500.

The experiment was performed according to the scheme: total experimental period chickens were divided into three phases according to the type of the feed mixtures:

a) the starter, it was intented for chickens from hatching to 18 days of age; during this time chickens received starter feed mixture HYD 01, in the experimental group it wasenriched with 4% alfalfa meal ,

b) growth, it was intented for chickens from days 19to 31 of age; chickens fed the growth feed mixture HYD 02during this time, in the experimental group it was enriched with 4% alfalfa meal,

c) final, it was intended for chickens from days 32 to 38 of age; chickens fed a final feed mixture HYD 03during this time, in the experimental group it was enrichedwith 4% alfalfa meal.

The company Biofeed a. s. Kolárovo produced feed mixtures. The feed mixtures in powder form were used. Alfalfa was used to the mixture in the vegetation phase of pucks by drying. Chickens were slaughter at the Department of Evaluation and Processing of Animal Products. The collected meat samples were stored at -18 °C.

Preparation of the samples

The sample preparation was determinated by modified method of **Jung and Henke (1996)**.

This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetra-zolium chloride (I.N.T.) to form a red formazan dye. The superoxid dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the role of reduction of INT under the conditions of the assay **(Randox, 2009)**.

1 gram of sample was flushed with ice-cold 154 mM NaCl solution, subsequently, the sample is placed in

homogenization medium, consisting of 250mM mannitol, 70 mM sucrose, 1 mM EDTA and the solution is adjusted with Tris to pH 7.4.

After homogenization of tissue in homogenization solution, the sample was centrifuged 10 min at 800xg in a centrifuge SW 14 FROILABO (Meyzieu, France). The supernatant was used as a homogenate.

In cold environments, sonication was performed on the unit UP 100 H Ultrasonic Processor Companies Hielscher 300W (Hielscher, Germany) in three 10-second intervals. Finally, the sample was centrifugated at 14000xg in 10 minutes.

The resulting supernatant was pipetted into a test tube and stored at -70 °C until analysis.

The prepared sample is measured using a kit RANSOD superoxide dismutase, Diluent and Control from Randox. After preparation of the various reagents and calibration solutions are measured activity of superoxide dismutase by spectrophotometry at 505 nm and 37 °C.

The concentration of superoxide dismutase was read from the calibration curve dependence of absorbance and concentrations of superoxide dismutase.

The resulting value of the contents of superoxide dismutase was expressed as SOD units, $U.g^{-1}$ of muscle.

Statistical analysis

The data obtained from experiment were evaluated according to basic statistical characteristic (average, s = standard deviation, CV = coefficient of variation). Difference between groups was tested according to Scheffe´s test ($\alpha = 0.05$). It was used ANOVA in the programSAS version 9.1.

RESULTS AND DISCUSSION

Different authors **Suksombat and Buakeeree (2006), Al-Haweizy and Al-Sardary (2007), Bobko et al. (2012)** and **Tkáčová et al. (2015)** studied the influence of lucerne meal on production quality by adding different proportions of lucerne meal to feed mixtures. It has been found that the best contents of lucerne meal is 4% in a feed mixtures.

Superoxide dismutase activity has rising tendency during time storage as the control group and the experimental group (Figure 1).

The average activity of superoxide dismutase after time storage of meat 6 months at -18 °C was at the level of 14.57 $U.g^{-1}$ and in the control group reached almost the same value 14.08 $U.g^{-1}$.The difference in the activity of superoxide dismutase was not statistically significant (p >0.05) after time storage of meat 6 months at -18 °C between the groups.

The average activity of superoxide dismutase after time storage of meat 12 months was at the level of 46.3 $U.g^{-1}$ in group with a share of 4% lucerne meal and in control group 38.6 $U.g^{-1}$.The difference in the activity of superoxide dismutase was statistically significant (p <0.05) after time storage of meat 12 months of storage of the meat at -18 °C between the groups.

The average activity of superoxide dismutase was grouped with a share of 4% lucerne meal at the level of 52.30 $U.g^{-1}$ after time storage of meat 18 months of at -18 °C and in the control group 34.58 $U.g^{-1}$.The difference in the activity of superoxide dismutase was statistically significant (p <0.05) after time storage of meat 18 months at -18 °C between the groups.

Statistical diferences between all groups in the experiment are shown in Table 1.

Authors are explaining alfalfa contains in addition to compounds with antioxidant properties and also substances that have a negative impact on production - a high proportion of fiber, low energy value **(Dansk, 1971; Suksombat and Buakeeree, 2006)** compared to other feed. Alfalfa contains a high level of anti-nutritional factors - saponins **(Francis et al., 2002; Stochmal et al., 2001)**.

Few authors, however deals with the effect of lucerne meal feeding in chicken meat on oxidation.

Gibbs et al. (2013) argues, based on feed experiments that it is possible to influence the content of unsaturated fatty acids in chicken meat by adding fishmeal to the feed mixture, and thus the feeding can affect the subsequent oxidation of meat. **Hugo et al. (2009)** applied fish oil and sunflower oil into chicken feed mixture.

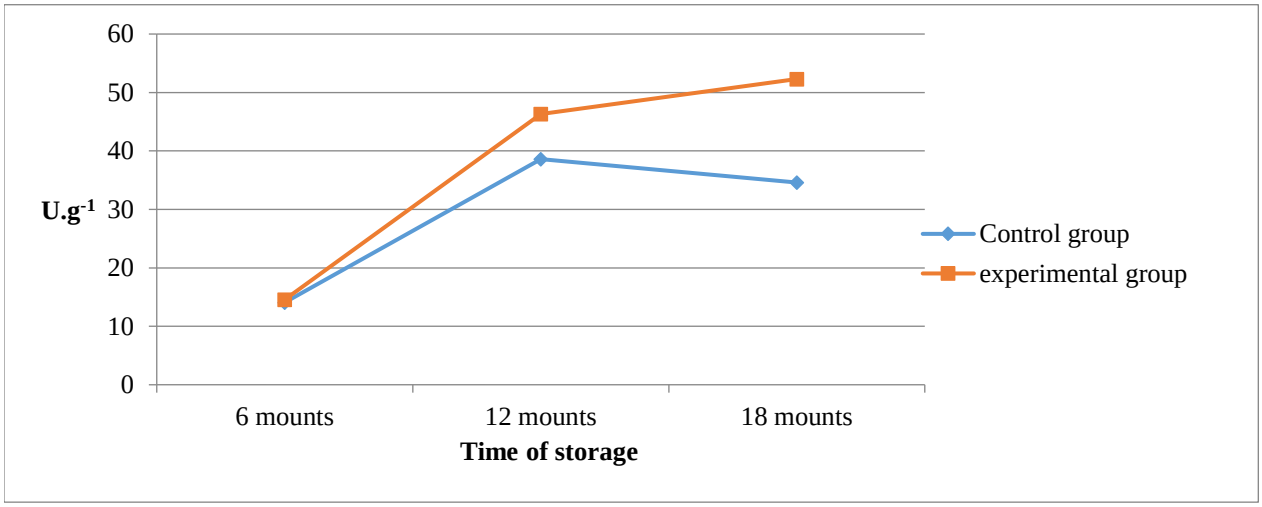

Figure 1 The activity of superoxide dismutase in dependente to different time storage of meat at -18 °C.

Table 1 Statistical evaluation of the differences of superoxide dismutase activity between the different time storage of meat at -18 °C and between groups of chickens.

F-test	$21,15^{+++}$					
Group						
	K 06	**E 06**	**K 12**	**E 12**	**K 18**	**E 18**
K 06		-	+++	+++	+++	+++
E 06			+++	+++	+++	+++
K 12				-	-	-
E 12					-	-
K 18						+++

The effect of dietary supplementation with extracted alfalfa meal (2 g per 1 kg diet) on oxidative stability studied **Karwowska et al. (2007)**. The results did not indicate the influence of dietary supplementation with extracted alfalfa meal on the changes of lipid oxidation of smoked ham. During the storage period (14 days) of control and experimental ham slight changes of TBARs values were noted.

Dong et al. (2011) indicates in their work polysavone modulates antioxidation properties and modifies meat quality, but with no adverse effect on performance of broiler chickens.

Castellini et al. (2002) carried out the experiment with chickens. They fed chickens with feed mixture with 2.8% of dehydrated alfalfa meal. These organic chickens had lower levels of abdominal fat. Polyunsaturated fatty acids of n-3 series and TBA-RS were higher. A negative aspect was the higher level of TBA-RS in the muscles, probably due to greater physical activity.

Similarly, **Lu et al. (2006)** says the addition of the supplemental Mn did not influence ($p>0.05$) content of malondialdehyde (MDA)and activity of Mn-containing superoxide dismutase (MnSOD) in breast muscle and activity of malate dehydrogenase (MDH) and hormone sensitive lipase (HSL) activity in abdominal fat too.

The primary difficulty in assaying Superoxide dismutase (SOD) for its enzymatic activity consists in the free radical nature of its substrate O2•- which can only be supplied by generation within the assay medium. The substrate O2•- cannot easily be detected directly by conventional analytical tools **(Flohé and Ötting, 1984)**.

Barriere et al. (2001) performed experiment where they used *Staphylococcus xylosus* as starter culture in sausages. He was to characterize the roles of catalase and superoxide dismutase (SOD) in the inhibition of free fatty acid oxidation by the *S. xylosus*. SOD of *S. xylosus* contributed to the inhibition of lipid oxidation.

CONCLUSION

Alfalfa is interesting object examining not only for human nutrition but also in animal nutrition for their content of biologically active substances. It can be used for nutrition of chickens but only in limited quantities because a higher proportion 6% or more has a negative impact on production. On the basis of experimental results we can state that proportion of alfalfa in feed mixture can have positive effect on activity of superoxide dismutase. It is appropriate to include of alfalfa in chicken feed mixtures for its other benefits.

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