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# THE EFFECT OF DIETARY ALFALFA MEAL ON THE CHICKEN MEAT QUALITY

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## ABSTRACT

The purpose of this study was to investigate the effect of feed mixtures with proportion of alflafa meal 4% on body weight of broiler chickens, fat content their meat and oxidative stability meat fat under storage conditions. Final hybrid Cobb 500 chickens were used in the experiment. The broiler chickens were fed with feed mixtures starter from the 1st to the 18th day, grower from the 19th to the 31st day and finisher from the 32nd to the 38th ad libitum. An alfalfa meal of 4% was added in the feed mixtures of experimental group. In our experiment, we used alfalfa meal, which was made from Medicago sativa L. harvested in the bud's phase. The carcasses of broilers were stored at -18° C for a period of nine months. The average weight of chickens at the end of our experiment, the control group was 1685.6 g. In experimental group with addition of 4% alfalfa meal in feed mixtures, the chickens weighed 1709.6 g. The difference in body weight between the groups was not statistically significant ($p$ >0.05). The average fat content in chicken meat was lower in the group with a share of 4% alfalfa meal 2.33 g.100g$^{-1}$ compared to the fat content in meat chickens control group 2.59 g.100g$^{-1}$. Differences in fat content in meat between the groups were not statistically significant ($p$ >0.05). In our experiment, the average peroxide value of fat in meat under storage conditions 9 months at -18°C was lower in the group with a share of 4% alfalfa meal 2.42 μmol O2.g$^{-1}$ compared with an average value of peroxide number 5.79 μmol O2.g$^{-1}$ in the control group. Medicago sativa L. is an interesting object for research. It is characterized by high content of protein and biologically active substances that are effective for the promotion of health, and also an improvement the nutritional value and technological properties of the poultry food, when is used in feed mixtures.

**Keywords**: dietary alfalfa meal; chicken meat; storage; fat; peroxide number.

## INTRODUCTION

*Medicago sativa L.* is one of the cheapest sources of protein from the aspect of high yields and low production costs **(Radović et al., 2009)**. It is feedstuff with high fiber and with low metabolizable energy **(Donalson et al., 2005)**. *Medicago sativa L.* is a readily available, high protein, high fiber feedstuff with one of the slowest rates of passage through the avian system **(Garcia et al., 2000)**. *Medicago sativa L.* is valuable for chemical composition. It has a high crude protein content **(Lupašku, 1988)**, with well-balanced contents in amino acids **(Sen et al., 1998; Dinic et al., 2005; Markovic et al., 2007a; Ponte et al., 2004b; Jiang et al., 2012)**. Crude protein depends on the vegetative growth phase at harvest time and may vary within the range from 200 to 240 g.kg$^{-1}$ **(Radović et al., 2009)**.

*Medicago sativa L.* is an important source of vitamins **(Jiang et al., 2012)**, such as $\beta$-carotene and another 10 vitamins **(Lupašku, 1988; Kindschy, 1991; Sen et al., 1998)**, various microelements too. The animals need these nutrients for normal growth and development (**Marković et al., 2007b**).

The $\beta$-carotene, xanthophylls and flavonoids in alfalfa are responsible for the high antioxidant properties **(Aziz et al., 2005)**. In particular, $\beta$-carotene is an important bioactive substance in alfalfa that is a precursor of vitamin A and retinoid, which has been defined as an important molecule in animal nutrition **(Schweigert et al., 2002)**. Many authors investigated the effect of *Medicago sativa L.* on production properties, the color of subcutaneous fat and meat quality of broiler chickens **(Han and Parsons, 1990; Ponte et al., 2004b; Donalson et al., 2005)**.

By **Sen et al., 1998; Ponte et al., 2004a** carotenoids and xanthophylls have cause that poultry carcasses have desirable yellow color.

*Medicago sativa L.* also contains high levels (2 to 3% of dry matter) of saponins, which have been shown to have hypocholesterolemic, anticarcinogenic, antiinflammatory, and antioxidant properties **(Klita et al., 1996; Rao and Gurfinkel, 2000; Francis et al., 2002; Ponte et al., 2004a)**.

Alfalfa meal must be used in feed mixtures in limited quantities for maintain a high production of broiler chickens **(Guenthner et al., 1973)**.

*Medicago sativa L.* contains significant amounts of bioactive substances that exhibit antioxidant properties. Antioxidant is targeted against oxidation. The role of antioxidants is to protect lipids against radical peroxidation **(Lauro, 1991)**.

Lipid oxidation is a major cause of quality deterioration of processed and unprocessed foods. Secondary oxidation products include aldehydes, ketones, hydrocarbons, and alcohols, among others. secondary products of oxidation are generally odor-active, where as primary oxidation products are colorless and flavorless **(Akoh and Min, 2002)**.

Hydrogen peroxide can also arise directly from oxygen, for example by glucose oxidase **(Rybár, 2002)**. Hydrogen peroxide, which is produced by superoxide dismutase or by direct enzymatic production (amino oxidase, glucose oxidase, and other) has a very important role in the initiation of lipid peroxidation. Its other function is creating the hydroxyl free radicals which oxidize all biological molecules. It is managed by a glutathione peroxidase in tissue. Hydrogen peroxide can be decomposed also by catalase **(Kanner et al., 1987)**.

**Guenthner et al. (1973)** and **Dansk (1971)** recommend the using the limited quantity of alfalfa meal in feed mixtures, which attribute mainly to the high fiber content. Many studies includes the effect of the addition of alfalfa meal in feed mixtures of laying hens **(Güçlü et al., 2004; Mourao et al., 2006; Olgun and Yildiz, 2015; Varzaru et al., 2015)**, but few authors investigate the addition of alfalfa meal in broiler feed mixtures **(Carasco and Bellof, 2013)**.

The purpose of this study was to investigate the effect of feed mixtures with proportion of alflafa meal 4% on body weight of broiler chickens, fat content their meat and oxidative stability meat fat under storage conditions.

## MATERIAL AND METHODOLOGY

Final hybrid Cobb 500 chickens were used in the experiment. The hybrid of the chickens is intended for meat production. The experiment was conducted on a commercial poultry farm. In poultry hall was created space for experimental broiler chickens in accordance with the requirements of Council Directive 43/2007/EC. Directive determines the maximum density of housing chickens on deep litter, which are intended for meat production. The litter consisted of wood chips, which were covered with adjusted straw. Space was divided into two equal parts. In each section were placed 100 birds of one-day-old. Own experimental feeding technology was used in the experiment. The broiler chickens were fed with feed mixtures starter from the 1$^{st}$ to the 18$^{th}$ day, grower from the 19$^{th}$ to the 31$^{st}$ day and finisher from the 32$^{nd}$ to the 38$^{th}$*ad libitum*. An alfalfa meal of 4% was added in the feed mixtures of experimental group. The feed mixtures were produced by Act. no. 440/2006 Coll. on feed mixtures in according to requirements nutrient content and energy for broiler chickens. The feed mixtures were without antibiotics and coccidiostats in control and experimental groups.

Carcase dissection was carried out at Department of animal products evaluation and processing, FBFS, SUA in Nitra. The *c*arcasses of broilers were stored at -18 °C for a period of nine months. *C*hemical analyzes meat samples were carried out at Department of food hygiene and safety, FBFS, SUA in Nitra. Lipid extraction was carried out using a device Det Gras N Selecta P (JP Selecta S.A., Barcelona, Spain). Peroxide value was determined in fat so obtained. The peroxide value is determined by measuring the amount of iodine, which is formed by the reaction of the hydroperoxides formed in fat with iodide ion under acidic conditions. A thiosulphate concentration of 0.01 mol.L$^{-1}$ was used for analysis. The liberated iodine is titrated with sodium thiosulphate 0.01 mol.l$^{-1}$. Based on the usage of sodium thiosulfate in the analyzed sample and in blank test it determined the amount of $O_2$ μmol.g$^{-1}$.

Statistical evaluation of the results was carried out by the system program SAS Enterprise Guide version 1.5. We were used the Student's t-test to compare statistical difference between two groups of data.

## RESULTS AND DISCUSSION

*Medicago sativa L.* is an important feed material that is used in nutrition for all kinds of animals intended for food production. It is characterized by a relatively high content of essential nutrients and certain biologically active substances. Currently, the extract from alfalfa meal begins to use in human medicine. Alfalfa meal is sources of many minerals and vitamins, especially vitamins of group B, vitamin C and E, contains flavonoids, phenolic acids, xanthophylls, carotenoids and other. It has different effects, such as the ability to eliminate uric acid in patients with gout; potassium can help to excrete sodium, and to participate in the reduction of cholesterol levels in blood and tissue. It participates in the acid-base balance, in inhibition the activity of inflammatory enzymes and acts as an antioxidant.

We investigated the effect of alfalfa meal on meat quality of broiler chickens in our experiment. **Hunt and Bethke (2011)** reported variation in riboflavin content of up to 25% depending on the phase of vegetation, which plays an important role in selecting the right collection green *Medicago sativa L.* – correct phase of vegetation for the purpose of producing alfalfa meal. In our experiment, we

**Table 1**Statistical evaluation of body weight.

| Index | Group | |
|---|---|---|
| | Control | Experimental |
| SD | 139.82 | 166.85 |
| $c_v$, % | 8.3 | 9.76 |
| t-test | $p$ >0.05 | |

Note:Control group – without alfalfa meal, Experimental group – 4% alfalfa meal in feed mixture, *SD – standard deviation, $c_v$ – coefficient of variation*

**Table 2** Average dry mater and fat content of chicken meat* and peroxide number.

| *Group* | | *Index* | | |
|---|---|---|---|---|
| | | Dry mater, $g.100g^{-1}$ | Fat**, $g.100g^{-1}$ | Peroxide number, $\mu molL\ O_2.g^{-1}$ |
| *Control* | Mean | 27.89 | 2.59 | 5.79 |
| | SD | 1.85 | 0.81 | 1.24 |
| | $c_{v,\%}$ | 6.62 | 31.07 | 17.76 |
| *Experimental* | Mean | 28.56 | 2.33 | 2.42 |
| | SD | 1.54 | 0.52 | 0.75 |
| | $c_{v,\%}$ | 5.37 | 22.14 | 7.4 |

Note: *Chicken meat was stored under storage conditions at -18 °C 9 months, **(**Tkáčová, 2013)** Control group – without alfalfa meal, Experimental group – 4% alfalfa meal in feed mixture, SD – standard deviation, $c_v$ – coefficient of variation.

used alfalfa meal, which was made from *Medicago sativa L.* harvested in the buds stage.

**Dong et al., (2007)** investigated the effect of alfalfa extract obtained in the growing phase during flowering. Natural extract of *Medicago sativa* L. contains polysaccharides (18.63%), triterpenoids, saponins (5.58%) and flavonoids (5.89%). The experiment results showed that the extract of alfalfa decreased deposition of abdominal fat ($p$ <0.05) and increased immunity (humoral immunity and cellular immunity) without adversely affecting the production of chickens intended for meat production. These authors give a direct relation to fat storage and immunity.

The average weight of chickens at the end of our experiment, the control group was 1685.6 g. The Similar results found **Haščík et al., (2010)**. A body weight of their experimental chickens Cobb 500 at their age 42 days was 1629.15 g, while the chickens were fed a standard commercial feed. The similar results of body weight of broiler chickens at age 42 days found also **Liptaiová et al., (2011)**. Their experimental broiler chickens weighted 1591.0, 1603.0, 1651.0 and 1698.0 g, respectively. In our experimental group with addition of 4% alfalfa meal in feed mixtures, the chickens weighed 1709.6 g. The difference in body weight between the groups was not statistically significant ($p$ >0.05). Higher body weight of broiler chickens of the same age found **Angelovičová et al., (2012)** in their experiments. Final body weight of their experimental broiler chicken was 2010.24 g and 2019.12 g, respectively.

**Jiang et al., (2012)** in a study they found that the addition of alfalfa meal did not have any effect on growth performance of Muscovy ducks from the 14th to the 49th day of age. Ducks given 3, 6, and 9% alfalfa meal had significantly higher dressing percentage and lower abdominal fat percentage compared with those given no alfalfa meal. Ducks given 9% alfalfa meal had higher breast meat percentage compared with those given no alfalfa meal. In other experiments, it would be appropriate to investigate also fiber, proteins and saponins of alfalfa meal from aspect of its use in feed mixtures. Their content can be a significant factor affecting the results of experiments. *Medicago sativa L.* is rich in fiber content, and is most often added to poultry diets as a source of xanthophylls for pigmentation, or as a source of so – called unidentified growth factors **(Leeson and Summers, 2005)**.

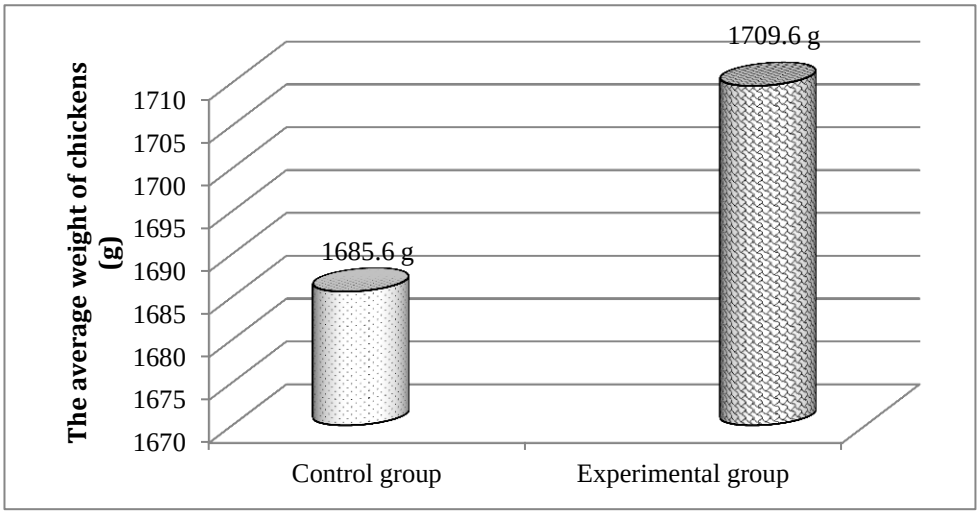


**Figure 1** Average final body weight of broiler chickens.

Note: Control group – without alfalfa meal, Experimental group – 4% alfalfa meal in feed mixture.

***Fat content and* peroxide number of chicken meat**

In our experiments, we used meat samples for determining the dry matter, which consisted of the same amount of breast and thigh muscles, and skin of 1 $cm^2$ with subcutaneous tissues.

The average dry matter content in the meat of chickens for meat production was in the group with a share of 4% alfalfa meal 28.56 $g.100g^{-1}$ and in control group 27.89 $g.100g^{-1}$. The differences in dry matter content of meat between the groups were not statistically significant ($p$ >0.05).

The average fat content in chicken meat was lower in the group with a share of 4% alfalfa meal 2.33 $g.100g^{-1}$ compared to the fat content in meat chickens control group 2.59 $g.100g^{-1}$. Differences in fat content in meat between the groups were not statistically significant ($p$ > 0.05) **(Tkáčová, 2013)**. In contrast to our experiment, in which we studied the fat content of the chicken meat, **Angelovičová and Semivanová (2012)** investigated the fat content in the breast muscle. A breast muscle contained lower fat 0.88 $g.100g^{-1}$, 1.05 $g.100g^{-1}$, respectively, than chicken meat. **Dong et al.,** (**2007**) investigated the effect of alfalfa extract obtained in the growing phase during flowering. Natural extract of *Medicago sativa L.* contained polysaccharides (18.63%), triterpenoids saponins (5.58%) and flavonoids (5.89%). The results of experiment showed that the extract of *Medicago sativa L.* decreased deposition of abdominal fat ($p$ <0.05) and increased immunity (humoral immunity and cellular immunity) without adversely affecting production. These authors state that between fat storage and immunity is a direct relation. Interesting results related to investigation of the alfalfa meal effect 4% in feed mixtures of broiler chickens obtained **Bobko et al.,** (**2012**) on the baking losses of meat. Baking losses were lower (30.72%) in group with application of alfalfa meal in comparison with control group (31.66%), without significant differences between groups ($p$ >0.05). **Haščík et al., (2010)** noted that the baking losses are often influenced by the chemical composition of muscle tissue, especially by the fat in muscle of animals.

In our experiment, the average peroxide value of fat in meat under storage conditions 9 months at -18 °C was lower in the group with a share of 4% alfalfa meal 2.42 μmol $O_2.g^{-1}$ compared with an average value of peroxide number 5.79 μmol $O_2.g^{-1}$ control group. **Tichivangana and Morrissey (1985), Ruiz et al., (1999)** reported that the rate of oxidation of fats in meat also depends on the presence of oxidants and antioxidants, which puts high emphasis on quality selection of raw materials – selection of *Medicago sativa L.* for the alfalfa meal production. Many studies were focused on the impact of *Medicago sativa L.* on the egg yolk quality of laying hens, but little attention is paid to the impact of *Medicago sativa L.* on meat quality of broiler chickens. This may be due to the fact that *Medicago sativa L.* is rich in fiber content, which may adversely affect the body weight gains. **Laudadio et al., (2014)** eliminated this property in experiment. The combination of sieving and air classification of alfalfa meal was effective in separating protein and fiber from starting material. Low-fiber alfalfa meal was found to contain appreciable content of nutrients; in fact, the sieving and air classification processes improved crude protein and reduced crude fiber and neutral detergent fiber level compared with untreated meal. This mixture was used in feed mixture for laying hens. We hypothesize for the future adjustment path alfalfa meal by the way, and its use for broiler chickens. Some authors investigated the effect of alfalfa meal in combination with other additives. **Ponte et al., (2004a)** investigated the potential use of cellulase and xylanase to increase the nutritional value of *Medicago sativa L.* for broiler chickens. They found that a high proportion of alfalfa meal 20% for the purpose of application of cellulase and xylanase in chickens causes no apparent health problems and contributes significantly to the coloration of subcutaneous fat chicks, especially yellow pigments, while red and rosé unwanted pigments are significantly reduced. **Ziegelhoffer et al., (1999)** investigated transgene *Medicago sativa L.*, which had incorporated the genes in their bacterial cellulose gene. The authors recommended based on the results of their experiment, this type of *Medicago sativa L.* as suitable for the production of alfalfa meal.

**Hunt and Bethke (2011)** show the variations in the content of riboflavin up to 25% depending on the vegetative growth phase.

Further research is needed regarding alfalfa meal, as relates to the effect of the dietary fiber and a saponin and its effect on the growth ability of broiler chickens, as well as the possibility reducing of the fiber and increasing the protein.

Antioxidants already in low concentrations significantly delay or prevent oxygenation of oxidizable constituents **(Halliwell and Gutteridge, 2001)**.

The fatty acids deposited in animal tissues are derived from various sources: from endogenous synthesis or directly from the feed, and from microbial synthesis or modification in the digestive tract. In non-ruminants such as pigs and poultry the dietary fatty acids more directly influence the body fat composition, making nutrition an effective tool to manipulate animal lipid composition **(Scheeder, 2006)**.

In meat, triacylglycerols, phospholipids, and cholesterol are the main substrates for lipid oxidation **(Márquez-Ruiz et al., 2014)**.

Some of these secondary products can be toxic to humans and are responsible for the undesirable rancid odor typical of oxidized oils **(Decker et al., 2010; Kołakowska and Bartosz, 2014)**.

Oxidation may be initiated by the formation of lipid peroxides. Initial phases of lipid oxidation can be detected by measuring the peroxide value, which quantifies the levels of peroxides and hydroperoxides formed at that stage **(Bennett et al., 2014)**.

## CONCLUSION

*Medicago sativa L.* is an interesting object for research. It is characterized by high content of protein and biologically active substances that are effective for the promotion of health, and also an improvement the nutritional value and technological properties of the poultry food, when is used in feed mixtures. *Medicago sativa L.* uses in feed mixtures for poultry as alfalfa meal. The use this feed material in

feed mixtures for poultry is restricted by high fiber content. Further researches needed regarding alfalfa meal, as relates to the effect of the dietary fiber and a saponins and its effect on the growth ability of broiler chickens, as well as the possibility reducing of the fiber and increasing the protein and using biologically active substances.

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