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INFLUENCE OF PHYTOESTROGENS ON SKELETAL MUSCLE STRUCTURE

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Abstract

Constant increase of meat quantity along with ensuring its high quality are nowadays being the priority requirements of the market towards modern meat production. With selection and animal nutrition as the basic mechanisms regulating the quantity and quality of meat, in recent years more attention has been devoted to investigations of the effects of different chemical compounds on muscle tissue, while monitoring their potential negative effects on both animals and humans as the end consumers. A group of compounds that is being increasingly studied in the last years are phytoestrogens – substances of plant origin with chemical structure very similar to estrogen, capable of causing either estrogenic or anti-estrogenic reactions in the organism. The most studied phytoestrogens are daidzein and genistein, and due to their ability to mimic estrogen in the body, they are thought to be able of influencing growth and carcass composition in farm animals. This paper gives an overview of the newer results on the effects of phytoestrogens genistein and daidzein on skeletal muscle tissue in farm animals.

Key words: *daidzein, muscle growth, muscle structure, phytoestrogens*

Introduction

Modern meat production, as one of the most important branches within animal production is facing very difficult tasks – at the same time it has to settle often opposite demands of the market: to increase the production so it could meet the needs of end customers, not to undermine basic biological and physiological possibilities of animals by selection, to keep the quality of meat at high level both for end consumers and meat industry, to speed up the process of meat production, while the costs of production should not exceed the limit of profitability. Having in mind that regulation of muscle growth and differentiation of muscle cells includes a number of factors: environmental, genetical, and intrinsic factors such as hormones and growth factors, the results of scientific research within these fields need to be an integral part of the meat production process.

The influence of estrogen on muscles

Ruminants are the only animal species in which an unambiguous positive effects of estrogen on muscle growth, daily gain increasment, feed utilization with minimal increase in food consumption and decrease of fat deposition are observed. After diethylstilbestrol, DES, was synthesized in late 30ies of the twentieth Century, the effects of this chemical on

production characteristics in domestic animals have been investigated, and hence increased daily gain in heifers, sheep and poultry were determined, DES was patented as stilbestrol. It was used until 1979., when it was forbidden as potentially carcinogenic. However, in some countries, similar compounds (17 β estradiol, estradiol benzoate, zeranol, dienestrol) are still being used as growth promoters in cattle production.

Estrogens have less effects in non-ruminants. The mechanism of action of estrogen on the growth of pigs is not fully understood. In intact males - boars, administration of estrogen increases food intake and its utilization, thereby increasing growth, reducing the undesirable characteristic smell of the meat, but to a smaller extent, increases the amount of adipose tissue (Hancock et al., 1991). Rempel and Clapper (2002), and Hilleson-Gayne and Clapper (2005) found the dependence of IGF concentrations and 17 β -estradiol, where the application of estradiol led to an increase in the concentration of IGF in serum (Rempel and Clapper, 2002), and reduction of estradiol concentration in serum led to a reduction of the IGF concentration (Hilleson-Gayne and Clapper, 2005). According to these authors, therefore, estradiol might indirectly, through IGF axis, have an impact on the growth of pigs. These authors observed the most pronounced effects of estradiol on changes in the IGF concentration in boars.

On the other hand, by examining the effect of estrogens *in vitro* - in pig muscle cell cultures, Mau et al. (2008) have observed almost no effect on the proliferation of muscle cells at physiological concentrations, but the inhibitory effect of estrogen on proliferation of muscle cells, if the estrogen was administered in supraphysiological concentrations, was determined. Tsai et al. (2007) came to similar conclusions, and confirmed the *in vivo* reduction of body weight and the level of IGF in muscles of ovariectomized female rats with implants containing 17 β estradiol. Enns and Tiidus (2010), observed the stimulatory effect of estrogen to rebuild muscle tissue in humans after injury through the activation and proliferation of satellite cells. It is possible that different results with respect to the effects of estrogen are a consequence of different gender used in the different experiments, or use of estrogen in combination with anabolic agents.

Plant hormones – phytoestrogens

Phytoestrogens are a group of chemical compounds of plant origin, which have either estrogenic or antiestrogenic effects in the organism. These compounds have been identified in more than 300 different plants (Tham et al., 1998), such as garlic, parsley, soybean, clover, alfalfa, wheat, barley, rice, carrot, potato, apple, pear, grape, etc. In the food standardly used both for human consumption and for animal nutrition, the most represented two categories of phytoestrogens are isoflavones and lignans. In addition to these two groups, there is a third group of phytoestrogens - coumestanes. In contrast to a widely present lignans, isoflavones are found almost exclusively in legumes. Especially rich source of these compounds is soy - plant species inevitable in animal nutrition (Tham et al., 1998; Reinli and Block, 1996).

Two compounds from the group of isoflavones which are now studied the most are daidzein and genistein. A huge attention was directed towards investigation of these isoflavones effects after the discovery of Akyame et al. (1987), who have observed that genistein is a potent inhibitor of protein tyrosine kinases - a group of enzymes which play a key role in the formation and uncontrolled growth of cancer cells. At the same time, the increase of awareness of more and more frequent occurrence of various diseases especially hormone-dependent tumors in Western countries compared to the eastern countries, especially Japan and China. It led the scientific population towards extensive research of

soy, a plant which dominates in the diet of humans in the Far East, and consequently phytoestrogens which are found in soy, and potentially could be used in the prevention and treatment of tumors. Total isoflavone content, and the amount of genistein and daidzein in particular soy products are shown in Table 1.

Table 1. *The amount of isoflavones in different soy products, mg isoflavones/kg feed*

Product	Total isoflavones	Daidzein	Genistein	Reference
Soybean 1	1176	365	640	Tham et al, 1998
Soybean 2	4215	1355	2676	
Roasted soybean	2661	941	1426	
Soy flour	2014	412	1453	
Tofu	532	238	245	
Tempeh	865	405	422	
Miso	647	272	281	
Soybean meal	/	395-488	506-695	Fritsche i Steinhart, 1999
Soybean hulls	/	< 0,1	18,4	
Soybean flakes	/	363-475	1275-1547	

Common characteristics of all phytoestrogens, are that their chemical structure is very similar to that of estrogen (Figure 1), and that in organism they have the ability to cause either estrogen, or anti-estrogenic response, depending on the concentration, the concentration of endogenous estrogen and individual characteristics of an individual, particularly gender and hormonal status (Tham et al., 1998).

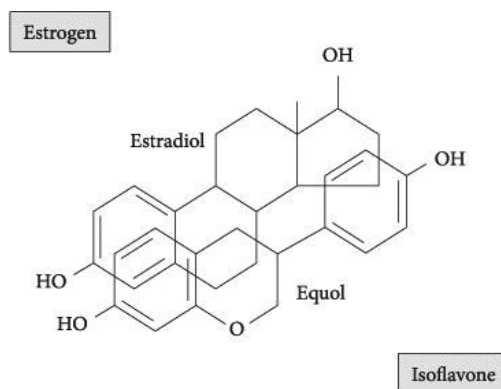


Figure 1. The similarity of the chemical structure of estrogens and isoflavones

These functions phytoestrogens exhibit binding to estrogen receptors ER in tissues: originally known, "classical" estrogen receptors ER α and newly found ER β , possibly have different roles in gene regulation, and also the presence of these receptors in various tissues differs. Thus, in rats the presence of ER α in the uterus, testis, ovary, kidney, epididymis, thyroid gland was observed, and ER β were the most present in the prostate, lung, bladder, brain (Kuiper et al., 1996. a, b, Setchell and Cassidy, 1999). In humans, ER α were determined in the testes, kidneys, and adrenal glands, both ER α and ER β were determined in blood vessels, mammary gland, uterus, ovaries, while the presence of only ER β was

found in the brain, lungs, thyroid gland, prostate, bladder, bones (Setchell and Cassidy, 1999). It is very important to stress the fact that the affinity of different estrogenic compounds towards two types of estrogen receptors varies, and specifically phytoestrogens have significantly higher affinity for ER β than to ER α (Kuiper et al. 1998).

Investigations of the presence and role of estrogen receptors in muscle tissue are of recent date. Wiik et al. (2009) reported the existence of both types of receptors in the muscles of humans, of both sexes and various ages. Their research showed the presence of ER in the nuclei of muscle fibers, and in the capillaries surrounding the fibers. The same results are shown by Kalbe et al. (2007), confirming for the first time the presence of ER α and ER β in several muscles of pigs. This means that the muscle tissue is a target tissue for the estrogen action, both endogenous, as well as those compounds that mimic estrogen, including isoflavones. Two compounds from the group of isoflavones whose effects on muscles are now studied intensively are daidzein and genistein.

The influence of isoflavones on muscle tissue

Due to the ability to mimic estrogen, it is possible that isoflavones could affect growth and carcass composition in farm animals. However, the results of authors who have examined the impact of isoflavones on muscle tissue in different animal species, the growth and carcass quality in general, are very different.

According to Lee et al. (2005) and Bidner et al. (1972), the use of different estrogenic compounds in the pig production leads to reduction in carcass fat and increase of muscularity. Payne et al. (2001) examined the effect of three levels of isoflavones in food in castrates and gilts fattening. Castrates received: 1. group: soybean meal (1.14 mg/g total isoflavones); 2. group: soy protein (0.06 mg/g total isoflavones); 3. group: soy protein with addition of isoflavones to the level as in the first group. Gilts received: 1. group: soybean meal (1.14 mg/g total isoflavones); 2. group: soybean meal plus supplement of isoflavones in concentrations twice higher than soybean meal; 3. group: soybean meal plus supplement of isoflavones in concentrations five times higher than in soybean meal. Authors monitored daily gain and food consumption, the cross section of MLD, fat thickness, percentage of muscle in the carcass and technological properties of meat (pH₂₄, meat temperature, meat color, hardness, etc.). Looking at the whole fattening period, isoflavones did not affect daily gain and daily food consumption in castrates, but in the final phase of fattening, the average daily weight gain and food consumption were significantly higher ($p < 0.10$) in animals fed soy protein concentrate. Hull length, weight and percentage of lean meat, and lean meat daily gain were increased, while fat thickness decreased in castrates who received additional isoflavones in the diet. However, between barrows fed soybean meal, and barrows fed soy protein with added isoflavones there were no significant differences. Technological properties of meat did not differ ($p > 0.10$) in barrows from different experimental groups. In gilts from three experimental groups there were no differences in any of the observed characteristics. Based on the results, Payne et al. (2001) concluded that isoflavones reduce fat and increase the amount of lean meat in fattening barrows but not in gilts, and that the addition of isoflavones above the amount normally found in soybean meal has no effect on the improvement of the carcass characteristics and meat quality.

Jiang et al. (2007) investigated the effects of different doses of isoflavones (0, 10, 20, 40, 80 mg/kg) in male broilers fed the same basal diet without soybean meal. The results showed that the addition of the isoflavones of 10 and 20 mg/kg increased the average daily weight gain and food consumption. Addition of 40 mg/kg isoflavones increased the water holding capacity of meat, and the pH value of the meat. Based on these results, Jiang et al.

(2007) concluded that the addition of isoflavones in the diet of male broilers had a positive impact on growth and meat quality.

Rehfeldt et al. (2007) investigated the effects of adding isoflavones daidzein to sows diet during late gestation on the properties of the muscle tissue in the offspring. Results of this study did not showed statistically significant differences in litter size, piglet birth weight and percentage of muscle, depending on the daidzein addition in sows diet. The interaction treatment x litter size showed a tendency of reducing muscle tissue in favor of the skin in piglets whose mothers received daidzein. The percentage of body fat in piglets originating from mothers treated with daidzein was higher ($p = 0.04$). Addition of daidzein had no effect neither on the weight of *m. semitendinosus* in piglets, nor on the number of fibers in the muscle cross section. Body weight at the end of fattening did not differ in slaughter pigs whose mothers were treated with daidzein compared to progeny of untreated sows, and also there was no difference in the amount of lean meat, backfat thickness and percentage of meat in the carcass. As to the histological features of *m. semitendinosus*, there was no difference in the size of the muscle cross section, the total number of muscle fibers, and the number of nuclei per fiber, but a difference was observed in the presence of different fiber types within the muscle. The offspring of treated sows had a higher number of fast twitch glycolytic (FTG) fibers and a lower number of fast twitch oxidative (FTO) fibers compared to the offspring of untreated sows. Also, a significant difference was observed depending on the gender: barrows had a significantly higher number of muscle fibers ($p=0.04$), smaller diameter ($p=0.03$) and a higher proportions of STO ($p=0.06$) and FTG ($p=0.03$) fibers compared to females. Based on these results authors pointed at a marginal effect of daidzein addition to sows diet during late pregnancy on characteristics of muscle tissue in the offspring.

According to Adamovic (2013) addition of different doses of daidzein in diet of pregnant sows from day 85 of gestation until the end of pregnancy, had no effect on the morphological characteristics of muscle: weight, length, volume and cross-section of the muscle in newborn piglets and slaughter pigs at the end of fattening. In this study, treatment of pregnant sows with daidzein during the late stages of pregnancy did not have statistically significant effects on the histological characteristics of *m. semitendinosus* neither in newborn piglets, nor in slaughter pigs at the end of fattening.

However, in experiments with cultures of muscle cells (*in vitro*), it was shown that isoflavones inhibit the growth and development of muscle cells. Jones et al. (2005) found that phytoestrogens, genistein in particular, at a concentration of ≥ 1 mmol / l, inhibits proliferation of rat muscle cells *in vitro*, but does not affect protein degradation. Similar results were obtained by Ji et al. (1999), who found that genistein strongly inhibits proliferation and fusion of myoblasts in rats, that the inhibition strength is dependent on the dose of genistein, and that the effective doses are already from 1 $\mu\text{mol/l}$. Authors have not observed adverse effects of genistein on protein degradation. Also, it was noticed that the negative effect of genistein on myoblast fusion is present only when genistein was added during the first 24 hours of initiation of miotubules forming. If genistein was added later, after the first 24 hours of initiation, it had no effect on the fusion of myoblasts. Regarding the synthesis of myosin, authors observed a detrimental effect of genistein at high doses and long exposure. At low doses (1 $\mu\text{mol/l}$), which correspond to a concentration in the serum of humans and rats in which soy is present in the diet, genistein increased the volume of myosin synthesis.

Mau et al. (2008) studied the direct effects of genistein and daidzein on proliferation of muscle cell cultures derived from newborn piglets. In this research doses of isoflavones

0.1, 1, 10 and 100 μM were applied, which could normally be measured in the serum after consuming infant feeding soy based formula, or plant feeds usually used in the pig diet. The results of this study showed that the effects of isoflavones depend on the dose of isoflavones used, and much stronger inhibitory effect of genistein, compared to daidzein, on the proliferation of muscle cells. Such an effect of genistein has been shown already at a dosage of 1 μM . On the other hand, daidzein, even at considerably higher doses - up to 10 μM , did not have a detrimental effect on the growth of pig muscle cells.

Similar research on the cultures of muscle cells of pigs was conducted by Rehfeldt et al. (2009). The same doses of genistein and daidzein (0.1, 1, 10, 20, and 100 $\mu\text{mol/l}$) were applied and 17 β -estradiol (0.1, 1 nmol/l) during the last 26 hours of cell cultivation. Myogenic differentiation was not changed under the action of any of the concentrations of isoflavones or estradiol. At first sight this is inconsistent with the findings of Ji et al. (1999), but these authors found an inhibitory effect of genistein on the proliferation and myoblast fusion when it was administered during the initiation. Therefore Rehfeldt et al. (2009) conclude that the inhibitory effect of genistein is dependent on the stage of development of cell cultures, and therefore, if it is added later, genistein as well as daidzein and estradiol, have no effects on differentiation. However, these authors have confirmed the inhibitory effects of genistein, depending on the administered dose, and absence of effects of daidzein and 17 β -estradiol in the synthesis of proteins. It has been found that low doses of genistein, daidzein, and 17 β -estradiol decrease the degradation of proteins in cultured muscle cells, which could potentially contribute to increased accumulation of proteins in the pig skeletal muscles.

Conclusion

Most authors agree that application of high doses of isoflavones, especially genistein, would have a detrimental effects on protein synthesis and, if applied during initiation of muscle cells differentiation, it would inhibit normal development of muscle cells. However, some authors report positive effects of isoflavones on daily gain, lean meat content, fat reduction, muscle fiber types proportion etc., which seem to be dependant on the species, gender, dose of isoflavone used, and the moment of application. Since soy, as a very rich source of isoflavones, makes the two-thirds of protein requirements in animal feed, further investigations of possible positive as well as negative effects of those compounds on the muscle tissue should be made, in respect to meat quantity and quality. Also, since soy-based formulas are widely used in newborn children nutrition, the results from already conducted and some future experiments on animals would be very important for evaluation of possible effects of isoflavones in humans.

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