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Effect of Baikal Skullcap Root (*Scutellaria baicalensis radix*) on Cholesterol Level and Meat Quality in Rabbits Fed a Cholesterol Rich Diet

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The aim of this study was to evaluate the effect of the Baikal skullcap root (*Scutellaria baicalensis radix*) on the cholesterol level and chemical composition of the hind leg muscles of rabbits. Thirty two White New Zealand rabbits were assigned to four groups. Group C consisted of control animals which were fed a basal mixture for rabbits. Group CH received the same basal diet with a 1% (w/w) pure cholesterol supplement. Group CH+SR received the basal diet with two supplements: 1% (w/w) pure cholesterol and 9% (w/w) skullcap root. Group SR received the basal diet with a 9% (w/w) skullcap root supplement. After 6 weeks rabbits were slaughtered and, total cholesterol as well as dry matter, protein, fat, ash and pH_{24} were determined in samples of hind leg muscles. Using a Baikal skullcap root with hypercholesterolemic diet (group CH+SR) caused significant reduction ($P \leq 0.05$) in total cholesterol level in comparison with hypercholesterolemic diet (CH group). The addition of Baikal skullcap root to the food of rabbits significantly increased the muscle protein content ($P \leq 0.05$) in comparison with C and CH groups. Moreover, supplementation with Baikal skullcap root (CH+SR) decreased about 15.6 % ($P \leq 0.05$) fat level in comparison to CH group. No significant effects were seen in dry matter content, ash, and pH_{24} value of hind leg muscles of experimental rabbits

Key words: Rabbit, Baikal skullcap root, cholesterol, muscle chemical composition.

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The relationship between health and diet enriched in vegetable derived products as sources of therapeutic agents has recently been extensively studied (BIELAŃSKI *et al.* 2007; JASPRICA *et al.* 2007; BARTKOWIAK *et al.* 2007). Considerable interest has arisen in the possibility that the consequences of several diseases may be prevented by improving the dietary intake of natural nutrients with the antioxidant properties of plant phenolics and flavonoids (LAM *et al.* 2007).

Baikal skullcap (*Scutellaria baicalensis* Georgi) is an interesting medicinal plant because of a particularly high content of flavonoids which serve as modifiers of inflammatory processes, e.g. against bacterial infections as well as having antiviral, antitumor, antioxidative properties, hepatoprotecive and lipid preventive effects (BOCHORAKOVA *et al.* 2003; CHAN *et al.* 2000; HARRIS 2008; WO NIAK *et al*. 2004; YAGHOUBI *et al.* 2008). The most important flavonoids are: baicalin, baicalein and wogonside. The quantity of these compounds in the skullcap plant is exceptionally large, on average 15-20 % of flavones are found in roots, 12-17 % in baicalin, and 3-4 % in wogonside (OSZMIAŃSKI J. 2002; TANG & EISENBRANDT 1992). These compounds have a potentially important function in preventing free radical induced diseases such as atherosclerosis. Cholesterol feeding has often been used to evaluate serum or tissue cholesterol levels in the study of the etiology of hypercholesterolemia-related disturbances. Hypercholesterolemia is one of the most important risk factors that are considered to predispose to various chronic diseases of the circulatory system (LÓPEZ REVUELTA *et al.* 2006; KIM *et al.* 2008). In previous studies the beneficial effect of Baikal skullcap root on lowering the total plasma cholesterol and LDL-cholesterol in hypercholesterolemic rabbits was observed (KRÓLICZEWSKA *et al*. 2010).

The high content of unsaturated lipids in muscle results in an increased oxidative deterioration (ENGBERG *et al.* 1996), which may have adverse effects on health (ADDIS *et al.* 1996). Several dairy products and milk powder are reported to contain oxidized cholesterol after processing. Fresh meat and fresh meat products contain zero or trace amounts of cholesterol oxides. Oxysterols contained in cooked meat range from 180-1900 ug/g. Both synthetic and natural antioxidants may protect against oxysterols, some of which have demonstrated efficiency in inhibiting cholesterol oxidation in different experimental models (VALENZUELA *et al*. 1995).

In the present study, we assess the effect of skullcap root on the cholesterol level in muscles of rabbits fed a high cholesterol diet as well as on meat quality indices such as protein, fat, ash content, and meat pH.

Material and Methods

Thirty-two White New Zealand rabbits were used for the study. For the first two weeks the animals were only fed a commercial basal mixture that meets rabbit dietary requirements (Table 1; DE BLAS 1998). This commercial basal mixture consisted of triticale, oat, wheat meal, wheat bran, sunflower meal, barley germ, dried alfalfa, limestone, salt, sodium bicarbonate and a 0.5% mineral-vitamin premix. After an adaptation period, the rabbits were randomly assigned to four groups of 8 animals each.

Table 1

The nutritional values of the commercial basal mixture for rabbits used in this study

Ingredient	Amount
Metabolizable energy (ME; MJ/kg)	9.218
Crude protein (%)[1]	16.0
Crude fibre (%)[1]	16.0
Crude ash (%)[1]	10.0
Crude fat (%)[1]	2.8
Methionine (%)	0.27
Lysine (%)	0.61
Calcium (%)	1.3
Available phosphorus (%)	0.5
Natrium (%)	0.27
Vitamin A [j.m.]	10,000
Vitamin D_3 [j.m.]	1000
Vitamin E [mg]	40

[1]According to AOAC

[2]Provided per kg of diet: vit. A, 12 000 IU, vit. D_3, 2500 IU, vit. E, 20 mg, vit. Bi, 1.5 mg, vit. B_2, 7.5 mg, vit. B_6, 4.5 mg, vit. B_{12}, 30 mg, vit. K_3 3 mg, nicotinic acid, 45 mg, pantothenic acid, 15 mg, folic acid,0.8 mg biotin, 0.08 mg, vit. C, 10 mg, choline chloride, 450 mg.

The groups were designated to C, CH, CH+SR and SR groups. Group C consisted of the control animals, which were fed a basal mixture for rabbits. It had also been used in the adaptation period. Group CH received the same basal diet with 1% (w/w) pure cholesterol supplement using Cholesterol 95 GC obtained from Sigma Aldrich. Group CH+SR received the basal diet with two supplements: 1% (w/w) pure cholesterol and 9% (w/w) skullcap root. Group SR received the basal diet with 9% (w/w) skullcap root supplement. The dried roots were crushed using a laboratory mill and then mixed with the basal diet to yield a 9% (w/w) skullcap root content. The diets and fresh water were provided *ad libitum*. After 6 weeks of feeding the respective diets the experimental period was terminated and rabbits were slaughtered. For 24 hours the carcasses were stored at 4°C and the pH_{24} was measured. Next, the samples of hind leg muscles were stored in freezer at -20°C for further chemical analysis.

The basic chemical analysis of the muscles was determined using standard methods: the crude protein content (CP) by the Kjeldahl method, according to Polish Standard PN-75/A-04018; dry matter (DM) by the thermal method (105şC), according to Polish Standard PN-ISO 1442:2000; crude ash (CA) acc. to Polish Standard PN-72/A-82245; crude fat (CF) by the Soxhlet method, according to Polish Standard PN-ISO 1444:2000; and the pH value acc. to Polish Standard PN-ISO 2917. Total cholesterol (free and esterified) content in collected samples of muscles was determined by enzymatic analysis (KARKALAS *et al*. 1982) using a commercially available kit from Boehringer Mannheim Farb, R-Biopharm AG, Darmstadt, Germany, Cat. No 0139050) and then was read using a Beckman DU 640 Spectrometer.

All data were analyzed by one-way ANOVA (Statistica for Windows ver. 9.0, STATISTICA for Windows, StatSoft, Inc., Tulsa, OK, USA) to test the effects of the dietary treatments with the significance level at $P<0.05$. All data were expressed as means ± SD.

Results and Discussion

Results of the total cholesterol content in rabbit muscles are presented in Figure 1. The high dose of Baikal skullcap root with hypercholesterolemic diet (group CH+SR) caused significant reduction ($P<0.05$) in total cholesterol level in hind leg muscles in comparison with the hypercholesterolemic diet (CH group).

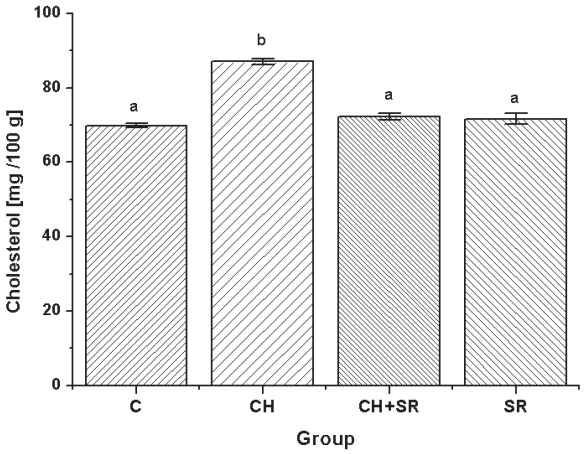

Fig. 1. Total cholesterol content in muscles of rabbits [mg/100g]. C – control basal diet; basal diet + cholesterol 1g/100g diet (CH); basal diet + cholesterol 1g/100g + 9g/100g skullcap root (CH+SR); basal diet + 9g/100g skullcap root (SR). Mean values with letters a and b are significantly different ($P \leq 0.05$).

Few studies have reported on the total cholesterol content of rabbit meat in a hypercholesterolemic diet. The cholesterol content of the body is determined by genetic and environmental factors, with nutrition playing an important role (KOWALSKA 2008). In the present study, a cholesterol rich diet did not cause excessive accumulation in meat. Cholesterol content in muscles of rabbits ranged from 69.86 mg/100g (group C) to 87.06 mg/100g (group CH). According to data by SZKUCIK & PYZ-ŁUKASIK (2009) total cholesterol content in rabbit meat ranged from 32 to 50 mg/100g of muscle tissue and had a lower value than beef, pork or chicken (LUKEFAHR *et al.* 1989). Our results are in agreement with data reported by BIELAŃSKI *et al.* (2000). They showed that the meat cholesterol level is higher and ranged between 120 mg/100g meat for Californian rabbit to 145 mg/100g for New Zealand White rabbits. Additionally, the highest total cholesterol content (163.3 mg/100g) for rabbit meat was observed by LUKEFAHR *et al.* (1989).

In contrast to the results of HOLMES *et al.* 1984, in the present study we observed that after feeding an atherogenic diet with the addition of skullcap root the tissue cholesterol level responded to the dietary modification. The positive effect of skullcap root in preventing cholesterol deposition in the muscles was observed in the CH+SR group, however no significant differences between cholesterol levels were observed between the SR and C groups (Fig. 1). This shows that flavonoids from skullcap do not decrease the physiological levels of cholesterol in the muscles.

The effects of Baikal skullcap root addition to the hypercholesterolemic diet of rabbits on the basic chemical composition of the meat are presented in Table 2. No significant effects were seen in dry matter content of hind leg muscles of experimental rabbits.

In our study, the rabbits on the skullcap root supplemented diets (group CH+SR and group SR) exhibited a remarkable elevation in protein content ($P \leq 0.05$) in comparison to C and CH groups. Similar results were obtained in previous study by KRÓLICZEWSKA *et al*. (2008) on broiler chickens. These authors found that the protein content was higher in the breast muscle of chickens fed the Baikal skullcap root supplemented diet than that of the control group ($P \leq 0.05$), whereas in the leg muscle, no significant effects were seen (KRÓLICZEWSKA *et al*. 2008). The protein is the most valuable constituent of the meat and its content in rabbit muscles is defined at a level of 22 to 23% (SZKUCIK & PYZ-ŁUKASIK 2009; DALLE-ZOTTE 2002). Similar results were obtained in the present study (Table 2). Regarding data obtained by SZKUCIK and LIBELT (2006) the protein level in rabbit meat depends on the part of the carcass. The lowest level

Table 2

The chemical composition of rabbit meat, means (n=8) SD

Parameter	Dietary treatments[1]			
	C	CH	CH+SR	SR
Dry matter (%)	25.64±0.32	25.90±0.27	26.10±0.21	25.86±0.23
Crude protein (%)	22.30±0.35^{a}	22.16±0.36^{b}	22.54±0.29^{c}	22.52±0.44^{c}
Crude fat (%)	1.78±0.21^{a}	2.11±0.18^{b}	1.96±0.14^{c}	1.83±0.19^{a}
Crude ash (%)	1.23±0.06	1.25±0.05	1.21±0.10	1.23±0.03
pH_{24}	5.82±0.10	5.85±0.21	5.86±0.15	5.84±0.11

[1] C, control basal diet; basal diet + cholesterol 1g/100g diet (CH); basal diet + cholesterol 1g/100g + 9g/100g skullcap root (CH+SR); basal diet + 9g/100g skullcap root (SR).
Mean values in the same raw with different superscript letters a, b and c are significantly different ($P \leq 0.05$).

protein content is found in the fore of the carcass (21 %), higher in the thigh (22.8 %) and the highest is in the saddle meat (23.9 %).

Fat is an essential component of meat determining its energetic value. Rabbit meat is regarded as a low-fat meat. Between individual muscles, the concentration of lipids varys typically from 1-2 % in the longissimus dorsi muscle to 3-4 % in the hind leg (DALLE-ZOTTE *et al*. 1996), but SZKUCIK and LIBELT (2006) showed that the concentration of lipids typically vary from 1 to 6 % and depend on carcass part. In the present study we estimated that the fat level in rabbit meat was between 1.78 (C group) to 2.11% (CH group). Supplementation with Baikal skullcap root (CH+SR) decreased the fat level by 15.6% ($P \leq 0.05$) in comparison to the CH group. The dietary fat inclusion level and its source are important in carcass and meat quality. Moreover, when higher fat inclusions in the diet are present, the fat content of the meat increases, while protein content decreases (DALLE-ZOTTE 2002; CHRIST *et al*. 1996; PLA & CERVERA 1997). Similar results were also obtained in the present study. The lowest protein content was measured in the rabbits receiving the high cholesterol diet with no skullcap root supplement (CH group) and simultaneously we observed the highest fat content in this group. The lowest protein content and the highest fat content were found in other studies in rabbits consuming sage extract. Moreover, lower protein content and higher fat content were found in musculus longissimus dorsi of rabbits fed diets supplemented with oregano extract and commercial phytoadditive XTRACT – a plant extract mixture composed of carvacrol, cinnamon-aldehyde and capsaicin (POGÁNY SIMONOVÁ *et al*. 2010).

pH is the most important factor of meat quality. LAWRIE (1991) reported that the lowering of pH in muscles is due to the accumulation of lactic acid after stunning and that both the rate and the extent of the post-mortem pH fall are influenced by intrinsic factors such as species, the type of muscles and variability between animals. HULOT & OUHAYOUN (1999) also indicated that it is one of the most significant postmortem changes that occur during the conversion of muscles to meat. This parameter affects the structure of proteins as well as water retention capacity of the tissue, and may modify the sensorial quality of the meat – mainly color and tenderness (BARRÓN *et al*. 2004; MAJ *et al*. 2008).

In the present study Baikal skullcap root had no effect on the pH which ranged from 5.82 to 5.86. These results are similar to those obtained by MAJ *et al*. (2008) being from 5.82 for New Zealand rabbits to 5.89 for Californian rabbits 24 h after slaughter. However, these authors asserted that for meat of good quality, pH should be in the range of 5.4 to 5.8 after 24 hours of meat storage. Additionally SZKUCIK and PYZ-ŁUKASIK (2006) assessed differentiation in the pH value which changes depending of the part of carcass. The lowest pH was measured in the saddle (5.56), the highest in the thigh (5.71) after 24 hours of meat storage. Our results are lower than values found in longissimus dorsi pH (6.03) by BARRÓN *et al.* (2004) who also reported pH_{24h}. Data from an earlier study indicate that the addition of skullcap root to the diet of broiler chickens also had no effect on breast and leg muscle pH (KRÓLICZEWSKA *et al*. 2008).

No significant differences were found in ash content (Table 2). Similar results were obtained in studies of POGÁNY SIMONOVÁ *et al*. (2010) after enrichment of rabbit diet with plant extracts. PEIRETTI and MEINERI (2009) reported that antioxidants of blue-green algae *Spirulina platensis* did not influence the chemical composition (dry matter, crude protein, ash) of the longissimus dorsi muscle of rabbits fed high fat diets.

In conclusions, results of the current study showed that feeding rabbits with Baikal skullcap can lower the cholesterol level in the muscles of rabbits fed a fat-enriched diet. Additionally, Baikal skullcap root did not worsen the chemical composition of the rabbit meat. The present work confirmed the positive effect of feeding Baikal skullcap on lipid content in hind leg muscle of rabbit.

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