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The influence of dietary supplementation with *Melissa officinalis* and combination of *Achillea millefolium* and *Crataegus oxyacantha* on oxidative stability of stored poultry meat*

S. Marcinčák[1,3], L. Mesarčová[1], P. Popelka[1], M. Čertík[2], J. Šimková[1], D. Marcinčáková[1], P. Maľa[1], P. Zachar[1] and M. Mártonová[1]

[1]The University of Veterinary Medicine and Pharmacy
Komenského 73, 041 81, Košice, Slovakia
[2]Slovak University of Technology, Faculty of Chemical and Food Technology
Radlinského 9, 812 37, Bratislava, Slovakia

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ABSTRACT

The effect of feeding common balm (*Melissa officinalis* L.) and combination of yarrow (*Achillea millefolium* L.) and common hawthorn (*Crataegus oxyacantha* L.) on sensory properties and oxidative stability (2-thiobarbituric method - TBA) of chilled and frozen chicken meat was investigated. The experiment was carried out on 90 one-day-old broiler chicks (ROSS 308) divided into three groups and fed for 41 days, as follows: control (C) with basal diet without supplementation, the second group (LB) with basal diet supplemented with ground common balm 20 g·kg^{-1}, and the third group (YH) with basal diet supplemented with ground yarrow 20 g·kg^{-1} and hawthorn 10 g·kg^{-1}. Supplementation with common balm, and especially with combination of yarrow and hawthorn, caused the significant reduction in lipid oxidation processes in chicken meat during chilling and freezing storage. In experimental groups (LB, YH) stored chilled or frozen significant lower amounts of TBA reactive products were found compared with control group ($P<0.05$). Thigh meat was more susceptible to lipid oxidation compared with breast meat. In addition, diets supplemented with plants had a positive effect on sensory quality of fresh or frozen (12 month) meat. On the other hand, organoleptic properties of breast muscles were not influenced by supplementation.

KEY WORDS: broiler chicken, herbs, meat storage, lipid oxidation

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[3] Corresponding author: e-mail: marcincak@uvm.sk

INTRODUCTION

Essential fatty acids play an important role in human nutrition as precursors to many biologically active substances. Broiler chicken meat has many desirable nutritional characteristics such as low lipid content and relatively high amount of polyunsaturated fatty acids (PUFAs) compared with fat of other slaughtered animals (Brenes and Roura, 2010). This is due to the relatively high content of phospholipids in the muscle cell membrane structures (Bystrický and Dičáková, 1998). A higher level of polyunsaturated fatty acids in muscle membranes is related to the increasing susceptibility of meat and meat products to fat oxidation. PUFAs undergo rapid oxidative changes which impair the organoleptic characteristics and shorten food shelf-life (Lopez-Bote et al., 1998). Lipid oxidation is one of the primary mechanisms of food quality deterioration, especially meat products (Gorelik et al., 2008). Therefore, there is a need to increase the antioxidation capacity of muscles which can be achieved by feeding with antioxidant active substances (Marcinčák et al., 2008; Mátéová et al., 2008).

Chicken meat containing higher amount of PUFAs is at risk that their profile will change during storage (Koreleski and Świątkiewicz, 2007). Using natural antioxidants in poultry fattening is a simple method to achieve a higher antioxidant stability, improvement of sensory properties (aroma and taste) and prolongation of storage (Windisch et al., 2008; Luna et al., 2010). A major source of natural antioxidants is the plant material (Smet et al., 2008). Many plants have beneficial multifunctional properties derived from their specific bio-active components. Because of possible 'synergy' between constituents, it remains unclear which components may stimulate endogenous digestive enzymes, act as an antioxidant, antimicrobial agent, or immunomodulator (Brenes and Roura, 2010; Nasir and Grashorn, 2010). Common balm, yarrow and hawthorn are known as herbs with high content of antioxidant active substances (Dastmalchi et al., 2008). Some molecules responsible for the antioxidative properties of natural plant extracts are flavonoids and phenolic compounds (Lahucky et al., 2010). The constituents responsible for the pharmacological effects of hawthorn preparations include flavonoids and oligomeric procyanidins. These compounds have lipid-lowering, antioxidant and anti-inflammatory properties which may protect organism against myocardial damage and arrhythmias (Ljubuncic et al., 2005).

The aim of this study was to investigate the effect of supplementation of diet with selected plants: common balm (*Melissa officinalis* L.), and combination of common yarrow (*Achillea millefolium* L.) and common hawthorn (*Crataegus oxyacantha* L.) on the oxidative stability and sensory properties of chicken meat (thigh and breast) stored under chilling (4°C) or freezing conditions (-18°C) for 14 days or 12 months, respectively.

MATERIAL AND METHODS

Experimental animals, diet and treatment

The experiment was carried out on 90 one-day-old unsexed broiler chicks (Ross 308). Broilers were reared in approved animal quarters and they were randomly divided into 3 groups, in 3 replications, each containing 10 birds. Each group was kept in pen with wood shavings. For 41 days all birds were fed *ad libitum* a commercial basal diet (Table 1): the starter, from day 1 to day 15, and finisher, from day 16 to day 41. The control group (C) received the basal diet (BD) only. The second group (LB) was fed with basal diet supplemented with

Table 1. Composition and calculated analysis of basal diets

Item	Starter	Finisher
Ingredients, %		
maize	51.3	49.0
wheat	8.0	10.0
wheat meal	7.0	4.0
soyabean meal	29.9	31.6
wheat bran	-	2.15
limestone	1.90	1.25
monocalcium phosphate	0.89	1.00
vitamin-mineral premix [1]	0.30	0.30
NaCl	0.36	0.30
L-lysine	0.10	0.15
DL-methionine	0.15	0.15
enzymatic preparation [2]	0.10	0.10
Calculated analysis, %		
linoleic acid	1.0	1.0
metabolizable energy, MJ·kg^{-1}	11.5	12.0
crude protein	17.5	19.0
crude fibre	5.0	4.0
ash	8.0	7.0
L-lysine	0.80	0.95
DL-methionine	0.35	0.40
methionine + cysteine	0.70	0.75
Ca	0.80	0.70
available P	0.50	0.50

[1] Premix supplied per kg of basal diet, IU: vit. A 8 000 000, vit. D_3 1 200 000; mg: vit. E 15 000; vit. K_3 3 000, vit. B1 1 500, vit. B6 8 000, niacin 15,000, choline chloride 50,000, pantothenic acid 50, pyridoxine 5, folic acid 2, biotin 0.2, I 2, Co 1, Cu 6.0, Fe 60, Zn 50, Mn 50; g: K 8.6, Cl^- 2; µg: cyanocobalamine, 30

[2] contained per kg of basal diet: α-amylase (EC 3.2.1.1) 200 U; endo-1,3(4)-β-glucanase (EC 3.2.1.6) 1,175 U; endo-1,4-β-glucanase (EC 3.2.1.4) 2,000 U; endo-1,4-β-xylanase (EC 3.2.1.8) 2,000 U; bacillolyzine (EC 3.4.24.28) 225 U; 6-fytase (EC. 3.1.3.26) 499.5 FYT

ground common balm in concentration 2.0% per 1 kg and the third group (YH) with basal diet supplemented with combination of ground yarrow (2.0%) and hawthorn (1.0%). All dry ground plants were purchased from Agrokarpaty Plavnica Ltd. (Slovakia).

During the trial, temperature was gradually decreased from 33°C on day 1 to 22°C on day 21 and then kept constant. The lighting programme was provided 24 h of continuous light per day. The humidity of the environment was 70%. Health status was evaluated daily. The experiment was approved by the Ethics Committee of the University of Veterinary Medicine and Pharmacy in Kosice (Slovakia).

Sample collection and analysis

On the day 41 of fattening, prior stunned, chickens were slaughtered, breast and thigh muscles were deboned and packed into polyethylene bags. Breast samples were stored only in freezer (-18°C) for 12 months. The thigh samples were stored in a refrigerator at 4°C for 14 days and also in a freezer (-18°C) for 12 months.

Oxidation of fat was determined by 2-thiobarbituric method (TBA) according to Marcinčák et al. (2004) and measured spectrophotometrically at 532 nm (Helios γ, v. 4.6, Thermospectronic, UK). Results were calculated as amount of malondialdehyde in 1 kg of sample.

Chilled breasts and thigh muscles stored 24 h after dressing and muscles stored 12 months in the freezer (-18°C) were used for the sensory evaluation under the guidance for sensory evaluation of poultry meat. Sensory analysis was conducted by 7- member panel, using five-point ranking system (Pribela, 2001). The system was applied for following four traits: taste, aroma, texture and juiciness, with maximum score 20 points.

Statistical analysis

Statistical analysis was performed by GraphPad Prism (2003), version 4.00 statistical software. Results were expressed as the least square means (mean) and standard deviation (±SD). Increasing amount of malondialdehyde in groups was compared by one-way ANOVA test during storage. Tukey's multiple comparison test was used to compare statistical differences among values and $P<0.05$ was considering as statistically significant. Values of the observed parameters, which are presented in Tables, are given as average values obtained from six replicates.

RESULTS AND DISCUSSION

Recently, interest in plant feed additives as an alternative growth a promoter has increased because of the ban on the use of antibiotic as feed additives (Windisch et al., 2008). The effect of herbs and their essential oils on the final weight of chickens has been described in several works (e.g., Barreto et al., 2008; Brenes and Roura, 2010). However, their effect on increasing total weight of chickens is inconsistent (Marcinčák et al., 2011). In our experiment, the highest average body weight on the day 41 of fattening was found in broiler chickens of the control group (2107 g). Body weight of broiler chickens from the experimental groups was slightly lower (LB-2063 g, YH-2087 g) compared with control, but statistical differences among groups were not significant (P>0.05).

A high oxidative stability of meat is important when attempting to avoid or delay products becoming rancid. Taking into account the character of lipid oxidation, effect of antioxidants is more pronounced in dietary application than the post-mortem addition to meat (Govaris et al., 2004). TBA value was expressed as an amount of malondialdehyde (MDA) which is the main secondary degradation product of polyunsaturated fatty acids. Determination of TBA value in thigh muscle stored at 4°C for 14 days are shown in Table 2.

Table 2. Results of TBA determination expressed as an amount of malondialdehyde (mg·kg^{-1}) during storage of thigh muscle under chilling conditions (4°C)

Group	Malondialdehyde, mg·kg^{-1}		
	1. day	7. day	14. day
Control	0.217 ± 0.024^{a3}	0.402 ± 0.094^{a2}	0.715 ± 0.081^{a1}
Common balm	0.148 ± 0.030^{a2}	0.252 ± 0.008^{b2}	0.414 ± 0.063^{b1}
Yarrow + hawthorn	0.133 ± 0.029^{a2}	0.237 ± 0.058^{b2}	0.382 ± 0.049^{b1}

^{a,b} - values with different superscripts in column are statistically different (P<0.05)
^{1,2,3} - values with different superscripts in row are statistically different (P<0.05)

The values of MDA in all samples were low. However, further storage gradually increased the MDA. The increase of MDA in the samples from experimental groups was significantly lower (P<0.05) than in control. A significant decrease in oxidative products in the experimental groups compared with control (P<0.05) was already noticeable on the 7th day of storage. Higher oxidative stability during storage was found in thigh muscle from chickens fed with combination of plants yarrow and hawthorn (P>0.05). Luna et al. (2010) investigated the oxidative stability of stored meat (4ºC, 10 days) from chicken fed with thymol and carvacol extracts in a dose of 150 mg·kg^{-1} for 42 days. They stated that the addition of extracts had no significant influence on the oxidative stability of breast muscle, as opposed to thigh muscle oxidative stability positively affected during storage.

The positive impact of adding rosemary and powder extract on oxidative stability of broiler meat were also found by Šperňáková et al. (2007) and of oregano by Govaris et al. (2004).

The antioxidant properties of methanolic and ethanolic extracts of *Melisa officinalis* have already been pointed out (Berasategi et al., 2011). They contain flavonoids and hydroxycinnamic acid derivates, rosmarinic acid is the major component (Dastmalchi et al., 2008). Extracts of *Crataegus oxyacantha* and *Achillea millefolium* are also rich in such polyphenolic compounds as flavonoids, proanthocyanidins and phenolcarbonic acids (Ljubuncic et al., 2005; Trumbeckaite et al., 2011). Studies have shown that flavonoids have the capacity to act as powerful antioxidants by scavenging free radicals and terminating oxidative reactions (Sáyago-Ayerdi et al., 2009). The protective properties of herbal phenolic compounds against lipid oxidation might arise at least in part from specific interactions with lipid metabolism rather than from an increment of total dietary antioxidative potential. This hypotesis is supported by observing improved oxidative stability of tissues in oregano fed chicken exposed to transport stress (Botsoglou et al., 2004).

The amount of malondialdehyde in breast muscles was determined only in frozen samples after one month and twelve months of storage (Table 3). Since the fat content in breast muscles is significantly lower than in thigh muscles (Aziza et al., 2010), the oxidative damage in frozen samples is lower in breast than in thigh muscles. The obtained results show that the amount of MDA in experimental groups was lower than in the control group ($P<0.05$) after one month of storage. After 12 months storage it was found that the amount of malondialdehyde increased ($P<0.05$) only in control group. Amount of MDA in breast muscles of the experimental groups (LB - 0.114 $mg \cdot kg^{-1}$, YH - 0.130 $mg \cdot kg^{-1}$; $P<0.05$) was significantly lower than in the control group (0.211 $mg \cdot kg^{-1}$).

Table 3. Results of TBA determination expressed as an amount of malondialdehyde ($mg \cdot kg^{-1}$) during storage of breast muscle under freezing conditions (-18°C)

Group	Malondialdehyde, $mg \cdot kg^{-1}$	
	1 month	12 months
Control	0.145 ± 0.017^{a1}	0.211 ± 0.027^{a2}
Common balm	0.085 ± 0.039^{b1}	0.114 ± 0.011^{b1}
Yarrow+hawthorn	0.084 ± 0.010^{b1}	0.130 ± 0.016^{b1}

^{a,b} - values with different superscripts in column are statistically different ($P<0.05$)

^{1,2} - values with different superscripts in row are statistically different ($P<0.05$)

TBA value in the thigh muscle of samples stored in a freezer (-18°C) for 12 months are shown in Table 4. After the first month of storage the values of TBA products in meat, expressed as the amount of MDA, were the same in

Table 4. Results of TBA determination expressed as an amount of malondialdehyde (mg·kg^{-1}) during storage of thigh muscle under freezing conditions (-18°C)

Group	Malondialdehyde, mg·kg^{-1}		
	1 month	6 months	12 months
Control	0.159 ± 0.012^{a1}	0.200 ± 0.048^{a1}	0.654 ± 0.041^{a2}
Common balm	0.104 ± 0.013^{a1}	0.122 ± 0.019^{b1}	0.489 ± 0.022^{b2}
Yarrow+hawthorn	0.103 ± 0.017^{a1}	0.097 ± 0.029^{b1}	0.318 ± 0.027^{c2}

^{a,b,c} - values with different superscripts in column are statistically different (P<0.05)
^{1,2} - values with different superscripts in row are statistically different (P<0.05)

both experimental groups (LB - 0.104, YH - 0.103 mg·kg^{-1}); higher level of TBA products were recorded in control (0.159 mg·kg^{-1}). After 6 months of storage, levels of TBA degradation products increased in samples control and common balm groups, but they were lower in experimental groups compared with control (P<0.05). The increase (P<0.05) in products of fat degradation, expressed as the amount of MDA, was also determined in all samples after the storage in a freezer for 12 months. The highest MDA values (P<0.05) were observed in the control group. In experimental groups lower decrease in degradation products and lower fed oxidation were found during storage in the samples of chicken fed with combination of yarrow and hawthorn herbs (P<0.05). Use plants (common balm, yarrow and hawthorn) had a significant impact on the reduction the oxidative processes in meat during storage in a freezer (P<0.05) while suggests a higher oxidative stability of meat during storage. Lopez-Bote et al. (1998) indicated similar results of TBA values which were achieved when meat of broilers with addition of antioxidants was stored under freezing conditions for four months. Florou-Paneri et al. (2006) investigated the effect of feeding the mixture with oregano and oregano extract on oxidative activity in turkeys. They reported that the dose of 10 g·kg^{-1} of oregano or 200 mg·kg^{-1} of oregano extract reduced fat oxidation, while at higher dosage the oxidation was lower.

Thigh meat is higher in lipids and triglycerides and more prone to lipid oxidation than breast meat (Aziza et al., 2010). When comparing oxidative damage of breast and thigh muscles during storage under freezing conditions (12 months), we may conclude that oxidative damage of lipids was significantly lower in breast than in thigh muscle. When feeding diet was supplemented with plants, oxidative damage was significantly reduced. The lower MDA values found in tissues of chicken given herbs supplementation are probably the results of various antioxidants that entered the blood stream, and were distributed and retained in the tissues, exhibiting antioxidant activity (Florou-Paneri et al., 2006; Marcinčák et al., 2008).

Fat in meat is important in terms of sensory, because it contains a wide range of aromatic and flavour substances. Results of the overall sensory evaluation are given in Table 5. Cooking method was used for sensory evaluation, where taste,

Table 5. Results of sensory evaluation of thigh muscle: fresh chilled (1 day after slaughter processing) and frozen meat (stored 12 months in a freezer at -18°C)

Group		Sensory evaluation	
		fresh	frozen
Control	breast	16.03 ± 2.30^{a}	15.65 ± 2.25^{a}
	thigh	16.96 ± 1.95^{a1}	15.80 ± 1.79^{b1}
Common balm	breast	15.80 ± 2.25^{a}	15.60 ± 2.33^{a}
	thigh	17.08 ± 1.72^{a1}	16.25 ± 1.22^{a12}
Yarrow+hawthorn	breast	16.13 ± 1.98^{a}	15.45 ± 2.50^{a}
	thigh	17.80 ± 1.43^{a1}	17.30 ± 1.11^{a2}

[a,b] - values with different superscripts in row are statistically different ($P<0.05$)

[1,2] - values with different superscripts in column are statistically different ($P<0.05$)

juiciness, tenderness and flavour of meat were evaluated (maximum total score was 20). When comparing sensory evaluation of breast and thigh muscles, thigh muscle was higher rated in all three groups.

The results show that the supplementing with plants has a positive impact on the sensory evaluation. This effect is noticeable after storage of thigh samples in a freezer for 12 months. The assessed parameters, taste, flavour and juiciness, were rated higher in experimental groups than in the control (data not presented).

Lipid oxidation in meat products (apart from microbial spoilage) is the primary process by which quality loss occurs. Products of lipid oxidation processes can adversely affect texture, colour, flavour, nutritive value and safety of meat products (Lahucky et al., 2010). Antioxidants have been utilized for many years to avoid or delay these lipid oxidation processes (Korimová et al., 1998). In the sensory analysis all analysed samples stored under freezing conditions for 12 months were worse compared with fresh samples. These differences were more pronounced in evaluation of thigh muscle. However, the added plants had a significantly positive effect on improving sensory quality of thigh samples after 12 months of freezing storage compared with control. Stored breast meat (6 months, -20°C) was found to be suitable for consumption, but the effect of additives (plant extracts of coneflower, thyme and sage) on the taste and smell of meat was not recorded (Koreleski and Świątkiewicz, 2007). Several authors stressed improved sensory characteristics of poultry meat after adding plants with antioxidant activity (Šperňáková et al., 2007; Marcinčák et al., 2008). Improvement of taste and flavour was found in meat of chickens fed with the diet supplemented with rosemary powder (Šperňáková et al., 2007).

CONCLUSIONS

Based on the obtained results, it may be concluded that the addition of common balm in a dose of 2%, and mainly a combination of yarrow (2%) and hawthorn (1%)

per kg of the diet has a significant impact on the reduction of oxidation processes in thigh and breast muscles stored under chilling (4°C) or freezing conditions (-18°C). The results show that the feeding of plants has a positive impact on the sensory traits. This effect is more pronounced after the storage of thigh samples in a freezer for 12 months.

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