TITLE: Proximate composition, cholesterol concentration and lipid oxidation of meat from chickens fed dietary spice addition (*Allium sativum, Piper nigrum, Capsicumannuum*)

AUTHORS: Puvača Nikola, Kostadinović Ljiljana, Popović Sanja, Lević Jovanka, Ljubojević Dragana, Tufarelli Vincenzo, Jovanović Rade, Tasić Tatjana, Ikonić Predrag, Lukač Dragan

CSIRO PUBLISHING

Animal Production Science
http://dx.doi.org/10.1071/AN15115

Proximate composition, cholesterol concentration and lipid oxidation of meat from chickens fed dietary spice addition (*Allium sativum, Piper nigrum, Capsicum annuum*)

N. Puvača[A,F], *Lj. Kostadinović*[B], *S. Popović*[B], *J. Lević*[B], *D. Ljubojević*[C], *V. Tufarelli*[D], *R. Jovanović*[E], *T. Tasić*[B], *P. Ikonić*[B] *and D. Lukač*[A]

[A]Faculty of Agriculture, Department of Animal Science, University of Novi Sad, Trg Dositeja Obradovića 8, 210000 Novi Sad, Serbia.
[B]Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia.
[C]Scientific Institute of Veterinary Medicine 'Novi Sad', Rumenački put 20, 21000 Novi Sad, Serbia.
[D]Department of DETO, Section of Veterinary Science and Animal Production, University of Bari 'Aldo Moro', Casamassima 3, 700100 Valenzano, Italy.
[E]Institute for Science Application in Agriculture, Bulevar Despota Stefana 68, 1000 Belgrade, Serbia.
[F]Corresponding author. Email: nikola.puvaca@gmail.com

Abstract. The effects of supplementing spices, including garlic, black pepper and hot red pepper, in broiler chicken diet on proximate composition, cholesterol content and lipid oxidation of breast and thigh with drumstick meat, skin and liver were investigated. Meat proximate composition included measurements of moisture, protein, fat and ash content. Cholesterol content of tissue homogenates was performed by high-performance liquid chromatography–DAD analyses, while lipid oxidation of white and red meat, as well as liver, was expressed as a concentration of thiobarbituric acid reactive substances (TBARS) (mg malondialdehyde (MDA)/kg tissue). For biological research, eight treatments with a total of 1200 broiler chickens of hybrid line Hubbard were formed, with four replicates. In the control treatment, the chickens were fed with commercial mixtures of standard composition and quality based on corn flour and soybean meal. Experimental treatments were fed with the same commercial mixtures, except with addition of spices. At the end of the experiment and on the basis of gained results, it can be concluded that the chickens in experimental treatments with hot red pepper achieved statistically significantly ($P < 0.05$) higher final body masses (2460.6 and 2442.4 g) than did the chickens in the control and other treatments. Black pepper showed a positive and significant ($P < 0.05$) influence on improving the protein content in breast meat (24 g/100 g), hot red pepper lowered the cholesterol concentrations in meat (24.7 g/100 g in red meat), skin (87.4 g/100 g) and liver (263.1 g/100 g), while black pepper significantly ($P < 0.05$) reduced lipid oxidation in breast (0.05 mg MDA/kg tissue) and thigh with drumstick (0.12 mg MDA/kg tissue). On the basis of obtained findings, it can be concluded that the dietary spice herbs had a positive influence on a proximate composition of chicken meat, cholesterol concentrations and lipid oxidation process.

Additional keywords: chemical composition, fat, meat, peroxidation, protein.

Received 10 March 2015, accepted 13 May 2015, published online 6 July 2015

Introduction

Spices and medicinal plants as a natural foodstuffs appeal to all who question safety of synthetic food additives and demand high-quality products that at the same time are safe and stable (Hedayati *et al.* 2013). Quality and healthfulness were reported to be some of the most important factors influencing consumer choice of food. Interest in plants, plant extracts and derived phytochemicals as dietary additives for poultry has increased during the past decades (Khan *et al.* 2012; Puvača *et al.* 2013). Moreover, spice herbs and aromatic plants possess many antioxidants that are effective in preventing oxidative changes and, thus, can minimise off-odour production in meat (Najafi and Torki 2010). The use of nutritional strategies in improving the proximate meat quality is a relatively new approach that has emerged at the interface of animal and food science from the fact that antibiotics as synthetic growth promoters have been banded for use. Nutritional approaches are often more effective than is direct addition of the additive to meat since the compound is preferably deposited where it is most needed (Govaris *et al.* 2004). The physiological ability to store excess energy or caloric intake in the form of fat is a trait that confers numerous advantages across the biological spectrum. Fat is the most efficient way for living organisms to store excess energy. Moreover, fat excludes water, which can account for much of

the mass of stored glycogen, the major starch and storage form of glucose in the body (Lawrence 2010). Saturated and monounsaturated fatty acids are common in animal fats, such as meat products (Ljubojević *et al.* 2015). Monounsaturated fatty acids are also abundant in certain vegetable oils. Monounsaturated oils are touted as the most favourable in the diet because they do not raise cholesterol as much as do saturated fats and they are less susceptible to spontaneous oxidation than are the polyunsaturated fatty acids (Puvača *et al.* 2014*b*). Through the ages cholesterol has evolved into an essential component of animals, being metabolised into a wide range of useful substances in the body such as steroid hormones, vitamin D and bile salts, which acts as detergents in the digestive system, emulsifying oils and fats to make it easier for lipases to do their job (Konjufca *et al.* 1997). Poultry are able to synthesise cholesterol from nutrients such as fatty acids, carbohydrates, or even protein, depending on the diet and the relative availability of each of these nutrient precursors (Shirzadegan and Falahpour 2014). Cholesterol is constantly being moved as it is needed in one organ or another for many vital processes (Asztalos *et al.* 2000). Nearly 50% of the mass of low-density lipoprotein (LDL) is composed of cholesterol, and LDL seems to be the lipoprotein that is most prone to spontaneous oxidation (Chen *et al.* 2008). Oxygen is essential for survival, and the reaction with oxygen is known as oxidation (Ciftci *et al.* 2010), but beside its essentiality for life, it can cause damage to various cells (Min and Ahn 2005). Three sensory and quality characteristics such as colour, texture and flavour are the main attributes affecting meat consumer acceptance, and lipid peroxidation is the primary cause of these quality deteriorations in meat and meat products (Kostadinović *et al.* 2015). Lipid peroxidation primarily occurs through a free radical chain reaction, and oxygen is the most important factor on the development of lipid peroxidation in meat (Ahn *et al.* 1993; Laudadio and Tufarelli 2011), through a free radical chain reaction that comprises three primary steps, namely, initiation, propagation and termination (Min and Ahn 2005). Lipid oxidation is an important determinant of shelf life of meat and meat products. Antioxidants are natural or synthetic substances used to prevent lipid oxidation. Meat protection, primarily against lipid components, is possible by adding antioxidants to feed mixtures (Marcinčák *et al.* 2005). This is a simple way to ensure oxidative stability of meat fats during the post-slaughter processing of carcasses and storage of meat.

On the basis of previous considerations, the aim of our experiment was to investigate the influence of garlic, black pepper and hot red pepper powders as natural-antioxidant dietary supplements in broiler chicken rations on meat proximate composition, cholesterol concentration and lipid oxidation.

Materials and methods

Animal housing and nutrition

Biological tests with chickens were carried out under production conditions at the experimental farm 'Pustara' at the Faculty of Agriculture in Novi Sad, Serbia. At the beginning of the experiment, a total of 1200 day-old Hubbard strain broilers were equally distributed into eight dietary treatments, with four replicates each. Dietary treatments in the experiments were as follows: CON (control diet), G-0.5 (garlic powder 0.5 g/100 g diet), G-1.0 (garlic powder 1.0 g/100 g), BP-0.5 (black pepper powder 0.5 g/100 g), BP-1.0 (black pepper powder 1.0 g/100 g), HRP-0.5 (hot red pepper 0.5 g/100 g), HRP-1.0 (hot red pepper 1.0 g/100 g) and MX-0.5 (mixture of spices at the ratio of 1 : 1 : 1 in total amount of 0.5 g/100 g). Feeding program included a three-phase diet as starter, grower and finisher, respectively (Table 1). During the whole trial, feed and water were provided *ad libitum*. Rearing and housing conditions were as previously described in detail by Puvača *et al.* (2015).

Sample collections

At the end of 42nd day of the experiment, 12 broiler chickens, six male and six female of an average bodyweight of each treatment group were selected for meat-quality evaluations. Before slaughtering, broiler chickens were starved for 12 h. On slaughter, dressed cold carcasses were dissected into primal cuts such as breast, thighs with drumsticks, wings, back, head, neck and legs, following the method prescribed by the Regulation on Poultry Meat Quality (Official Gazette of the SFRY No. 1/81 and 51/88 1988). After 24 h post-mortem, samples of breast (*Musculus pectoralis*) and thigh with drumstick (*Tibialis anterior* and *Biceps femoris*), skin (from breast and thigh with drumstick) and liver (right lobe) were further analysed for their proximate composition, cholesterol concentration and lipid oxidation.

Proximate-composition analyses

Proximate composition of breast, thigh with drumstick, skin and liver were determined according to the ISO recommended standards for moisture (ISO 1442 1997), protein (ISO 937 1978), fat (ISO 1443 1973) and ash (ISO 936 1998) contents. Moisture content of selected tissues was determined after drying the samples at 105°C for 24 h to constant weight. Crude protein concentration was determined by Kjeldahl method, and ash was determined after burning at 550 ± 25°C. Crude fat in chicken tissues was analysed using the Soxhlet apparatus (Sigma-Aldrich, Buchs SG, Switzerland) with ether as a solvent. Data presented are means of 12 measurements.

Analyses of cholesterol concentration

Standard solution of cholesterol (analytical standard min. 99.9%, Lot. No.: 01106ME-207, Sigma-Aldrich, Hamburg, Germany)

Table 1. Composition and energy content of the starter, grower and finisher diets excluding the added spices
Spice herbs were added on top of the basal diet

Nutrient	Diet mixtures		
	Starter	Grower	Finisher
Dry matter (% of as-fed basis)	89.4	89.3	89.4
Moisture (% of as-fed basis)	10.5	10.7	10.5
Crude protein (% of as-fed basis)	21.1	20.7	17.3
Crude fat (% of as-fed basis)	3.9	3.9	4.7
Crude fibre (% of as-fed basis)	3.5	3.5	3.6
Crude ash (% of as-fed basis)	5.0	4.8	5.6
Ca (% of as-fed basis)	0.8	0.9	1.1
P (% of as-fed basis)	0.6	0.6	0.5
Metabolisable energy (MJ/kg)	12.5	12.8	13.3

was prepared dissolving 10 mg of standard into 100 mL of hexane. This stock solution was used to prepare a series of standard solutions for calibration curve. The cholesterol extraction and the clean-up procedure were performed according to the method described by Carnevale de Almeida *et al*. (2006), with slight modifications. Briefly, ~2 g of sample was saponificated with 4 mL of 50% potassium hydroxide and 6 mL of 95% ethanol. The tubes were vortexed thoroughly after addition of each component, and then heated for 10 min at 60°C. After this, 5 mL of water was added and the samples were cooled. The non-saponifiable fraction was extracted three times using 10 mL of hexane. The hexane phase was evaporated to dryness using Rotavapor (Yamato Scientific America Inc., Santa Clara, CA, USA) at 28°C. The dry residue was dissolved in 1 mL of hexane and filtered through 0.45 µm pore size PTFE filter (GORE® Filtration Products, Plano, TX, USA) in vial before high-performance liquid chromatography (HPLC) analysis. HPLC studies were performed using an Agilent 1200 system (GenTech Scientific Inc., Arcade, NY, USA) equipped with a UV-visible detector (DAD), binary pump, a vacuum degasser and an auto sampler. System control and data analysis were processed with Chemstation Software (Agilent Technologies, GenTech Scientific Inc., Arcade, NY, USA). The chromatographic column Zorbax RX-SIL (1.8 µm particle size, 100 mm × 2.1 mm internal diameter) was obtained by Agilent (GenTech Scientific Inc., Arcade, NY, USA). The column was washed in hexane by isocratic elution at a flow-rate of 0.5 mL/min. The injection volume was 5 µL. The spectra were recorded at a wavelength of a 210 nm. The compounds were identified by chromatographic comparisons with authentic standards and against UV spectra comparison using a DAD detector. Quantification was based on the use of a calibration curve.

Thiobarbituric acid reactive substances (TBARS) assay

One gram each of the excised broiler breast, thigh with drumstick and liver was minced with scissors and homogenised in an ultraturax in three volumes of isotonic buffer (0.05 mol/L tris–HCl, 0.25 mol/L sucrose, pH = 7.5). The homogenates was filtered through gauze into ice-cold tubes and collected for further analysis (Chiu *et al.* 1976). The oxidative stability of broiler tissues was determined by using TBARS assay. Tissue homogenates (0.2 mL) were transferred into the tube and 2 mL of a tribromoanisole and 2,4,6-trichloroanisaole solution was added. The mixture was put in the mixer and 1.05 mL of ccHCl was added. Afterwards, the tube was filled with distilled water up to 50 mL. The mixture was incubated in a boiling water bath for 15 min to develop colour. After colour development, the samples were cooled in cold water for 10 min and then centrifuged for 10 min at 3000*g*. Spectrophotometric measurement was performed at 536 nm. The TBARS concentration was expressed in mg malondialdehyde (MDA) per kg of tissue.

Statistical analyses

Statistical analyses were conducted using statistical software program Statistica 12 for Windows (StatSoft, Inc., Tulsa, OK, USA), to determine whether variables differed among treatments. Significant effects were further evaluated using ANOVA, least-square means (LSM) and standard errors of least-square means (SE_{LSM}). Fisher's l.s.d. *post-hoc* multiple-range test with Bonferroni corrections was used to ascertain differences among treatments. A significance level of $P = 0.05$ was used.

Results and discussion

Proximate composition

The results given in Table 2 show that there were no significant ($P > 0.05$) differences in moisture content in both breast and thigh with drumstick meat. The highest concentration of protein in breast meat was observed in Treatment BP-1.0, which was significantly ($P < 0.05$) different from Treatments G-0.5, CON and HRP-1.0. The lowest amount of fat was observed in Treatment BP-1.0. This was expected, considering the negative correlations between the concentrations of protein

Table 2. Least-square means of chicken final bodyweight, carcass weight after slaughtering and cooling and proximate composition of breast meat and thigh with drumstick (g/100 g)

CON, control treatment; G-0.5, treatment with addition of dietary garlic powder at 0.5 g/100 g; G-1.0, treatment with addition of dietary garlic powder at 1.0 g/100 g; BP-0.5, treatment with addition of dietary black pepper powder at 0.5 g/100 g; BP-1.0, treatment with addition of dietary black pepper powder at 1.0 g/100 g; HRP-0.5, treatment with addition of dietary hot red pepper powder at 0.5 g/100 g; HRP-1.0, treatment with addition of dietary hot red pepper powder at 1.0 g/100 g; MX-0.5, treatment with addition of dietary garlic, black pepper and hot red pepper powder mixture at 0.5 g/100 g in equal ratios of 1 : 1 : 1. SE_{LSM}, standard errors of least-square means. Means within a column followed by the same letter or with no letter are not significantly different (at $P = 0.05$)

Experimental treatment	Chicken weight		Breast meat				Thigh with drumstick			
	End of trial (42 days) (n = 150)	Carcass after slaughtering and cooling (g) (n = 12)	Moisture (n = 12)	Protein (n = 12)	Fat (n = 12)	Ash (n = 12)	Moisture (n = 12)	Protein (n = 12)	Fat (n = 12)	Ash (n = 12)
CON	2075.8d	1746.2bc	73.8a	22.1b	0.55a	1.12bc	74.7a	18.6b	2.59e	1.0b
G-0.5	2371.1b	1906.2a	73.4a	22.3b	0.48b	1.19ab	74.3a	18.7b	3.74a	1.06a
G-1.0	2336.1bc	1835.1ab	73.9a	22.6ab	0.30c	1.18ab	72.7a	19.8ab	2.79d	1.09a
BP-0.5	2076.5d	1649.8c	73.3a	22.6ab	0.28c	1.16abc	74.2a	19.4ab	2.99c	1.0b
BP-1.0	2077.8d	1706.0c	72.3a	24.0a	0.16d	1.24a	72.5a	20.0a	2.26f	1.07a
HRP-0.5	2460.6a	1950.7a	71.8a	22.7ab	0.49b	1.17ab	74.3a	19.5ab	3.24b	1.08a
HRP-1.0	2442.4a	1957.1a	72.6a	21.8b	0.18d	1.06c	73.3a	20.6a	3.68a	1.06a
MX-0.5	2297.8c	1894.8a	72.4a	22.5ab	0.27c	1.17ab	74.8a	19.7ab	2.13f	1.08a
Pooled SE_{LSM}	23.96	45.34	0.81	0.53	0.02	0.02	0.81	0.44	0.04	0.02

and fat. The highest fat concentration in breast meat was recorded in the control treatment, which was significantly ($P < 0.05$) different from the other treatments. Meat ash concentration was almost the same in all groups, but with significant differences ($P < 0.05$) between individual dietary treatments.

The highest concentration of protein in thigh with drumstick was observed in the dietary treatment with addition of hot red pepper (HRP-1.0). It is interesting to notice that the same treatment also contained highest concentration of fat, together with Treatment G-0.5. This could be explained by the influence of capsaicin as the active compound in hot red pepper on the metabolism of fat and the highest utilisation from the feed which is incorporated in the body. The lowest concentration of protein in thigh with drumstick, as in the breast meat, was observed in control treatment, with the fat concentration of 2.59 g/100 g, which could be a sign of a positive influence of dietary spice-herb addition on the nutritive quality of chicken meat. From the results presented in Table 3, unlike for breast and thigh with drumstick, significant ($P < 0.05$) differences in moisture content of skin and liver can be observed. The highest concentration of skin protein was observed in Treatments BP-0.5 and BP-1.0, which were significantly ($P < 0.05$) different from Treatments CON, MX-0.5 and HRP-0.5. The lowest concentration of skin fat was observed in Treatment HRP-0.5, while the highest skin fat concentration was recorded in control treatments CON and G-0.5, which were significantly ($P < 0.05$) different from the other treatments.

The highest concentration of protein in liver was observed in the dietary treatment including dietary spice-herb mixture (MX-0.5), while the lowest was observed in the experimental treatment BP-0.5. As for the thigh and drumstick, the same tendency was observed for the liver fat concentration. In our experiment, moisture content of meat from breast and thigh with drumstick was almost equal among dietary treatments, while the content of moisture in skin and liver showed significant differences. Similarly, Gardzielewska *et al.* (2003) reported that broiler chicken feed supplementation with echinacea, garlic and ginger resulted in no effect on meat moisture content. In the present trial, the highest protein concentration of breast and thigh with drumstick meat was observed in Treatments BP-1.0 and HRP-1.0, respectively. The highest protein concentration of skin and liver was also observed in treatments with black pepper powder, as well as in MX-0.5 treatment. From this fact, it can be noticed that the dietary addition of black pepper and hot red pepper to chicken ration led to significant improvement of proximate meat, skin and liver quality. In a previous study by Puvača *et al.* (2014*a*), addition of garlic powder to chicken diet at a concentration of 2.0 g/100 g resulted in improved protein content (22.9 g/100 g) in breast meat compared with unsupplemented diet (21.8 g/100 g). Souza *et al.* (2011) reported that protein concentration of chicken breast meat ranged from 22.48 to 22.61 g/100 g without significant differences. Majewska *et al.* (2009) reported that concentration of protein of ostrich meat was influenced by the different muscle patties. Onibi *et al.* (2009) reported a significant influence of spice herbs in broiler nutrition on fat content, where the thigh muscle had the highest fat concentration (82.9 g/kg), followed by drumstick muscle (66.9 g/kg), whereas the breast muscle had the lowest (49.1 g/kg). Lowest fat concentration of breast meat in the current study was recorded in chickens fed 1.0 g/100 g of black pepper powder and in red meat of chicken fed with mixture of garlic, black pepper and hot red pepper powder at 0.5 g/100 g. Similarly, fat deposition has been reported to be higher in red meat than in breast meat (Onibi 2006). It seems that the skin has the highest concentration of fat deposition. The highest fat concentration in skin was observed in the control treatment and lower level of garlic powder treatment. Investigation of Ogunmola *et al.* (2013) showed a differing fat concentration in poultry, ranging from 3.70% in chickens to 18.1% in turkeys. Marcinčáková *et al.* (2011) investigated the influence of dietary supplementation of *Melissa officinalis* and combination of *Achillea millefolium* and *Crataegus oxyacantha* in chicken ration on meat quality and reported no significant influence on fat concentration in breast meat, while a significant decrease in the fat concentration of thigh meat was observed. Addition of black pepper powder in diet had high influence on ash content of breast meat, as well as did the addition of dietary spice-herb mixture on liver ash concentration.

Cholesterol concentration

Significant ($P < 0.05$) influence of dietary garlic, black pepper and hot red pepper on cholesterol concentration of breast, thigh with drumstick, skin and liver is shown in Table 4. The highest concentration of cholesterol was observed in breast, thigh with drumstick, skin and liver of chickens fed the control treatment.

Table 3. Least-square means of proximate composition of skin and liver (g/100 g)

See Table 2 for explanation of experimental treatments. Means within a column followed by the same letter or with no letter are not significantly different (at $P = 0.05$)

Experimental treatment	Skin				Liver			
	Moisture	Protein	Fat	Ash	Moisture	Protein	Fat	Ash
CON	52.0a	11.5bc	41.0a	0.67a	74.4ab	15.3a	2.18e	1.20bc
G-0.5	45.9de	12.2ab	41.3a	0.63ab	73.6b	15.6ab	3.49b	1.22b
G-1.0	48.9bc	12.1ab	37.0cd	0.65ab	75.5ab	15.4a	3.24c	1.16c
BP-0.5	50.7ab	12.7a	36.5d	0.58c	73.6b	14.5b	2.27e	1.17bc
BP-1.0	46.7cde	12.7a	38.5b	0.65ab	76.2a	15.9a	1.98f	1.20bc
HRP-0.5	44.4e	11.0c	36.2d	0.57c	73.1b	15.2ab	3.15c	1.16c
HRP-1.0	45.3de	12.2ab	36.4d	0.65ab	73.6b	15.1ab	2.56d	1.21bc
MX-0.5	47.6cd	11.5bc	38.4bc	0.61bc	73.4b	16.1ab	3.83a	1.32a
Pooled SE_{LSM}	0.82	0.28	0.53	0.02	0.81	0.36	0.03	0.02

Table 4. Least-squares means of cholesterol concentration (mg/100 g) in breast, thigh with drumstick, skin and liver

See Table 2 for explanation of experimental treatments. Means within a column followed by the same letter or with no letter are not significantly different (at $P = 0.05$)

Experimental treatment	Cholesterol content			
	Breast	Thigh with drumstick	Skin	Liver
CON	68.8a	84.0a	137.7a	565.5a
G-0.5	60.5b	52.9b	122.3b	502.9b
G-1.0	41.4e	46.4b	97.5d	474.0d
BP-0.5	54.3c	37.8c	122.2b	493.6c
BP-1.0	40.1e	30.7d	100.6c	393.2e
HRP-0.5	53.0c	25.8d	95.5d	335.9g
HRP-1.0	41.2e	24.7d	87.4f	263.1h
MX-0.5	44.1d	24.8d	93.7e	345.5f
Pooled SE_{LSM}	0.81	2.33	0.75	2.09

The lowest concentration of cholesterol in breast meat was observed in chickens from treatments with a higher inclusion of spice herbs at 1.0 g/100 g of black pepper, hot red pepper and garlic, with no significant ($P > 0.05$) differences among them, but significant ($P < 0.05$) if compared with other treatments. Addition of spice herbs to chicken diet at lower concentrations resulted also in a significant ($P < 0.05$) reduction of cholesterol in breast meat, compared with control dietary treatment. The highest influence in lowering cholesterol concentration in thigh with drumstick was found in treatments with higher levels of spice addition, followed by the spice-mixture treatment. As in breast meat, significant ($P < 0.05$) differences were observed also in red meat. All spice herbs led to significant ($P < 0.05$) reduction in skin cholesterol concentration, and the highest reduction was recorded in Treatment HRP-1.0. As the main target organ for the studies of xenobiotic, liver showed the highest cholesterol deposition compared with breast meat, thigh with drumstick and skin. Among all dietary spice-herb treatments from T2 to T8, a statistically significant ($P < 0.05$) reduction of cholesterol, compared with control treatment, was observed. The available literature is limited regarding changes in muscle cholesterol due to the effect of cholesterol-reducing agents such as black pepper and hot red pepper, especially in poultry. Konjufca *et al.* (1997) reported higher cholesterol concentrations in thigh than breast meat when garlic powder was added to chicken feed. A possible explanation could be that the cholesterol is usually associated with adipose tissue, which is more abundant in thigh than in breast meat. Moreover, thigh meat has a much greater content of slow-twitch fibres than has breast meat, which has many more big mitochondria, and the metabolic rate of slow-twitch fibres is much faster in comparison to fast-twitch fibres. In our experiment, garlic powder significantly decreased cholesterol concentrations in breast meat, thigh with drumstick, skin and liver compared with control treatment, while the peppers had avhigher influence than did garlic. Other researchers have also confirmed the positive influence of dietary garlic powder in broiler nutrition on reduction of cholesterol concentrations in meat (Stanaćev *et al.* 2012) and blood (Shahriari *et al.* 2009; Issa and Abo Omar 2012; Puvača *et al.* 2015). Results have shown a high effect of lowering cholesterol by black pepper powder. Under the influence of black pepper, cholesterol concentration in breast meat, thigh with drumstick, skin and liver were reduced significantly ($P < 0.05$), compared with the control and garlic treatments, but with no significant differences compared with hot red pepper treatments, except in skin and liver. These positive effects of black and hot red pepper can be attributed to several antioxidative compounds (Nabil Alloui *et al.* 2014). Next to the antioxidative compounds black pepper constituents include essential oils (α- and β-pinene, limonene, and β-caryophyllene), piperine, eugenol, the enzyme lipase and minerals (Singletary 2010). In the present study, it was confirmed that black pepper lowers cholesterol in tissues of chicken, while others (Shahverdi *et al.* 2013; Abou-Elkhair *et al.* 2014) have confirmed lowering effects on cholesterol in blood and serum of chickens. Al-Kassie *et al.* (2011) reported that the black pepper in concentrations of 0.50%, 0.75% and 1.0% in broiler diet depressed the concentrations of cholesterol, haemoglobin and red blood cells and the ratio of H : L (heterophils/lymphocytes-ratio). The greatest lowering effect of cholesterol in our study was observed with dietary hot red pepper and spice mixtures. Hot red pepper (HRP-1.0) reduced cholesterol concentration the most in breast meat, thigh with drumstick, skin and liver. Lower amount of hot red pepper (HRP-0.5) also showed significant lowering effect compared with the control and garlic powder treatments. Dietary spice mixture (MX-0.5) also showed a significant cholesterol-lowering effect in white and red meat, as well in skin tissue and liver. All dietary spices in the present experiment showed a positive influence on investigated traits, where the dietary increase of spices increased the cholesterol reduction potential. Interesting fact is that the lower concentration of dietary spice mixture had almost the same influence as did the higher concentration of individual spices, or even a greater influence. This can lead to a conclusion that these specific spices act synergistically. The obtained results on meat cholesterol are in accordance with the results of Al-Kassie *et al.* (2012) who used hot red and black pepper separately and in combination. The same authors stated that addition of these spices to broiler diet led to a reduction in the concentration of cholesterol, red blood cells and whole blood count and the ratio of H : L. The results of Shahverdi *et al.* (2013) showed that the inclusion of hot red pepper in broiler diet decreased cholesterol, triglyceride and glucose concentrations and the H : L ratio in broiler blood plasma, which is in accordance with the earlier investigation of Al-Kassie *et al.* (2011). Hot red pepper showed the best cholesterol-lowering effect in the present experiment. Advantage of hot red pepper in broiler nutrition is that the chickens can consume capsaicin without an adverse effect on feed intake due to their insensitivity to the irritant of capsaicinon sensory neurons and lack of vanilloid receptors for capsaicin.

Lipid oxidation

Table 5 shows breast and thigh with drumstick meat and liver antioxidant indices of broiler chickens. The concentration of MDA was significantly lower ($P < 0.05$) in the treatments supplemented with different levels of dietary spice herbs than in the control treatment. Concentration of MDA in all investigated

Table 5. Least-square means of concentration of thiobarbituric acid reactive substances (TBARS) (mg malondialdehyde/kg tissue) of breast, thigh with drumstick and liver 24 h *post-mortem*

See Table 2 for explanation of experimental treatments. Means within a column followed by the same letter or with no letter are not significantly different (at $P = 0.05$)

Experimental treatment	TBARS		
	Breast	Thigh with drumstick	Liver
CON	0.68a	1.37a	1.89a
G-0.5	0.38b	0.34d	0.48e
G-1.0	0.36c	0.27e	0.53c
BP-0.5	0.10g	0.29e	0.37f
BP-1.0	0.05h	0.12f	0.13h
HRP-0.5	0.23d	0.39c	0.50d
HRP-1.0	0.17f	0.36d	0.31g
MX-0.5	0.20e	0.43b	0.62b
Pooled SE_{LSM}	0.01	0.01	0.01

tissues was significantly ($P < 0.05$) affected by dietary treatments. In this experiment, lipid peroxidation in tissues of broiler chickens fed diet supplemented with garlic, black pepper and hot red pepper powder was significantly lower ($P < 0.05$) than in tissues of broiler chickens in CON treatment. Additionally, MDA concentration decreased with increasing level of spice herbs in feed. This showed that the presence of spice herbs has an impact of lowering lipid peroxidation. These measurements were used since they reflect the effect of garlic, black pepper and hot red pepper on antioxidant activities. First, MDA can endogenously reflect lipid peroxidation, which is the consequence of diminished antioxidant protection as the concentrations of reactive oxygen species (ROS) increase. Dietary addition of black pepper to chicken ration showed the highest reduction in MDA concentration in tissues ($P < 0.05$). The digestive tract itself is considered to be a major site of free-radical production in poultry and some of them might be delivered via portal blood system into the liver and meat. In present study, results showed that dietary spice herbs have an effect on the liver function and the concentration of MDA in liver and meat. The reported results indicated that these spices have meat- and liver-protective effects.

Lipids are an important component of meat and contribute to several desirable sensory characteristics of meat and meat products. Lipids enhance the flavour and aroma profile of meat and also increase the tenderness and juiciness (Gorelik *et al.* 2008). However, it is generally accepted that lipid oxidation is the primary process responsible for quality deterioration of meat during storage. In present experiment, spice herbs showed a positive influence in lowering lipid oxidation process, especially with addition of black pepper powder. Recently, Kostadinović *et al.* (2015) reported that the concentration of MDA was significantly lower and GSHPx activity higher in the group supplemented with medicinal plant (*Artemisia absinthium*) than in unsupplemented group, and found lower lipid peroxidation in blood and plasma of broiler chickens fed diet supplemented with this medicinal plant. All meat and fish products are prone to oxidation. Among meat products, poultry meat is considered to be more prone to the development of oxidative rancidity than is red meat. This fact can be explained by the higher content of phospholipids in poultry meat. It has been demonstrated that oxidation of meat starts by a peroxidation of the phospholipid fraction. Due to the high degree of polyunsaturated lipids, the phospholipids are most prone to oxidation. The lowest concentration of MDA in breast meat, thigh with drumstick and liver was observed in Treatment BP-1.0, whereas the highest was observed in the control treatment. Lipid oxidation reduces meat quality by several ways, including, for example, off-flavour formation, drip loss and colour changes. During lipid oxidation, polyunsaturated fatty acids are degraded to volatile short-chain oxidation products, which lead to off-odour and off-flavour formation (Jensen *et al.* 1998). Oxidative processes can also affect the ability of the membranes to hold water and may contribute to drip loss (Weber and Antipatis 2001). In the present study, it has been shown that the dietary addition of spice herbs to chicken ration can serve as natural antioxidants, playing an important role in meat stability. Feeding poultry with higher level of natural dietary antioxidants provides the poultry industry with a simple method for improvement of the oxidative stability, sensory quality, shelf life and acceptability of poultry meats. Additionally, MDA concentration decreased with increasing concentration of spice herbs in feed. This showed that the presence of spice herbs has an impact of lowering lipid peroxidation. Results of thiobarbituric value method showed that supplementation with lemon balm, and mainly the combination of hawthorn and yarrow in broiler diet influenced the reduction of lipid-oxidation processes in thigh (Marcinčáková *et al.* 2011). Many investigations have shown that flavonoids have the capacity to act as powerful antioxidants by scavenging free radicals and terminating oxidative reactions. Luna *et al.* (2010) added thymol and carvacrol to broiler diet at a dose of 150 mg/kg for 42 days and noted that the addition of plants had a positive effect on the oxidative stability during storage of red meat. Similar positive effects of medicinal plants and aromatic herbs on oxidative meat stability were observed by Šperňáková *et al.* (2007) using rosemary powder and by Govaris *et al.* (2004) with oregano powder in broilers diet. According the Kostadinović *et al.* (2015), liver is an organ with a central metabolic role in the organism, often referred to as 'the main laboratory' since it performs the major detoxification tasks, and, for that reason, the liver is the prime target for the study of the metabolism of xenobiotic and other substances. Lipid peroxidation is a reaction between polyunsaturated fatty acids and oxygen, which is initiated by radical intermediates and active oxygen species produced by normal metabolic reactions or during metabolism of chemicals. Antioxidant enzymes counteract excessive formation and deleterious effects of reactive oxygen metabolites. For example, superoxide dismutase (SOD) catalyses the conversion of superoxide anion radical to H_2O_2, catalase reduction of H_2O_2 to water, while glutathione peroxidase (GSHPx) acts in conjunction with other enzymes to reduce H_2O_2 and to terminate lipid peroxidation (Halliwell and Chirico 1993). Spice herbs in the present experiment significantly reduced concentrations of MDA in liver, which indicates that spices have liver protective effects. Considering the character of lipid oxidation, it is necessary to feed the chickens with black pepper and hot red pepper at an early stage, so as to protect meat from the beginning of the oxidation process. This reduces the oxidative damage of fats in meat, extends shelf life, and also increases meat safety.

Conclusions

From reported findings, it can be concluded that the dietary addition of garlic, black pepper and hot red pepper to broiler diet resulted an in improvement of meat composition, lower cholesterol content, as well as decrease in lipid oxidation of meat. Overall, the addition of black pepper powder increased meat protein content, hot red pepper reduced cholesterol level in tissues, whereas mixture of the three spices acted synergistically, lowering cholesterol and decreasing lipid-oxidation process. Furthermore, this trial showed significant effect of selected spice herbs in chicken nutrition as powerful antioxidants in alteration of chicken meat quality and safety. Thus, spices, medicinal plants and aromatic herbs acted as very effective natural instruments, and could usefully serve as alternative to antibiotics, growth promoters and to improve meat quality; however, further investigation of their beneficial mechanisms is necessary.

Acknowledgements

The paper is a part of the research work on the Project III 46012 financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

References

Abou-Elkhair R, Ahmed HA, Selim S (2014) Effects of black pepper (*Piper nigrum*), turmeric powder (*Curcuma longa*) and coriander seeds (*Coriandrum sativum*) and their combinations as feed additives on growth performance, carcass traits, some blood parameters and humoral immune response of broiler chickens. *Asian-Australasian Journal of Animal Sciences* **27**, 847–854. doi:10.5713/ajas.2013.13644

Ahn DU, Wolfe FH, Sim JS (1993) The effect of metal chelators, hydroxyl radical scavengers, and enzyme systems on the lipid peroxidation of raw turkey meat. *Poultry Science* **72**, 1972–1980. doi:10.3382/ps.0721972

Al-Kassie GAM, Al-Nasrawi MAM, Ajeena SJ (2011) The effects of using hot red pepper as a diet supplement on some performance traits in broiler. *Pakistan Journal of Nutrition* **10**, 842–845.

Al-Kassie GAM, Butris GY, Ajeena SJ (2012) The potency of feed supplemented mixture of hot red pepper and black pepper on the performance and some hematological blood traits in broiler diet. *International Journal of Advanced Biological Research* **2**, 53–57.

Asztalos B, Lefevre M, Wong L, Foster TA, Tulley R, Windhauser M, Zhang W, Roheim PS (2000) Differential response to low-fat diet between low and normal HDL cholesterol subjects. *Journal of Lipid Research* **41**, 321–328.

Carnevale de Almeida J, Perassolo MS, Camargo JL, Bragagnolo N, Gross JL (2006) Fatty acid composition and cholesterol content of beef and chicken meat in Southern Brazil. *Brazilian Journal of Pharmaceutical Sciences* **42**, 109–117.

Chen YJ, Kim IH, Cho JH, Yoo JS, Wang Q, Wang Y, Huang Y (2008) Evaluation of dietary l-carnitine or garlic powder on growth performance, dry matter and nitrogen digestibilities, blood profiles and meat quality in finishing pigs. *Animal Feed Science and Technology* **141**, 141–152. doi:10.1016/j.anifeedsci.2007.05.025

Chiu DTY, Stults FH, Tappel AL (1976) Purification and properties of rat lung soluble glutathione peroxidase. *Biochimica et Biophysica Acta* **445**, 558–566. doi:10.1016/0005-2744(76)90110-8

Ciftci M, Simsek UG, Yuce A, Yilmaz O, Dalkilic B (2010) Effects of dietary antibiotic and cinnamon oil supplementation on antioxidant enzyme activities, cholesterol levels and fatty acid compositions of serum and meat in broiler chickens. *Acta Veterinaria Brno* **79**, 33–40. doi:10.2754/avb201079010033

Gardzielewska J, Pudyszak K, Majewska T, Jakubowska M, Pomianowski J (2003) Effect of plant-supplemented feeding on fresh and frozen storage quality of broiler chicken meat. *Animal Husbandry Series of Electronic Journal of Polish Agricultural University* **6**.

Gorelik S, Ligumsky M, Kohen R, Kanner J (2008) The stomach as a 'bioreactor': when red meat meets red wine. *Journal of Agricultural and Food Chemistry* **56**, 5002–5007. doi:10.1021/jf703700d

Govaris A, Botsoglou N, Papageorgiou G, Botsoglou E, Ambrosiadis I (2004) Dietary versus post-mortem use of oregano oil and/or α-tocopherol in turkeys to inhibit development of lipid oxidation in meat during refrigerated storage. *International Journal of Food Sciences and Nutrition* **55**, 115–123. doi:10.1080/09637480410001666487

Halliwell B, Chirico S (1993) Lipid peroxidation: its mechanism, measurement and significance. *The American Journal of Clinical Nutrition* **57**, 715S–725S.

Hedayati M, Manafi M, Yari M, Vafaei P (2013) Effects of supplementing diets with an acidifier on performance parameters and visceral organ weights of broilers. *European Journal of Zoological Research* **2**, 49–55.

ISO 1442 (1997) 'International standards meat and meat products: determination of moisture content.' (International Organization for Standardization: Geneva, Switzerland)

ISO 1443 (1973) 'International standards meat and meat products: determination of total fat content.' (International Organization for Standardization: Geneva, Switzerland)

ISO 936 (1998) 'International standards meat and meat products: determination of ash content.' (International Organization for Standardization: Geneva, Switzerland)

ISO 937 (1978) 'International standards meat and meat products: determination of nitrogen content.' (International Organization for Standardization: Geneva, Switzerland)

Issa KJ, Abo Omar JM (2012) Effect of garlic powder on performance and lipid profile of broilers. *Open Journal of Animal Sciences* **2**, 62–68. doi:10.4236/ojas.2012.22010

Jensen C, Skibsted LH, Bertelsen G (1998) Oxidative stability of frozen stored raw pork chops, chill stored pre-frozen raw pork chops, and frozen stored pre-cooked sausages in relation to dietary $CuSO_4$, rapeseed oil and vitamin E. *Zeitschrift für Lebensmittel Untersuchung und Forschung. A* **207**, 363–368.

Khan RU, Nikousefat Z, Tufarelli V, Naz S, Javdani M, Laudadio V (2012) Garlic (*Allium sativum*) supplementation in poultry diets: effect on production and physiology. *World's Poultry Science Journal* **68**, 417–424. doi:10.1017/S0043933912000530

Konjufca VH, Pesti GM, Bakalli RI (1997) Modulation of cholesterol levels in broiler meat by dietary garlic and copper. *Poultry Science* **76**, 1264–1271. doi:10.1093/ps/76.9.1264

Kostadinović Lj, Lević J, Popović S, Čabarkapa I, Puvača N, Đuragić O, Kormanjoš Š (2015) Dietary inclusion of *Artemisia absinthium* for management of growth performance, antioxidative status and quality of poultry meat. *European Political Science* **79**, 1–10. doi:10.1399/eps.2015.75

Laudadio V, Tufarelli V (2011) Pea (*Pisum sativum* L.) seeds as an alternative dietary protein source for broilers: influence on fatty acid composition, lipid and protein oxidation of dark and white meats. *Journal of the American Oil Chemists' Society* **88**, 967–973. doi:10.1007/s11746-010-1742-3

Lawrence G (2010) 'The fats of life: essential fatty acids in health and disease.' (Rutgers University Press: New Brunswick, NJ)

Ljubojević D, Radosavljević V, Puvača N, Živkov Baloš M, Đorđević V, Jovanović R, Ćirković M (2015) Interactive effects of dietary protein level and oil source on proximate composition and fatty acid composition in common carp (*Cyprinus carpio* L.). *Journal of Food Composition and Analysis* **37**, 44–50. doi:10.1016/j.jfca.2014.09.005

Luna A, Lábaque MC, Zygadlo JA, Marin RH (2010) Effects of thymol and carvacrol feed supplementation on lipid oxidation in broiler meat. *Poultry Science* **89**, 366–370. doi:10.3382/ps.2009-00130

Majewska D, Jakubowska M, Ligocki M, Tarasewicz Z, Szczerbinska D, Karamucki T, Sales J (2009) Physicochemical characteristics, proximate analysis and mineral composition of ostrich meat as influenced by muscle. *Food Chemistry* **117**, 207–211. doi:10.1016/j.foodchem.2009.03.100

Marcinčák S, Popelka P, Bystrický P, Hussein K, Hudecová K (2005) Oxidative stability of meat and meat products after feeding of broiler chickens with additional amounts of vitamin E and rosemary. *Meso* **7**, 34–39.

Marcinčáková D, Čertík M, Marcinčák S, Popelka P, Šimková J, Klempová T, Petrovič V, Tučková M, Bača M (2011) Effect of dietary supplementation of *Melissa officinalis* and combination of *Achillea millefolium* and *Crataegus oxyacantha* on broiler growth performance, fatty acid composition and lipid oxidation of chicken meat. *Italian Journal of Animal Science* **10**, e43. doi:10.4081/ijas.2011.e43

Min B, Ahn DU (2005) Mechanism of lipid peroxidation in meat and meat products: a review. *Food Science and Biotechnology* **14**, 152–163.

Nabil Alloui M, Agabou A, Alloui N (2014) Application of herbs and phytogenic feed additives in poultry production: a review. *Global Journal of Animal Scientific Research* **2**, 234–243.

Najafi P, Torki M (2010) Performance, blood metabolites and immunocompetence of broiler chicks fed diets included essential oils of medicinal herbs. *Journal of Animal and Veterinary Advances* **9**, 1164–1168. doi:10.3923/javaa.2010.1164.1168

Ogunmola OO, Taiwo OF, Ayankoso AS (2013) The nutritive value of the meat quality of locally breed chicken, exotic chicken and turkey. *Journal of Applied Chemistry* **3**, 46–50.

Onibi GE (2006) Dietary oil quantity and vitamin E supplementation. II: effect on carcass and meat quality of broiler chickens. *Bowen Journal of Agriculture* **3**, 106–115.

Onibi GE, Adebisi OE, Fajemisin AN, Adetunji AV (2009) Response of broiler chickens in terms of performance and meat quality to garlic (*Allium sativum*) supplementation. *African Journal of Agricultural Research* **4**, 511–517.

Puvača N, Stanaćev V, Glamočić D, Lević J, Perić L, Stanaćev V, Milić D (2013) Beneficial effects of phytoadditives in broiler nutrition. *World's Poultry Science Journal* **69**, 27–34. doi:10.1017/S0043933913000032

Puvača N, Stanaćev V, Beuković M, Ljubojević D, Kostadinović Lj, Džinić N (2014*a*) Effect of phytogenic feed additive (*Allium sativum* L.) in broiler chicken nutrition on breast meat quality and tissues cholesterol content. *Proceedings of International scientific/professional conference, Agriculture in Nature and Environment Protection* **7**, 75–80.

Puvača N, Lukač D, Ljubojević D, Stanaćev V, Beuković M, Kostadinović Lj, Plavša N (2014*b*) Fatty acid composition and regression prediction of fatty acid concentration in edible chicken tissues. *World's Poultry Science Journal* **70**, 585–592. doi:10.1017/S0043933914000634

Puvača N, Kostadinović Lj, Ljubojević D, Lukač D, Lević J, Popović S, Novakov N, Vidović B, Đuragić O (2015) Effect of garlic, black pepper and hot red pepper on productive performances and blood lipid profile of broiler chickens. *European Political Science* **79**, 1–13. doi:10.1399/eps.2015.73

Shahriari A, Fatemi Tabatabaie R, Jafari RA, Ghorbanzadeh B (2009) Modulation of serum and liver triglyceride and abdominal fat pad weight by dietary garlic in male broilers. *International Journal of Veterinary Research* **3**, 101–105.

Shahverdi A, Kheiri F, Faghani M, Rahimian Y, Rafiee A (2013) The effect of use red pepper (*Capsicum annum* L.) and black pepper (*Piper nigrum* L.) on performance and hematological parameters of broiler chicks. *European Journal of Zoological Research* **2**, 44–48.

Shirzadegan K, Falahpour P (2014) The physicochemical properties and antioxidative potential of raw thigh meat from broilers fed a dietary medicinal herb extract mixture. *Open Veterinary Journal* **4**, 69–77.

Singletary K (2010) Black pepper overview of health benefits. *Nutrition Today* **45**, 43–47. doi:10.1097/NT.0b013e3181cb4539

Souza XR, Faria PB, Bressan MC (2011) Proximate composition and meat quality of broilers reared under different production systems. *Brazilian Journal of Poultry Science* **13**, 15–20.

Šperňáková D, Máté D, Rózańska H, Kováč G (2007) Effects of dietary use of rosemary powder and α-tocopherol on performance of chicken, inhibition of lipid oxidation during storage at chilling conditions and increasing of meat quality. *Bulletin of the Veterinary Institute in Pulawy* **51**, 585–589.

Stanaćev V, Glamočić D, Milošević N, Perić L, Puvača N, Stanaćev V, Milić D, Plavša N (2012) Influence of garlic (*Allium sativum* L.) and copper as phytoadditives in the feed on the content of cholesterol in the tissues of the chickens. *Journal of Medicinal Plants Research* **6**, 2816–2820.

Weber GM, Antipatis C (2001) Pork meat quality and dietary vitamin E. *International Virtual Conference on Pork Quality* **2**, 122–128.