Journal of Animal Physiology and Animal Nutrition

DOI: 10.1111/j.1439-0396.2011.01207.x

ORIGINAL ARTICLE

The effect of supplementation of clove and agrimony or clove and lemon balm on growth performance, antioxidant status and selected indices of lipid profile of broiler chickens

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Keywords
Poultry, herbal substances, body weight, feed conversion ratio, blood chemistry, lipid metabolism

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Received: 11 November 2010;
accepted: 27 June 2011

Summary

The study investigated the effects of diet supplementation with 1% clove flower buds powder combined with either 0.2% lemon balm extract or 0.2% agrimony extract (each of the two pulverized extracts supplied through drinking water) on body weight of broilers, total feed intake, feed conversion ratio and the carcass yield, activity of superoxide dismutase (SOD, EC 1.15.1.1) and glutathione peroxidase (GSH-Px, EC 1.11.1.9) in blood, concentration of sulfhydryl (−SH) groups, malondialdehyde (MDA), vitamin A and E, low-density lipoproteins in the blood plasma, serum cholesterol, total lipids, triglycerides and high-density lipoproteins in broiler chickens at 42 days of age. On the day of hatching, 120 male and female broilers of Cobb 500 were randomly divided into three groups. The control group (1st group) of broilers received a basal diet (BD) without any feed and water additive. Both experimental groups of chicks were fed BD enriched with clove (*Syzygium aromaticum* L.) powder at a dose of 10 g/kg DM for 42 days. Moreover, either lemon balm (*Mellisa officinalis* L.) extract or agrimony (*Agrimonia eupatoria* L.) extract diluted with drinking water (2:1000) was given to broilers in the 2nd and 3rd group respectively. The results indicated that feeding the diets enriched with selected herbal supplements failed to affect the growth performance of broiler chickens at 42 days of age. In addition, this supplementation had no influence on the activities of SOD and GSH-Px, concentration of vitamin A and selected lipid metabolism indices. On the other hand, we observed beneficial effects on some indices of the antioxidant status (increased concentration of −SH groups and vitamin E, decreased concentration of MDA) in the blood of broilers in both experimental groups in comparison with the control group of chickens ($p < 0.05$). Furthermore, a slightly better antioxidant capacity was found in the blood of broilers supplied the combination of clove and lemon balm compared to clove and agrimony (vitamin E, 11.26 ± 0.73 vs. 9.73 ± 0.64 μmol/L, $p < 0.05$ respectively). It could be concluded that supplementation of the diet with clove flower buds

powder combined with lemon balm extract or agrimony extract dissolved in drinking water has a potential to increase the antioxidant status but fails to influence either the growth performance or the selected lipid metabolism indices of broilers at the age of 42 days.

Introduction

The interest in phytotherapy or 'herbal medicine' has increased considerably in the recent years. Recent *in vitro* experiments indicate that herbs are a potential source of many phytochemicals as mentioned below. These biologically active constituents of herbs can posses many beneficial properties. Therefore, there has been considerable research effort to use herbal substances in poultry nutrition as natural growth performance enhancers instead of the antibiotic growth promoters which were banned in many European countries. Despite the large number of research articles so far published in this field, the growth-promoting effect of herb extracts has not been clearly proved as reviewed by (Franz et al., 2010). The growth performance can be affected by herbal substances in some ways: (i) their significant antibacterial effect can suppress pathogenic microflora in the gastrointestinal tract of broilers and thus reduce their mortality during the fattening period (Brenes and Roura, 2010); (ii) they can improve the taste and smell of feedstuffs and thus influence the feed intake and growth performance of poultry (Windisch et al., 2008); (iii) they can stimulate appetite and digestion (Barreto et al., 2008).

Clove (*Syzygium aromaticum* L.) is considered one of the most versatile herbs. Meanwhile, lemon balm (*Mellisa officinalis* L.) and agrimony (*Agrimonia eupatoria* L.) are herbs most commonly used in our traditional folk medicine for more than 200 years because of their beneficial effect on human health. Recently, a number of plants and their extracts have been used in poultry nutrition. Their functional substances (such as flavonoids, polyphenols and terpenoids) are mainly secondary metabolites synthesized by plants to deter herbivorous predators, repel competitors and attract pollinators. Like mentioning later, there is a growing evidence that these herbal substances can neutralise reactive free radicals, the by-products of all energy generating processes of the organism, that cause oxidative stress when left in the cells in excess. Phospholipids are biomolecules particularly susceptible to peroxidation (uncontrolled chain reactions) which is initiated by free radicals (McBride and Kraemer, 1999). Lipid hydroperoxides, peroxyradicals and hydrogen peroxide generated within the initiation and propagation of lipid peroxidation can induce further damage to proteins and DNA (Tirosh and Reznick, 2000).

To estimate the prooxidat/antioxidat status in an organism, the determination of activities of antioxidant enzymes (such as superoxide dismutase, gluthatione peroxidase) as well as concentrations of malondialdehyde, sulfhydryl groups and vitamins in blood are generally employed. Recent *in vivo* studies in poultry conducted by Faixova et al. (2009), Faix et al. (2009), Hoffman-Pennesi and Wu (2010) and Toghyani et al. (2010) indicated beneficial effects of dietary supplementation of some herbs and herb extracts (Cinnamomum zeylanicum oil, borneol, thymol, thyme oil and thyme) on blood chemistry and antioxidant status of broilers.

The aim of the present study was to determine the effect of diet supplementation with 1% clove buds powder combined with either 0.2% lemon balm extract or 0.2% agrimony extract supplied with drinking water on growth performance, antioxidant status and selected indexes of lipid metabolism in the blood of broilers at the age of 42 days.

Material and methods

Plant material

The clove (*Syzygium aromaticum* L.) flower buds powder was purchased from Mäspoma (Zvolen, The Slovak Republic). Pulverized extracts of agrimony (*Agrimonia eupatoria* L.) and lemon balm (*Mellisa officinalis* L.) were prepared by Calendula (Nova Bana, The Slovak Republic). Both herbs were collected in East Slovakia. The plant material consisted of leaves, flowered tops and stalks dried at 30–35 °C, ground, extracted with 50% ethanol and evaporated to obtain a powder (prescription and protocol of Calendula, The Slovak Republic).

Experimental animals, diets and treatments

The experiment was carried out on 120 one-day-old male and female broilers of Cobb 500 which were

randomly allotted to three groups (40 birds per group). Each group had four replicates (10 birds per pen). The broilers were kept in large pens on wood shavings. On the day of hatching, the room temperature was kept at 32 °C and then was gradually decreased by 3 °C/week to a final temperature of 23 °C on the day 21 which was then maintained constant. Continuous lighting regimen (24 h of light per day) was kept throughout the fattening period. Relative humidity in the room was maintained at 70%. The experiment was approved by the Ethical Committee of the University of Veterinary Medicine and Pharmacy in Kosice, The Slovak Republic.

All birds were fed *ad libitum* with the commercial standard diets for broilers 'Starter' from day 1 to 14, 'Grower' from day 15 to 29 and 'Finisher' from day 30 to 42. The control group of chickens received the basal diet (BD) only. The composition of all BDs is presented in Table 1. The diets for each group of broilers were obtained by mixing 99.0 kg of BD with 1.0 kg of clove flower buds powder. Each of the two pulverized extracts, agrimony and lemon balm, were added to drinking water (dilution ratio 2:1000). The broilers had free access to feed and water.

Table 1 Composition of the respective basal diets given to the broilers throughout the experiment

	Starter (1–14 day)	Grower (15–29 day)	Finisher (30–42 day)
Ingredients (%)			
Maize	51.3	49.0	52.8
Wheat	15.0	14.0	20.0
Soybean meal (46.5% CP, 1.5% fat)	29.9	31.6	23.4
Wheat bran	–	2.15	–
Limestone	1.9	1.25	1.75
Monocalcium phosphate	0.89	1.0	0.9
Vitamin-mineral premix*	0.3	0.3	0.3
NaCl	0.36	0.30	0.35
L-lysine	0.10	0.15	0.15
DL-methionine	0.15	0.15	0.25
Enzymatic preparation†	0.1	0.1	0.1
Nutrient level (%)			
Linoleic acid	1.0	1.0	1.0
Metabolizable energy (MJ/kg)	11.5	12.0	12.0
Crude protein	17.5	19.0	17.0
Crude fibre	5.0	4.0	4.0
Ash	8.0	7.0	7.0
L-lysine	0.8	0.95	0.95
DL-methionine	0.35	0.4	0.4
Methionine + cysteine	0.7	0.75	0.7
Calcium	0.8	0.7	0.7
Phosphorus	0.5	0.5	0.5

*Supplied per kg of basal diet: vitamin A 8 000 000 IU; vitamin D_3 1 200 000 IU; vitamin E 15 000 mg; vitamin K_3 3000 mg; vitamin B1 1500 mg; vitamin B6 8000 mg; niacin 15 000 mg; choline chloride 50 000 mg; pantothenic acid 50 mg; pyridoxine 5 mg; folic acid 2 mg; cyanocobalamine 30 μg; biotin 0.2 mg; I 2 mg; Co 1 mg, K 8.6 g; Cl^- 2 g; Cu 6.0 mg; Fe 60 mg; Zn 50 mg; Mn 50 mg.
†Supplied per kg of basal diet: α-amylase 200 U, endo-1,3,(4)-β-glucanase 1175 U, endo-1,4-β-glucanase 2000 U, endo-1,4-β-xylanase 2000 U, bacillolysin 225 U, 6-fytase 499.5 FYT.

Sample collection and analysis

None of broiler chickens in any group died during the trial. The body weight of broilers, total feed intake, feed conversion ratio and carcass yield were among the growth performance parameters studied. The body weight, total feed intake, feed conversion ratio and carcass yield of broilers in each group and in each replicate were recorded on day 42 of life.

On day 42 of age, ten randomly chosen broilers were slaughtered by decapitation in each group. The blood was collected into heparinised test tubes and centrifuged for plasma specimens at 1180 *g* for 15 min or the tubes were left in a standing position at room temperature for 30 min to obtain the blood serum. Blood and plasma samples for the analysis of antioxidant indices were frozen and stored at −65 °C. The haemoglobin content was analysed by a standard kit obtained from Randox Laboratories (Crumlin, Antrim, UK). The activity of blood glutathione peroxidase (GSH-Px, EC 1.11.1.9) was determined by the method of Paglia and Valentine (1967) using a RANSEL kit (Randox Laboratories, UK). The concentration of malondialdehyde in blood plasma was measured by the modified fluorimetric method according to Jo and Ahn (1998). The superoxide dismutase (SOD, EC 1.15.1.1) activity of erythrocytes was analysed by the method of Arthur and Boyne (1985) using a RANSOD kit from Randox Laboratories. Ellman's (1958) method was used to determine the concentration of sulfhydryl groups in the plasma. The levels of vitamin A and E were assessed using the HPLC technique according to Tuckova and Kastel (1999).

Low-density lipoproteins (LDL), cholesterol, total lipids and triglycerides were determined in blood plasma. Cholesterol, triglycerides and total lipids were determined by the method according to Tietz (1995), LDL level according to Bachorik (1997) and high-density lipoproteins (HDL) in blood serum by the method of Sugiuchi et al. (1995). All the samples were analysed immediately after processing by a spectrophotometric method using a biochemical analyser Cobas C111 (Roche Diagnostics, Rotkreuz, Switzerland).

Statistical analysis

The results were subjected to one-way analysis of variance (ANOVA) using Tukey's multiple comparison test for statistical analysis of results at a significance level of $p < 0.05$ (statistical software, version 4.00, GraphPad Prism 2003).

Results

The effect of diet supplementation with 1% clove buds powder combined with either 0.2% lemon balm extract or 0.2% agrimony extract (both pulverized extracts supplied through drinking water) on the body weight, total feed intake, feed conversion ratio and carcass yield are shown in Table 2. Our results showed no statistically significant differences ($p > 0.05$) in the growth performance indices (such as body weight, total feed intake, feed conversion ratio and carcass yield) between the groups of chickens because of the supplementation of diets with herbal substances and their addition to drinking water given.

The activities of superoxide dismutase, glutathione peroxidase and concentration of vitamin A in the blood plasma were not significantly affected by both treatments as compared to the control group. The concentration of sulfhydryl (−SH) groups was higher, whereas the concentration of malondialdehyde (MDA) was lower in both experimental groups of broiler chickens fed the basal diet (BD) supplemented with clove and lemon balm and/or clove and agrimony as compared with the control group receiving BD only (−SH groups, 0.27 ± 0.03 and/or 0.25 ± 0.02 vs. 0.17 ± 0.01 mmol/L; $p < 0.05$ respectively, and MDA, 0.63 ± 0.02 and/or 0.65 ± 0.01 vs. 0.71 ± 0.02 μmol/L; $p < 0.05$ respectively). In addition, the concentration of vitamin E was higher in the group supplemented with clove and lemon balm in comparison with that supplemented with clove and agrimony (11.26 ± 0.73 vs. 9.73 ± 0.64 μmol/L; $p < 0.05$ respectively) but both were higher than that found in the non-supplemented group ($p < 0.05$; Table 3).

Table 4 shows that the plasma levels of high-density lipoproteins and low-density lipoproteins, and serum levels of cholesterol, triglycerides and total lipids were not influenced by dietary supplementation of broilers

Table 2 The effects of diet supplementation with 1% clove flower buds (*Syzygium aromaticum* L.) powder combined with either 0.2% lemon balm (*Mellisa officinalis* L.) extract or 0.2% agrimony (*Agrimonia eupatoria* L.) extract, dissolved in drinking water, on growth performance of broilers at the age of 42 days

	Dietary treatment			
Index	CON	CLB	CA	SEM
Body weight (g per bird)	2059	2111	2197	48.53
Total feed intake (g per bird)	4108	4137	4086	47.86
Feed conversion ratio	2.05	1.99	1.89	0.06
Carcass yield (g)	1384	1409	1519	38.51

CON, basal diet (BD) only; CLB, BD supplemented with 1% clove flower buds powder and drinking water containing 0.2% lemon balm extract; CA, BD supplemented with 1% clove flower buds powder and drinking water containing 0.2% agrimony extract.

Table 3 The effects of diet supplementation with 1% clove flower buds (*Syzygium aromaticum* L.) powder combined with either 0.2% lemon balm (*Mellisa officinalis* L.) extract or 0.2% agrimony (*Agrimonia eupatoria* L.) extract added to drinking water on the antioxidant status of broilers at the age of 42 days

	Dietary treatment			
Index	CON	CLB	CA	SEM
SOD in RBC (μkat/g Hb)	1110	1165	1163	77.43
GPx in RBC (μkat/g Hb)	195.5	222	195.1	25.27
−SH groups in plasma (mmol/L)	0.17^{b}	0.27^{a}	0.25^{a}	0.02
MDA in plasma (μmol/L)	0.71^{a}	0.63^{b}	0.65^{b}	0.02
Vitamin A in plasma (μmol/L)	3.03	2.79	2.8	0.20
Vitamin E in plasma (μmol/L)	8.64^{c}	11.26^{a}	9.73^{b}	0.65

Hb, haemoglobin; SOD, superoxide dismutase; RBC, red blood cells; GPx, glutathione peroxidase; −SH groups, sulfhydryl groups; MDA, malondialdehyde; CON, basal diet (BD) only; CLB, BD with 1% clove flower buds powder and drinking water with 0.2% lemon balm extract; CA, BD with 1% clove flower buds powder and drinking water with 0.2% agrimony extract.
Mean values with different superscripts in a row signify that the means are significantly different at $p < 0.05$.

Table 4 The effects of diet supplementation with 1% clove flower buds (*Syzygium aromaticum* L.) powder combined with either 0.2% lemon balm (*Mellisa officinalis* L.) extract or 0.2% agrimony (*Agrimonia eupatoria* L.) extract dissolved in drinking water on the levels of high-density lipoproteins (HDL), low-density lipoproteins (LDL), cholesterol, total lipids (TL) and triglycerides (TG) in broilers at the age of 42 days

	Dietary treatment			
Index	CON	CLB	CA	SEM
HDL in serum (mmol/L)	1.73	1.68	1.7	0.05
LDL in plasma (mmol/L)	0.47	0.51	0.52	0.06
Cholesterol in plasma (mmol/L)	3.63	3.51	3.61	0.16
TL in plasma (g/L)	5.25	5.04	4.91	0.36
TG in plasma (mmol/L)	0.43	0.36	0.39	0.06

CON, basal diet (BD) only; CLB, BD with 1% clove flower buds powder and drinking water with 0.2% lemon balm extract; CA, BD with 1% clove flower buds powder and drinking water with 0.2% agrimony extract.

with 1% clove flower buds powder combined with either 0.2% lemon balm extract or 0.2% agrimony extract diluted in drinking water.

Discussion

The aim of this research was to find effective doses of herbal additives which could have a beneficial impact on the growth performance and health status of broilers. According to Ertas et al. (2005), the combination of some plants extracts (oregano, clove and anise essential oil mix) could present a better effect on the growth performance in poultry in comparison with their individual supplementation. Synergism among some herbal constituents was highlighted in the *in vitro* studies performed by Moleyar and Narasimham (1992), Didry et al. (1994), Montes-Belmont and Carvajal (1998). Moreover, Burt (2004) reported that an antagonistic effect has been expected as well. We presumed that potential synergistic effects between clove and lemon balm and/or clove and agrimony could result in beneficial effect on both the growth performance of broilers and their antioxidant status. Furthermore, to avoid potential interactions among the active substances of herbs given before the dietary intake, clove was mixed into the diets and agrimony extract and lemon balm extract were dissolved in drinking water.

It is known that clove posses the growth-promoting properties, such as antimicrobial (Ehrich et al., 1995; Baratta et al., 1998; Dorman et al., 2000) and appetite and digestion stimulating properties (Kamel, 2001; Çabuk et al., 2003). Its major constituent is an essential oil (up to 20%) characterized by the presence of up to 85.5% of eugenol (Viuda-Martos et al., 2010). Vanselow et al. (1985) found that the dietary supplementation of 2% clove flower buds meal had no effect on the performance of chickens but the addition of 5% resulted in a serious impairment of their performance. Moreover, there is no evidence that lemon balm extract or agrimony extract can affect the growth performance of broilers. Our study showed that supplementation of diet with 1% clove flower buds powder combined with 0.2% lemon balm extract or 0.2% agrimony extract, both pulverized extracts diluted in drinking water, had no effect on the growth performance of broilers. We presume that our findings were related to their inefficacy to influence the growth performance of broilers at the doses given by one of the three ways mentioned above. The study performed by Dalkiliç and Güler (2009) supports our previous presumption that clove flower buds powder is a raw material of lower degree of purity of its active ingredients in contrast with its extracts, because they found that the inclusion of up to 0.04% clove extract in the diet can be used as a natural growth promoter for broilers instead of antibiotics.

Terpenoids are the major constituents of lemon balm extracts (Khalid et al., 2008). They have strong antioxidant properties as observed in *in vitro* experiments performed by Lamiason et al. (1991), Campos and Lissi (1995), Hohman et al. (1999), Triantaphyllou et al. (2001), de Sousa et al. (2004), Ivanova et al. (2005), Venskutonis et al. (2005) and Dastmalchi et al. (2008). Furthermore, the extracts of both these herbs are a potent source of polyphenols (Heilerová et al., 2003; Correia et al., 2006; Marcincak et al., 2008) which could protect organisms against the oxidative stress. Polyphenols are the major constituents of agrimony extracts (Gião et al., 2009). Likewise, *in vitro* experiments conducted by Copland et al. (2003), Correia et al. (2007); Gião et al. (2007, 2008, 2009) and Venskutonis et al. (2007, 2008) showed that agrimony extracts posses a significant radical scavenging activity and have a potent antioxidant capacity. In addition, clove is also an important source of active substances with the antioxidant properties (Dragland et al., 2003). Wang et al. (2010) and Viuda-Martos et al. (2010) reported that clove essential oil showed the strongest antioxidant activity from among all tested herb extracts. As mentioned above, the active ingredients of both herb extracts used in our study are able to scavenge the free radicals *in vitro*. We observed that diet supplementation with 1% clove flower buds powder combined with either 0.2% lemon balm extract or 0.2% agrimony extract added to drinking water had a beneficial effect on the antioxidant status of broilers in comparison with the control group of broilers. Our explanation based on the sparing effects of the supplemented active herbal substances (e.g. polyphenols) on vitamin E and sulfhydryl (−SH) groups would be a speculation only. Anyhow, the higher concentrations of vitamin E and −SH groups may provide a more efficient scavenging of free reactive radicals as published by Surai et al. (1999) and Giotta and Wang (1972) respectively. Consequently, this could be the reason for decreased formation of malondialdehyde in the blood plasma of broilers observed in both experimental groups in our study. Furthermore, Heilerová et al. (2003) found that lemon balm had a better antioxidant activity compared to agrimony but no differences were found with respect of the amount of polyphenols between both herb extracts. Similarly, our experiment

revealed that the group of chickens supplemented with clove and lemon balm has the slightly better antioxidant status in comparison with the group of broilers supplemented with clove and agrimony.

Little is known about the effects of herb extracts on lipid metabolism of broilers. The effect of some functional herb substances (cyclic terpenes) on serum cholesterol and total lipids in poultry were reported by Qureshi et al. (1988) and Faixova et al. (2009) respectively. On the other hand, no changes in serum cholesterol and plasma lipids because of dietary supplementation of herb extracts (some monoterpenes and essential oils) to broilers were observed by Hood et al. (1978) and Lee et al. (2003) respectively. Najafi and Torki (2010) found that total cholesterol, triglycerides and high-density lipoproteins in the blood of broilers did not respond to the dietary supplementation of clove extract. Moreover, we lack the evidence indicating that lemon balm extract or agrimony extract can influence the lipid metabolism of broilers. Our experiments showed that neither supplementation of clove powder and lemon balm nor clove powder and agrimony induced changes in the selected indices of lipid metabolism in the blood of broilers at the age of 42 days.

In conclusion, supplementation of broiler diet with 1% clove flower buds powder in combination with either 0.2% lemon balm extract or 0.2% agrimony extract (both pulverized extracts dissolved in drinking water) had a beneficial effect on the antioxidant status of broilers in comparison with the control group. In addition, clove and lemon balm combination showed better antioxidant properties compared to clove and agrimony. On the other hand, no effect of this supplementation was reflected in growth performance and selected lipid profile indices of 42-day-old broilers.

Acknowledgements

The study was supported by the Grant Agency for Science VEGA of the Slovak Republic, Grant No. 1/0235/08 and Grant No. 1/0614/09.

References

Arthur, J. R.; Boyne, R., 1985: Superoxide-dismutase and glutathione-peroxidase activities in neutrophils from selenium deficient and copper deficient cattle. *Life Science* **36**, 1569–1575.

Bachorik, P. S., 1997: Measurement of low-density lipoprotein cholesterol. In: N. Rifai, G. R. Warnick, M. H. Dominiczak (eds), *Handbook of Lipoprotein Testing*. AACC Press, Washington, pp. 145–160.

Baratta, M. T.; Dorman, H. J. D.; Deans, S. G.; Figueiredo, A. C.; Barroso, J. G.; Ruberto, G., 1998: Antimicrobial and antioxidant properties of some commercial essential oils. *Flavour and Fragrance Journal* **13**, 235–244.

Barreto, M. S. R.; Menten, J. F. M.; Racanicci, A. M. C.; Pereira, P. W. Z.; Rizzo, P. V., 2008: Plant extracts used as growth promoters in broilers. *Brazilian Journal of Poultry Science* **10**, 109–115.

Brenes, A.; Roura, E., 2010: Essential oils in poultry nutrition: main effects and modes of action. *Animal Feed Science and Technology* **158**, 1–14.

Burt, S., 2004: Essential oils: their antibacterial properties and potential application in foods – a review. *International Journal of Food Microbiology* **94**, 223–253.

Çabuk, M.; Alçiçek, A.; Bozkurt, M.; Imre, N., 2003: Antimicrobial properties of the essential oil isolated from aromatic plants and using possibility as alternative feed additives. II. *National Animal Nutrition Congress* 18–20 September, pp. 184–187, Department of Poultry Science, Akhisar Vocational School of Celal Bayar University, Manisa-Turkey.

Campos, A. M.; Lissi, E. A., 1995: Evaluation of the antioxidant capacity of herbal teas by a procedure based on the bleaching of ABTS radical cations. *Boletín de la Sociedad Chilena de Química* **40**, 375–381.

Copland, A.; Nahar, L.; Tomlinson, C. T. M.; Hamilton, V.; Middleton, M.; Kumarasamy, Y.; Sarker, S. D., 2003: Antibacterial and free radical scavenging activity of the seeds of *Agrimonia eupatoria*. *Fitoterapia* **74**, 133–135.

Correia, H.; Gonzalez-Paramas, A.; Amaral, M. T.; Santos-Buelga, C.; Batista, M. T., 2006: Polyphenolic profile characterization of *Agrimonia eupatoria* L. by HPLC with different detection devices. *Biomededical Chromatography* **20**, 88–94.

Correia, H. S.; Batista, M. T.; Dinis, T. C. P., 2007: The activity of an extract and fraction of *Agrimonia eupatoria* L. against reactive species. *Biofactors* **29**, 91–104.

Dalkiliç, B.; Güler, T., 2009: The Effects of Clove Extract Supplementation on Performance and Digestibility of Nutrients in Broilers. *Firat University Journal of Health Sciences* **23**, 161–166.

Dastmalchi, K.; Dorman, H. T. D.; Oinonen, P. P.; Darwis, Y.; Laakso, I.; Hiltunen, R., 2008: Chemical composition and *in vitro* antioxidative activity of a lemon balm (*Melissa officinalis* L.) extract. *LWT-Food Science and Technology* **41**, 391–400.

Didry, N.; Dubreuil, L.; Pinkas, M., 1994: Activity of thymol, carvacrol, cinnamaldehyde and eugenol on oral bacteria. *Pharmaceutica Acta Helvetiae* **69**, 25–28.

Dorman, H. J. D.; Surai, P.; Deans, S. G., 2000: *In vitro* antioxidant activity of a number of plant essential oils

and phytoconstituents. *Journal of Essential Oil Research* **12**, 241–248.

Dragland, S.; Senoo, H.; Wake, K.; Holte, K.; Blomhoff, R., 2003: Several Culinary and Medicinal Herbs are Important Sources of Dietary Antioxidants. *Journal of Nutrition* **133**, 1286–1290.

Ehrich, J.; Bauermann, U.; Thomann, R., 1995: Antimicrobial effect of CO_2 spice extracts from ummer savory to cinnamon. *Lebensmitteltechnik* **27**, 51–53.

Ellman, G. L., 1958: Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics* **82**, 70–77.

Ertas, O. N.; Güler, T.; Çiftçi, M.; Dalkiliç, B.; Simsek, Ü. G., 2005: The Effect of an Essential Oil Mix Derived from Oregano, Clove and Anise on Broiler Performance. *International Journal of Poultry Science* **4**, 879–884.

Faix, Š.; Faixová, Z.; Plachá, I.; Koppel, J., 2009: Effect of *Cinnamomum zeylanicum* Essential Oil on Antioxidative Status in Broiler Chickens. *Acta Veterinaria Brno* **78**, 411–418.

Faixova, Z.; Piesova, E.; Makova, Z.; Takacova, J.; Cobanova, K.; Leng, L.; Faix, S., 2009: Effects of borneol on blood chemistry changes in chickens. *Acta Veterinaria (Beograd)* **59**, 177–184.

Franz, C.; Baser, K. H. C.; Windisch, W., 2010: Essential oils and aromatic plants in animal feeding – a European perspective. A review. *Flavour and Fragrance Journal*, **25**, 327–340.

Gião, M. S.; González-Sanjosé, M. L.; Rivero-Pérez, M. D.; Pereira, C. I.; Pintado, M. E.; Malcata, F. X., 2007: Infusions of Portuguese medicinal plants: dependence of final antioxidant capacity and phenolic content on extraction features. *Journal of the Science of Food and Agriculture* **87**, 2638–2647.

Gião, M. S.; González-Sanjosé, M. L.; Muniz, P.; Rivero-Pérez, M. D.; Kosinska, M.; Pintado, M. E.; Malcata, F. X., 2008: Protection of deoxyribose and DNA from degradation brought about by aqueous extracts of several wild plants. *Journal of the Science of Food and Agriculture* **88**, 633–640.

Gião, M. S.; Pereira, C. I.; Fonseca, S. C.; Pintado, M. E.; Malcata, F. X., 2009: Effect of particle size upon the extent of extraction of antioxidant power from the plants Agrimonia eupatoria, Salvia sp and Satureja Montana. *Food Chemistry* **117**, 412–416.

Giotta, G. J.; Wang, H. H., 1972: Reduction of nitroxide free radicals by biological materials. *Biochemical and Biophysical Research Communications* **46**, 1576–1580.

GraphPad Prism, 2003: *GraphPad Prism Version 4.00 for Windows*. GraphPad Software, San Diego, CA.

Heilerová, Ľ.; Bučková, M.; Tarapčík, P.; Šilhár, S.; Labuda, J., 2003: Comparison of antioxidative activity data for aqueous extracts of lemon balm (*Melissa officinalis* L.), oregano (*Origanum vulgare* L.), thyme (*Thymus vulgaris* L.), and agrimony agrimony (*Agrimonia eupatoria* L.) obtained by conventional methods and the DNA-based biosensor. *Czech Journal of Food Sciences* **21**, 78–84.

Hoffman-Pennesi, D.; Wu, C., 2010: The effect of thymol and thyme oil feed supplementation on growth performance, serum antioxidant levels, and cecal Salmonella population in broilers. *Journal of Applied Poultry Research* **19**, 432–443.

Hohman, J.; Zupko, I.; Redei, D.; Csanyi, M.; Falkay, G.; Mathe, I.; Janicsak, G., 1999: Protective effects of the aerial parts of *Salvia officinalis*, *Melissa officinalis* and *Levandula angustifolia* and their constituents against enzyme dependent and enzyme independent lipid peroxidation. *Planta Medica* **65**, 576–578.

Hood, R. L.; Bailey, W. M.; Svoronos, D., 1978: The effect of dietary monoterpenes on the cholesterol level of eggs. *Poultry Science* **57**, 304–306.

Ivanova, D.; Gerova, D.; Chervenkov, T.; Yankova, T., 2005: Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *Journal of Ethnopharmacology* **96**, 145–150.

Jo, C.; Ahn, D. U., 1998: Fluorometric analysis of 2-thiobarbituric acid reactive substances in turkey. *Poultry Science* **77**, 475–480.

Kamel, K. C., 2001: Tracing modes of action and the roles of plant extracts in non-ruminants. In: P. C. Garnsworthy, J. Wiseman (eds), *Recent Advances in Animal Nutrition*. Nottingham University Press, Nottingham, UK, pp. 135–150.

Khalid, K. A.; Hu, W.; Cai, W., 2008: The Effects of Harvesting and Different Drying Methods on the Essential Oil Composition of Lemon Balm (*Melissa officinalis* L.). *Journal of Essential Oil Bearing Plants* **11**, 342–349.

Lamiason, J. L.; Petitjean Freytet, C.; Carnat, A., 1991: Medicinal Lamiaceae with antioxidant properties, a potential source of rosmarinic acid. *Pharmaceutica Acta Helvetiae* **66**, 185–188.

Lee, K. W.; Everts, H.; Kappert, H. J.; Frehner, M.; Losa, R.; Beynen, A. C., 2003: Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. *British Poultry Science* **44**, 450–457.

Marcincak, S.; Popelka, P.; Soltysova, L., 2008: Polyphenols and antioxidative capacity of extracts from selected Slovakian plants. *Acta Scientiarum Polonorum Medicina Veterinaria* **7**, 9–14.

McBride, J.; Kraemer, W. J., 1999: Free radicals, exercise and antioxidants. *Journal of Strength Conditioning Research* **13**, 175–183.

Moleyar, V.; Narasimham, P., 1992: Antibacterial activity of essential oil compounds. *International Journal of Food Microbiology* **16**, 337–342.

Montes-Belmont, R.; Carvajal, M., 1998: Control of *Aspergillus flavus* in maize with plant essential oil and their components. *Journal Food Protection* **61**, 616–619.

Najafi, P.; Torki, M., 2010: Performance, Blood Metabolites and Immunocompetence of Broiler Chicks Fed Diets Included Essentioal Oils of Medicinal Herbs. *Journal of Animal and Veterinary Advances* **9**, 1164–1168.

Paglia, D. E.; Valentine, W. N., 1967: Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory Medicine* **70**, 158–169.

Qureshi, A. A.; Mangels, W. R.; Din, Z. Z.; Elson, C. E., 1988: Inhibition of hepatic mevalonate biosynthesis by the monoterpene, d-limonene. *Journal of Agricultural and Food Chemistry* **36**, 1220–1224.

de Sousa, A. C.; Alviano, D. S.; Blank, A. F.; Alves, P. B.; Alviano, C. S.; Gattass, C. R., 2004: *Melissa officinalis* L. essential oil: antitumoral and antioxidant activities. *Journal of Pharmacy and Pharmacoligy* **56**, 1–5.

Sugiuchi, H.; Uji, Y.; Okabe, H.; Irie, T., 1995: Direct measurement of high-density lipoprotein cholesterol in serum with polyethylene glycol-modified enzymes and sulfated α-cyclodextrin. *Clinical Chemistry* **41**, 717–723.

Surai, P. F.; Noble, R. C.; Speake, B. K., 1999: Relationship between vitamin E content and susceptibility to lipid peroxidation in tissues of the newly hatched chick. *British Poultry Science* **40**, 406–410.

Tietz, N. W. (ed.), 1995: *Clinical Guide to Laboratory Tests*, 3rd edn. WB Saunders company, Philadelphia.

Tirosh, O.; Reznick, A. Z., 2000: Chemical bases and biological relevance of protein oxidation. In: C. K. Sen, L. Packer, O. O. P. Hanninen (eds), *Handbook of Oxidants and Antioxidants in Exercise*. Elsevier, Amsterdam, pp. 89–114.

Toghyani, M.; Tohidi, M.; Gheisari, A. A.; Tabeidian, S. A., 2010: Performance, immunity, serum biochemical and hematological parameters in broiler chicks fed dietary thyme as alternative for an antibiotic growth promoter. *African Journal of Biotechnology* **9**, 6819–6825.

Triantaphyllou, K.; Blekas, G.; Boskou, D., 2001: Antioxidant properties of water extracts obtained from herbs of the species Lamiaceae. *International Journal of Food Science and Nutrition* **52**, 313–317.

Tuckova, M.; Kastel, R., 1999: The methodology for the simultaneous analysis of vitamins A, E, and beta-carotene in animal blood plasma by gradient and isocratic methods of HLPC. *Folia Veterinaria* **43**, 85–91.

Vanselow, D. G.; Lowry, J. B.; Kompiang, I. P., 1985: Preserving poultry feeds in the humid tropics: the use of calcium propionate, clove leaf meal and a vapour barrier. *Journal of Stored Products Research* **21**, 7–11.

Venskutonis, P. R.; Gruzdiene, D.; Trizite, D.; Trizite, G., 2005: Assessment of antioxidant activity of plant extracts by different methods. *Acta Horticulturae* **677**, 99–107.

Venskutonis, P. R.; Škėmaitė, M.; Ragažinskienė, O., 2007: Radical scavenging capacity of Agrimonia eupatoria L. and Agrimonia procera Wallr. *Fitoterapia* **78**, 166–168.

Venskutonis, P. R.; Škėmaitė, M.; Sivik, B., 2008: Assessment of radical scavenging capacity of *Agrimonia* extracts isolated by supercritical carbon dioxide. *Journal of Supercritical Fluids* **45**, 231–237.

Viuda-Martos, M.; Navajas, Y. R.; Zapata, E. S.; Fernández-López, J.; Pérez-Álvarez, J. A., 2010: Antioxidant activity of essential oils of five spice plants widely used in a Mediterranean diet. *Flavour and Fragrance Journal* **25**, 13–19.

Wang, H. F.; Yih, K. H.; Huang, K. F., 2010: Comparative Study of the Antioxidant Activity of Forty-five Commonly Used Essential Oils and their Potential Active Components. *Journal of Food and Drug Analysis* **18**, 24–33.

Windisch, W.; Schedle, K.; Plitzner, C.; Kroismayr, A., 2008: Use of phytogenic products as feed additives for swine and poultry. *Journal of Animal Science* **86**, 140–148.