Volume 53(Suppl.1): 2009
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Effect of rosemary and garlic oil supplementation on glutathione redox system of broiler chickens

Zsolt Ancsin, Márta Erdélyi, Miklós Mézes*

Department of Nutrition, Szent István University, Gödöllő, Hungary

ABSTRACT The purpose of present study was to investigate the effect of rosemary and garlic oils on the lipid peroxidation and glutathione redox system in the blood and liver of broiler chicken. Day-old Hubbard broiler chickens (n=200) were fed with commercial broiler feed (control) and supplemented with garlic oil (0.25 g kg^{-1}), rosemary oil (1.5 g kg^{-1}) or their combination (0.25 g kg^{-1} garlic oil and 1.5 g kg^{-1} rosemary oil). At the end of the growing period (42 days of age) blood and liver samples of 10 animals were taken from each group to determine malondialdehyde and reduced glutathione content and glutathione peroxidase activity. There were no significant differences in the blood plasma or in red blood cell haemolysates among the groups, but garlic oil supplementation increased significantly reduced glutathione content and both essential oils the glutathione peroxidase activity in liver. However, combination of the two oils caused increase of malondialdehyde content of liver together with significantly higher glutathione peroxidase activity as compared to the control. Due to the beneficial effect on glutathione redox system both essential oils – used solely – can be used to reduce the effects of oxidative processes in physiological conditions **Acta Biol Szeged 53(Suppl.1): (2009)**

KEY WORDS

garlic oil,
glutathione,
glutathione peroxidase,
malondialdehyde,
rosemary oil

Phytobiotics or essential oils have importance as natural antioxidants both in animal and human nutrition but their antioxidant capacity is measurable in comparison with synthetic antioxidants only at relatively high doses (Cuvelier et al. 1990) and it was proven mostly only in Fe^{2+}/ascorbate system (Bozin et al. 2006, 2007). The main biologically active components of rosemary oil are carnosol, carnosic acid and its esters (Boutekedjiret et al. 2003). Some previous experiments showed that phenolic compounds of rosemary oil, such as carnosic acid act as antioxidants such as α-tocopherol (McCarthy et al. 2001) and its antioxidant activity was higher than some synthetic antioxidants in vitro (Richheimer et al. 1996). It was also found that rosemary oil improve meat (McCarthy 2001; Smet et al. 2005; Govaris et al. 2007) and egg (Galobart et al. 2001) quality, improve the resistance of the polyunsaturated fatty acids and cholesterol against oxidative damage.

The main active component of garlic oil is alliin (S-alylcysteine sulfoxide) or its derivatives which are showed antioxidant capacity in vitro (Yamasaki et al. 1994). Among the different, lipid soluble organic sulphur components of garlic, such as diallyl-disulphide (DADS), S-ethyl-cysteine or n-acetyl-cysteine (NAC) also have antioxidant properties in vitro (Dwivedi et al. 1998) and significantly decreased the rate of lipid oxidation and oxymioglobin formation in minced beef which also proved their antioxidant properties (Yin and Cheng 2003).

Accepted June 19, 2009
*Corresponding author. E-mail: Mezes.Miklos@mkk.szie.hu

Materials and Methods

A total of 200 day-old Hubbard broiler chickens were divided randomly into four treatment groups. Control group was fed with commercial diet without added antioxidants, while the feed of the treatment groups supplemented with 1.5 g kg^{-1} rosemary oil, 0.25 g kg^{-1} garlic oil or combination of them (1.5 g kg^{-1} rosemary oil and 0.25 g kg^{-1} garlic oil), respectively. The feeding trial lasted for 42 days and at the end of growing period 10 animals from each group was exterminated and blood and liver samples were taken.

Blood samples were taken after cervical dislocation into EDTA–Na_2 containing tubes. Blood plasma and blood cells were separated by centrifugation (2500 g, 20 min). Red blood cells were haemolysed with 1:9 volume of redistilled water and by freezing (-20°C, 18 hours) and thawing (25 °C, 30 min). Liver samples were taken from the distal part of the right lobe and store at -18°C until analysis. Liver homogenates were made with 9-fold cold (4°C) physiological saline (0.65 % w/v NaCl). Determination of malondialdehyde content was carried out from the crude homogenate, while other parameters determined from the 10,000 g supernatant fraction of the homogenate.

Malondialdehyde content of blood plasma and red blood cells (RBC) haemolysate was determined according to Placer et al. (1966) as modified by Matkovics et al. (1988) while in liver homogenate according to Mihara et al. (1980). Reduced glutathione (GSH) content of blood plasma, RBC haemolysate and liver homogenate was measured as described by

Table 1. Malondialdehyde and reduced glutathione content and glutathione peroxidase activity of blood plasma of chickens fed with different essential oils (mean ±S.D.).

	Control	Garlic oil	Rosemary oil	Garlic and rosemary oil
MDA (μmol L^{-1})	4.41 ± 0.77	4.28 ± 0.53	4.54 ± 2.09	4.02 ± 0.97
GSH (mmol L^{-1})	6.61 ± 2.03	4.86 ± 1.19	5.52 ± 0.37	6.02 ± 0.89
GSHPx (U g^{-1} protein)	10.94 ± 2.94	8.24 ± 3.89	8.44 ± 0.47	9.23 ± 1.58

Table 2. Malondialdehyde and reduced glutathione content and glutathione peroxidase activity of RBC haemolysate of chickens fed with different essential oils (mean ±S.D.).

	Control	Garlic oil	Rosemary oil	Garlic and rosemary oil
MDA (μmol L^{-1})	9.23 ± 1.32	10.26 ± 1.01	10.26 ± 1.24	9.51 ± 1.88
GSH (mmol L^{-1})	10.82 ± 4.84	10.64 ± 2.56	9.32 ± 1.15	8.50 ± 3.18
GSHPx (U g^{-1} protein)	5.86 ± 1.65	6.87 ± 2.23	6.65 ± 0.96	6.10 ± 1.52

Sedlak and Lindsay (1968), while glutathione peroxidase (E.C. 1.11.1.9) according to Matkovics et al. (1988). Enzyme activity expressed to protein content which was determined by biuret method (Weichselbaum 1948) for blood plasma and RBC haemolysate or with Folin-phenol reagent for liver homogenate (Lowry et al. 1951).

Statistical evaluation of the data was performed using paired LSD test (Statistica™ 4.5, Statsoft Inc., 1993).

Results and Discussion

There were no significant differences in any of the measured parameters in blood plasma or RBC haemolysate (Tables 1 and 2). These results can be explained with the fast absorption, metabolic conversion and excretion of essential oil constituents that was mentioned by Kohlert et al. (2000). Additionally these results suggest that the effect of essential oils manifested mainly in tissues, but not in blood, possibly because of their lipid soluble characteristics. Lee et al. (2004) reported that in spite of the short half-life of the essential oils, those are able to accumulate in chicken tissues - mainly in liver and kidney - if the administration is continuous without withdrawal periods.

Malondialdehyde content of liver homogenates were not modified by the two essential oils if those were applied separately. However, combined treatment caused significantly higher malondialdehyde concentration (Table 3). That result can be explained by the findings of Skibola and Smith (2000) and also with those of Liu (2003), who found that excess amount of essential oils in the diet, may have not have anti-oxidant but pro-oxidant effect.

Glutathione content was significantly higher in liver homogenate as effect of garlic oil supplementation (Table 3). There are two explanation of that result. First, garlic oil contains organic sulphur compounds which may react with 5,5'-dithiobis-2 nitrobensoic acid used as colour complex reagent for the determination of reduced glutathione as non-protein sulphydryl group. Second, garlic oil prevents oxidation of glutathione through its active components, such as S-allylcysteine, that lead to functional recovery (Sener et al. 2007). Additionally garlic oil possibly improves the

Table 3. Malondialdehyde and reduced glutathione content and glutathione peroxidase activity of liver homogenates of chickens fed with different essential oils (mean ±S.D.).

	Control	Garlic oil	Rosemary oil	Garlic and rosemary oil
MDA (μmol g^{-1})	6.78 ±0.80^{a}	6.30 ± 1,23^{ab}	6.19 ± 1.05^{ab}	7.88 ± 1.14^{b}
GSH (mmol g^{-1})	0.85 ± 0.09^{a}	0.96 ± 0,11^{b}	0.91 ± 0.10^{ab}	0.97 ± 0.17^{ab}
GSHPx (U g^{-1} protein)	0.77 ± 0.09^{a}	0.90 ± 0,20^{b}	1.22 ± 0.14^{b}	1.18 ± 0.24^{b}

^{a,b} Different superscripts in the same row means significant difference at $P<0.05$ level

absorption of amino acids, such as methionine or cysteine, from the gastro-intestinal tract which are the limiting factors of glutathione synthesis (Wang et al. 1997).

Glutathione peroxidase activity was significantly higher in the 10,000 g supernatant fraction of liver homogenates of all three treated groups as compared to the control (Table 3). Glutathione peroxidase activity depends on the presence of its active centre, selenium (Sun et al. 1998), also its substrates, reduced glutathione (Németh et al. 2004) and oxygen free radicals which are first increase and at continuously high level decrease the enzyme activity (Holovska et al. 1996). The results suggest that garlic or rosemary oil as antioxidants decrease the oxygen free radical formation therefore increase the enzyme activity and the same effect was found if those were applied together, and based on the significantly higher malondialdehyde content, provoke free radical formation but only at low extent which increase the enzyme activity. Auroma et al. (1992) reported that carnosic acid appears to scavenge H_2O_2, but it could also act as a substrate for the peroxidase system, also there are some reports about the possible pro-oxidant effects of some garlic oil constituents (Amagase et al. 2001).

In conclusion the results showed that both garlic and rosemary oils have antioxidant properties and those have positive effect on the glutathione redox system of liver in chickens, but using together in a much higher amount those have opposite, pro-oxidant, effect.

References

Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y (2001) Intake of garlic and its bioactive components. J Nutr 131 (Suppl. 3):955S-962S.

Aruoma OI, Halliwell B, Aeschbach R, Löligers J (1992) Antioxidant and pro-oxidant properties of active rosemary constituents: carnosol and carnosic acid. Xenobiotica 22:257-68.

Boutekedjiret C, Bentahar F, Belabbes R, Bessiere JM (2003) Extraction of rosemary essential oil by steam distillation and hydrodistillation. Flavour Fragrance J 18:481-484.

Bozin B, Mimica-Dukic N, Simin N, Anackov G (2006) Characterization of the volatile com-position of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. J Agric Food Chem 54:1822-1828.

Bozin B, Mimica-Dukic N, Samojlik I, Jovin E. (2007) Antimicrobial and antioxidant properties of rosemary and sage (Rosmarinus officinalis L. and Salvia officinalis L., Lamiaceae) essential oils. J Agric Food Chem 55:7879-7885.

Cuvelier ME, Berset C, Richard H (1990) Use of a new test for determining comparative antioxidant activity of butylated hydroxyanisoe, butylated hydroxytoluene, alpha- and gamma-tocopherols and extract from rosemary and sage. Sci Aliments 10:797-806.

Dwivedi C, Rohlfs S, Jarvis D, Engineer F (1992) Chemoprevention of chemically-induced skin tumor development by diallyl sulfide and diallyl disulfide. Pharm Res 9:1668-1670.

Galobart J, Barroeta AC, Baucells MD, Codony R, Ternes W (2001) Effect of dietary supplementation with rosemary extract and alpha-tocopheryl acetate on lipid oxidation in eggs enriched with omega 3-fatty acids. Poult Sci 80:460-467.

Govaris A, Florou-Paneri P (2007) The inhibitory potential of feed supplementation with rosemary and/or alpha-tocopheryl acetate on microbial growth and lipid oxidation of turkey breast during refrigerated storage. LWT Food Sci Technol 40:331-337.

C. Kohlert I, van Rensen R, März G, Schindler EU, Graefe M, Veit M (2000) Bioavailability and pharmacokinetics of natural volatile terpenes in animals and humans. Planta Med 66:495-505.

Lee KW, Everts H, Beynen AC (2004) Essential oils in broiler nutrition. Int J Poult Sci 3:738-752.

Liu RH (2003) Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr 78: 517S-520S.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275.

Matkovics B, Szabó L, Sz Varga I (1988) Determination of enzyme activities in lipid peroxidation and glutathione pathways (In Hungarian). Laboratóriumi Diagnosztika 15: 248-250.

McCarthy TL, Kerry JP, Kerry JF, Lynch PB, Buckley DJ (2001) Evaluation of the antioxidant potential of natural food / plant extracts as compared with synthetic antioxidants and vitamin E in raw and cooked pork patties. Meat Sci 57:45-52.

Mihara M, Uchiyama M, Fukuzawa K (1980) Thiobarbituric acid value of fresh homo-genate of rat as parameter of lipid peroxidation in ageing, CCl_4 intoxication and vitamin E deficiency. Biochem Med 23:302-311.

Németh K, Mézes M, Gaál T, Bartos Á, Balogh K, Husvéth F (2004) Effect of supplementation with methionine and different fat sources on the glutathione redox system of growing chicken. Acta Vet Hung 52:369-378.

Placer ZA, Cushman LL, Johnson BC (1966) Estimation of product of lipid peroxidation (malonyldialdehyde) in biochemical systems. Anal Biochem 16:359-364.

Richheimer SL, Bernart MW, King GA, Kent MC, Bailey DT (1996) Antioxidant activity of lipid-soluble phenolic diterpenes from rosemary. J Am Oil Chem Soc 73:507-514.

Sedlak I, Lindsay RH (1968) Estimation of total, protein-bound and nonprotein sulfhydryl groups in tissues with Ellmann's reagent. Anal Biochem 25:192-205.

Sener G, Sakarcan A, Yegen BC. (2007) Role of garlic in the prevention of ischemia-reperfusion injury. Mol Nutr Food Res. 51:1345-1352.

Skibola CF, Smith MT (2000) Potential health impacts of excessive flavonoid intake. Free Radic Biol Med 29:375-383.

Smet K, Raes K, Huyghebaert G, Haak L, Arnouts S, De Smet S. (2005) Influence of feed enriched with natural antioxidants on the oxidative stability of broiler meat. Proc. 17th European Symposium on the Quality of Poultry Meat, Doorwerth, pp. 99-106.

Yamasaki T, Li L, Lau B (1994) Garlic compounds protect vascular endothelial cells from hydrogen peroxide-induced oxidant injury. Phytother Res 8:408-412.

Wang ST, Chen HW, Sheen LY, Lii CK (1997) Methionine and cysteine affect glutathione level, glutathione-related enzyme activities and the expression of glutathione S-transferase isozymes in rat hepatocytes. J Nutr 127:2135-2141.

Weichselbaum TE (1948) An accurate and rapid method for the determination of protein in small amounts of serum and plasma. Am J Clin Pathol 16:40-43.