# Influence of dietary vitamin E and copper on fatty acid profile and cholesterol content of raw and cooked broiler meat

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**ABSTRACT**: We examined the influence of a diet containing 4% of rapeseed oil, 35 mg or 126 mg copper and supplement of 100 mg vitamin E per 1 kg on fatty acid profile and cholesterol content in raw and cooked broiler leg meat. Copper was added to feed mixtures as $CuSO_4.5H_2O$. The fatty acid profile and cholesterol content were also determined in raw, stewed and roasted meat of broilers receiving the same diet but with a supplement of 20 mg vitamin E. The results showed that 126 and 35 mg Cu/kg significantly increased oleic acid content. Monounsaturated fatty acids accounted for 51% and 52% of all fatty acids. The relatively low copper concentration of 35 mg/kg feed with a major portion of soybean, wheat and maize and vitamin E supplementation of 100 mg/kg reduced ($P < 0.01$) EPA by 17%, DPA by 16% and DHA by 11 and 15% ($P < 0.05$). All tested copper doses reduced cholesterol: 126 mg Cu by 22%, 35 mg by 6% and 126 mg in combination with 35 mg copper in relation to broiler age by 7% ($P < 0.05$); none of the cooking procedures influenced cholesterol. Meat cooking in experiment 1 did not influence the content of any fatty acid. Copper × cooking interactions were insignificant for all fatty acids and cholesterol. In experiment 2 more changes in the numerical values of fatty acids were caused by stewing compared to roasting. Among the valuable fatty acids n-3 long chain acids were influenced by both cooking procedures to the largest extent. Docosahexaenoic acid was reduced to about 60% of the original value ($P < 0.05$). On the other hand, a decrease in myristic acid by 14% in stewed meat and 12% in roasted meat ($P < 0.05$) was positive.

**Keywords**: broiler; copper sulphate; leg meat; cooking; fatty acids; cholesterol

The lipid fraction of meat can be modified by cooking. Lipid oxidation is in relation to cooking procedure, temperature, time, water content and pressure. Peroxidation processes are related to lipid characteristics and prooxidant to antioxidant ratio in meat. Copper, whose higher doses are often supplemented to diets for its growth stimulation in pigs, was described as a factor increasing lipid oxidation (Love, 1985) or, on the contrary, decreasing lipid oxidation (Sato and Hegarty, 1971). Copper sulphate at doses of 50 or 100 mg/kg feed inhibited lipid oxidation in cooked beef, and copper chloride at a dose of 150 mg Cu/kg strongly inhibited the oxidation (Sato and Hegarty, 1971). Currently, the antioxidative effect of copper as a microelement in antioxidative enzymes such as superoxide dismutase, glutathione peroxidase and catalase is recognized (Wieleba and Pasternak, 2001). Lauridsen *et al*. (1999) reported that vitamin E, Cu and rapeseed oil improved the antioxidative status of live pigs. Lauridsen *et al*. (2000) did not prove the prooxidative effect of 175 mg copper/kg feed in the mitochondria of two muscles in pigs. In the last years anticholesterolaemic effects of copper were examined in poultry (Bakalli *et al*., 1996). Copper is related to lipid metabolism (Amer and Elliot, 1973). Skřivan *et al*. (2002) reported a statistically significant decrease in total lipids in breast muscles, skin from breast muscles and skin from femoral muscles with fat as a result of higher dietary supplementation of copper in broiler chicks. The effects of copper on the fatty acid profile in poultry

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or pig meat have not been examined thoroughly until now and partial results are not identical. Some authors stated that meat cooking also had different effects on fatty acid content in the adipose component. Duckett and Wagner (1998) found out an increase in stearic acid in total lipids of cooked beef while the contents of linoleic, linolenic and arachidonic acid decreased. Smith *et al*. (1989) and Harris *et al*. (1992) did not detect any changes in the fatty acid profile in sirloin after cooking. Vitamin E supplement improved fat quality by reducing saturated fatty acids (SFA) and by increasing polyunsaturated fatty acids (long-chain n-3 PUFA) both in raw and cooked meat. Higher uptake of n-3 fatty acids through human food without adequate antioxidative protection can lead to an increase in free radicals and oxidation products (Dal Bosco *et al*., 2001). Low dietary copper also increases faecal free radical production in healthy men.

Echarte *et al*. (2001) reported that frying increased the PUFA/SFA ratio in pork and accelerated cholesterol oxidation. The ratio of fatty acid classes was considerably influenced by sunflower oil used for frying. Dal Bosco *et al*. (2001) compared the content of fatty acids in raw rabbit meat and in meat after 3 cooking procedures (cooking in a polyethylene bag in water bath, frying in sunflower oil and roasting). Standard cooking procedures terminated when the temperature inside meat samples reached 80°C. Cooked meat had a significantly lower content of linoleic acid compared to fried meat. The most marked and significant decrease against raw meat was measured in all studied n-3 polyunsaturated fatty acids (LNA, EPA, DPA and DHA). As among the polyunsaturated fatty acids the n-3 class has the highest weight in thrombogenic index (Ulbricht and Southgate, 1991), the index considerably increased after meat cooking. Myristic acid insignificantly decreased and monounsaturated fatty acids (MUFA) insignificantly increased as a result of all tested cooking procedures. Mainly myristic acid and also palmitic acid exert hypercholesterolaemic effects (Williams *et al*., 1999). The n-3 and n-6 polyunsaturated fatty acids are more susceptible to oxidation than monounsaturated acids. Dietary administration of monounsaturated fatty acids not only reduces membrane and meat lipid oxidation but also modifies the relative proportion of volatile aldehydes generated upon heating (Lopez-Bote *et al*., 1997). Diets for humans in which saturated fat is partially replaced by MUFA can achieve significant reductions in total and LDL-cholesterol concentration, even when total fat and energy intakes are maintained (Williams *et al*., 1999). Many monounsaturated fatty acids are contained in rapeseed oil that is cheap and easily available. In comparison with lard dietary rapeseed oil administered to chickens significantly decreased SFA and increased PUFA in intramuscular and abdominal fat of chickens (Skřivan *et al*., 2000).

The objective of experiment 1 was to determine the effects of various copper doses supplemented to feed with rapeseed oil and vitamin E in combination with a gentle procedure of heat treatment of broiler leg meat on fatty acid profile and cholesterol content. Experiment 2 was aimed at the effect of a less gentle procedure of heat treatment (stewing and roasting) on the content of fatty acids if vitamin E is on the usual nutrition level.

## MATERIAL AND METHODS

### Animals and diets

Cockerels of broiler hybrid Ross were kept from day 1 to day 42 of age in littered boxes. Chick density per floor unit area, microclimatic conditions in the facility, feeding and drinking space complied with the technological procedure of the given breeding firm. Feeds with a major portion of soybean, maize and wheat contained 4% rapeseed oil (Table 1). In experiment 1 a supplement of 100 mg α-tocopheryl acetate per kg was used because high doses of copper were administered while in experiment 2 a usual supplement of 20 mg α-tocopheryl acetate was used. Copper in experiment 1 was supplied by copper sulphate pentahydrate to amount to 126 mg of total copper content per kg feed in group II, 35 mg/kg in group III and 126 mg/kg in group IV until 14 days of broiler age, and only to 35 mg/kg from 14 to 42 days of age. Group I (control) received 13 mg Cu/kg of feed.

Eight broilers of the same live weight were chosen from each group of experiment 1 at 42 days of age. The broilers were stunned electrically and sacrificed. Dark meat with skin was taken for analyses. Meat from one leg was analysed in raw condition and meat from the other leg was analysed after cooking. Twenty-four broilers at the age of 42 days were sacrificed in experiment 2. Leg meat with skin of 8 broilers was processed in raw condition and the same amount after stewing or roasting. Determined characteristics were dry matter, proteins, fat, cholesterol and fatty acids.

Table 1. Composition of the basal diet for experiments 1 and 2

| Ingredients | g/kg |
|---|---|
| Wheat | 290 |
| Maize | 290 |
| Soybean meal | 325 |
| Fish meal | 20 |
| Rapeseed oil | 40 |
| Limestone | 15 |
| Dicalcium phosphate | 12 |
| Sodium chloride | 2 |
| DL-methionine | 1 |
| Vitamin-mineral mix* | 5 |
| Composition by analysis | |
| Dry matter | 904 |
| Crude protein | 224 |
| Fat | 65 |
| Fibre | 36 |
| Calcium | 9.2 |
| Phosphorus | 5.6 |
| Copper (mg/kg) | 13 |
| ME, MJ/kg | 12.62 |

*the vitamin/mineral premix provided per kg of diets: vitamin A – 12 000 IU, vitamin $D_3$ – 500 IU, vitamin E – 100 mg (experiment 1), 20 mg (experiment 2), vitamin $K_3$ – 3 mg, $B_1$ – 3 mg, $B_2$ – 5 mg, $B_6$ – 4 mg, $B_{12}$ – 0.04 mg, niacin amide – 40 mg, Ca pantothenate – 12 mg, biotin – 0.15 mg, folic acid – 1.5 mg, choline-Cl – 250 mg, ethoxyquin – 100 mg, Mn – 80 mg, Zn – 60 mg, Fe – 50 mg, I – 1.2 mg, Se – 0.25 mg

## Cooking procedures

**Experiment 1**. Broiler leg meat with skin was cooked in polyethylene bags in water bath at 85ºC until its internal temperature reached 80°C, and subsequently cooled immediately for 15 min before each determination. Juice drippings were mixed with homogenized meat before the samples were taken for analyses.

**Experiment 2**. Stewing: weighed leg meat with skin was put into a preserve jar with Omnia lid and sealed. The jars were placed into a laboratory drier, and after the temperature of 100°C was reached, they were heated another 60 minutes.

Roasting: weighed leg meat with skin was put into roasting glass pans with Simax lids. 50 ml of drinking water and 0.5% of cooking salt were added. The pans were placed in an electric oven heated to 180°C and measurements of temperature and time started. Roasting was terminated in 75 minutes from the beginning of measurement because the temperature at 153°C did not change in the time interval of 60–75 minutes.

## Analyses

Total lipids for the fatty acid analyses of raw and cooked meat were extracted from leg muscles with 2 : 1 chloroform-methanol according to the method of Folch *et al*. (1957). Alkaline trans-methylation of fatty acids was carried out according to ISO 5509. Gas chromatographic analysis of methyl esters was performed using a Hewlett-Packard 5890 gas chromatograph equipped with programmed HP-Innova capillary column (180° to 240°C) and an Fl detector. To determine cholesterol lipids were saponified and the unsaponified matter was extracted according to Nollet (1996). Silyl derivatives were separated and quantified on a gas chromatograph equipped with SAC-5 capillary column (Supelco), operated isothermally at 285°C. Copper was determined by atomic absorption spectrophotometry (Perkin Elmer Atomic Absorption Spectrophotometer 500).

Protein content was analysed with Kjeltec apparatus (Tecator AB, Höganös, Sweden) using the factor 6.25. Dry matter content was determined after the samples were dried at 105°C for 16–18 hours. Analyses of fat were carried out after hydrolysis, using petroleum ether for extraction (Soxlet System H+ equipment, Tecator AB). Each leg sample was analysed both raw and cooked.

## Statistical analysis

Data of experiment 1 were processed by two-way analysis of variance with copper supplement × cooking interaction using the GLM procedure (SAS Institute, 1989). The used statistical model comprised fixed effects of copper supplement (four levels) and cooking (two levels). Data of experiment 2 were processed by one-way analysis of variance. Significant effects of treatments were

determined by Duncan's multiple range test. As the differences in dry matter content between raw, stewed and roasted meat were large, protein, fat and cholesterol contents were calculated per absolute dry matter and these values were statistically processed.

## RESULTS

### Experiment 1

**Chemical composition of leg meat.** Cooking increased dry matter content although meat was cooked in sealed polyethylene bags and juice drippings were mixed with meat before the sample collection. As dry matter content increased, protein content also increased while fat content decreased insignificantly (Table 2). The effect of copper sulphate supplements on the summary content of subcutaneous and intramuscular fat was also evidenced. But the differences are not significant either, as a result of higher variability. On the contrary, a significant reduction in cholesterol was measured. All copper supplements reduced the cholesterol concentration in leg meat but cooking did not influence it.

**Fatty acid pattern of leg meat.** The relative ratios of fatty acids in leg meat lipids were not influenced by cooking but by copper supplements (Table 3). An increase in MUFA was a positive effect of higher dietary lipid content, not only of the highest dose 126 mg but also of 35 mg of copper. Oleic acid and eicosanoic acid contributed to this increase to the largest extent. As a result of rapeseed oil use monounsaturated fatty acids increased above 1/2 of fatty acid content in meat. Among the saturated fatty acids, myristic acid decreased at a dose of 126 mg copper, but it increased in the treatment 126 mg copper until 14 days of age and 35 mg copper from 14 to 42 days of age. Vitamin E at an amount of 100 mg per 1 kg feed with 4% rapeseed oil did not prevent the oxidation of n-3 and n-6 PUFA; a highly significant decrease was measured in alpha-linolenic, eicosapentaenoic and docosapentaenoic acid (LNA, EPA and DPA).

### Experiment 2

**Chemical composition of leg meat**. A large increase in dry matter in stewed and roasted meat was reflected in a similarly marked increase in protein, fat and cholesterol content. When the values for original dry matter were expressed as the values of 100% dry matter, the differences between the groups were blurred and only a difference in fat content between stewed and roasted meat was statistically significant on the lowest level 0.05. The content of dry matter, proteins and cholesterol in raw meat corresponded to experiment 1 but fat content increased, which could be related with the lower supplement of vitamin E.

**Fatty acid pattern of leg meat.** Cooking decreased the content of myristic acid, statistically significantly in stewed meat. The content of n-6 and particularly of n-3 polyunsaturated fatty acids also decreased while the decrease was relatively

Table 2. Concentration of dry matter, protein, fat and cholesterol in raw and cooked leg meat from broilers, fed different contents of dietary copper

| Copper (mg/kg) | 13 | | 126 | | 35 | | 126/35 | | Statistical effects | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meat | Raw | Cooked | Raw | Cooked | Raw | Cooked | Raw | Cooked | Copper | Meat | Copper × meat | SEE |
| Dry matter (%) | 28.9 | 29.7 | 28.1 | 28.7 | 28.6 | 29.4 | 28.2 | 29.3 | ns | ** | ns | 4.54 |
| Protein (%) | 17.7 | 19.1 | 17.8 | 18.7 | 17.6 | 18.9 | 17.8 | 18.8 | ns | *** | ns | 2.67 |
| Fat (%) | 9.2 | 8.7 | 8.4 | 8.0 | 9.2 | 8.5 | 8.5 | 8.6 | ns | ns | ns | 4.71 |
| Cholesterol (mg/kg) | 724.2 | 735.1 | 565.7 | 576.7 | 681.4 | 693.7 | 673.5 | 684.4 | * | ns | ns | 15.31 |

SEE = standard error of estimation; ns = not significant

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Table 3. Changes of the fatty acid profile (% of total methyl esters present) of raw and cooked leg meat from broilers, fed different contents of dietary copper

| Copper (mg/kg) | 13 | | 126 | | 35 | | 126/35 | | Statistical effects | | | SEE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Meat | R | C | R | C | R | C | R | C | copper | meat | copper × meat | |
| C 14:0 | 0.48 | 0.48 | 0.46 | 0.46 | 0.48 | 0.49 | 0.51 | 0.52 | *** | ns | ns | 0.013 |
| C 15:0 | 0.10 | 0.10 | 0.10 | 0.10 | 0.09 | 0.09 | 0.10 | 0.10 | ns | ns | ns | 0.004 |
| C 16:0 | 17.00 | 17.13 | 16.63 | 16.85 | 16.79 | 17.09 | 16.71 | 17.04 | ns | ns | ns | 0.349 |
| C 17:0 | 0.16 | 0.15 | 0.16 | 0.15 | 0.14 | 0.14 | 0.14 | 0.14 | ns | ns | ns | 0.007 |
| C 18:0 | 5.39 | 5.54 | 5.54 | 5.57 | 5.31 | 5.26 | 5.35 | 5.31 | ns | ns | ns | 0.189 |
| C 20:0 | 0.13 | 0.13 | 0.14 | 0.12 | 0.13 | 0.11 | 0.12 | 0.12 | ns | ns | ns | 0.007 |
| C 14:1 | 0.09 | 0.09 | 0.08 | 0.08 | 0.09 | 0.09 | 0.09 | 0.09 | ns | ns | ns | 0.005 |
| C 16:1 | 3.68 | 3.73 | 3.52 | 3.81 | 3.62 | 3.89 | 3.38 | 3.84 | ns | ns | ns | 0.210 |
| C 18:1 | 46.42 | 46.63 | 47.42 | 47.43 | 47.02 | 47.70 | 46.09 | 46.40 | *** | ns | ns | 0.308 |
| C 20:1 | 0.66 | 0.68 | 0.71 | 0.69 | 0.71 | 0.69 | 0.66 | 0.65 | ns | ns | ns | 0,019 |
| C 22:1 | 0.06 | 0.06 | 0.05 | 0.06 | 0.05 | 0.07 | 0.05 | 0.06 | ns | ns | ns | 0.005 |
| C 18:2 n-6 | 19.39 | 19.02 | 19.08 | 18.83 | 19.56 | 18.49 | 20.14 | 19.45 | ns | ns | ns | 0.452 |
| C 18:3 n-6 | 0.25 | 0.24 | 0.24 | 0.23 | 0.23 | 0.22 | 0.24 | 0.23 | ns | ns | ns | 0.008 |
| C 20:2 n-6 | 0.17 | 0.17 | 0.17 | 0.16 | 0.16 | 0.15 | 0.17 | 0.16 | ns | ns | ns | 0.008 |
| C 22:2 n-6 | 0.02 | 0.03 | 0.01 | 0.01 | 0.01 | 0.02 | 0.02 | 0.02 | ns | ns | ns | 0.003 |
| C 20:3 n-6 | 0.17 | 0.17 | 0.18 | 0.17 | 0.15 | 0.15 | 0.17 | 0.16 | * | ns | ns | 0.008 |
| C 20:4 n-6 | 0.74 | 0.68 | 0.72 | 0.67 | 0.59 | 0.60 | 0.76 | 0.70 | * | ns | ns | 0.041 |
| C 22:4 n-6 | 0.13 | 0.12 | 0.13 | 0.12 | 0.11 | 0.11 | 0.15 | 0.14 | * | ns | ns | 0.011 |
| C 18:3 n-3 | 3.93 | 3.75 | 3.67 | 3.50 | 3.92 | 3.67 | 4.04 | 3.84 | ** | ns | ns | 0.105 |
| C 20:3 n-3 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.05 | 0.04 | ns | ns | ns | 0.002 |
| C 20:5 n-3 | 0.23 | 0.22 | 0.21 | 0.19 | 0.20 | 0.19 | 0.23 | 0.21 | ** | ns | ns | 0.010 |
| C 22:5 n-3 | 0.32 | 0.31 | 0.30 | 0.29 | 0.27 | 0.26 | 0.35 | 0.33 | ** | ns | ns | 0.018 |
| C 22:6 n-3 | 0.42 | 0.47 | 0.37 | 0.42 | 0.33 | 0.40 | 0.41 | 0.45 | * | ns | ns | 0.026 |
| Others | 0.02 | 0.06 | 0.07 | 0.05 | 0.00 | 0.08 | 0.07 | 0.00 | | | | |
| SFA | 23.26 | 23.53 | 23.03 | 23.25 | 22.94 | 23.18 | 22.93 | 23.23 | ns | ns | ns | 0.432 |
| MUFA | 50.91 | 51.19 | 51.78 | 52.07 | 51.49 | 52.44 | 50.27 | 51.04 | ** | ns | ns | 0.429 |
| PUFA | 25.81 | 25.22 | 25.12 | 24.63 | 25.57 | 24.30 | 26.73 | 25.73 | ns | ns | ns | 0.614 |
| PUFA n-6 | 20.87 | 20.43 | 20.53 | 20.19 | 20.81 | 19.74 | 21.65 | 20.86 | ns | ns | ns | 0.649 |
| PUFA n-3 | 4.94 | 4.79 | 4.59 | 4.44 | 4.76 | 4.56 | 5.08 | 4.87 | * | ns | ns | 0.142 |
| PUFA : SFA | 1.11 | 1.07 | 1.09 | 1.06 | 1.11 | 1.05 | 1.17 | 1.11 | ns | ns | ns | 0.044 |
| PUFAn-6 : PUFAn-3 | 4.22 | 4.27 | 4.47 | 4.55 | 4.37 | 4.33 | 4.26 | 4.28 | ** | ns | ns | 0.073 |

R = raw; C = cooked; SEE = standard error of estimation; ns = not significant; SFA = the sum of saturated fatty acids; MUFA = the sum of monounsaturated fatty acids; PUFA = the sum of polyunsaturated fatty acids

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Table 4. Concentration of dry matter, protein, fat and cholesterol in raw, stewed and roasted leg meat from broilers

| Meat | 1. Raw | 2. Stewed | 3. Roasted | Significance | | | SEE |
|---|---|---|---|---|---|---|---|
| | | | | 1:2 | 1:3 | 2:3 | |
| Dry matter (%) | 30.1 | 36.0 | 41.2 | *** | *** | *** | 10.19 |
| Protein (%)[a] | 17.5 | 21.2 | 24.3 | ns | | | |
| Protein (%)[b] | 58.3 | 58.9 | 60.0 | ns | ns | ns | 5.79 |
| Fat (%)[a] | 11.3 | 13.3 | 14.2 | ns | | | |
| Fat (%)[b] | 37.5 | 37.1 | 34.4 | ns | * | ns | 5.14 |
| Cholesterol (mg/kg)[a] | 742.6 | 949.6 | 1 094.9 | ns | | | |
| Cholesterol (mg/kg)[b] | 2 465.5 | 2 696.9 | 2 660.8 | ns | ns | ns | 42.03 |

[a]in starting dry matter; [b]in absolute dry matter; SEE = standard error of estimation; ns = not significant

*$P < 0.05$; ***$P < 0.001$

highest in long-chain fatty acids (EPA, DPA and DHA). The lower total proportion of n-3 PUFA was a cause of the significantly wider ratio of n-6 : n-3 PUFA. On the contrary, total monounsaturated fatty acids increased insignificantly, mainly thanks to oleic acid.

## DISCUSSION

Identical diets in both experiments contained rapeseed oil with a high proportion of oleic acid. Total dietary copper content in experiment 1 was 35 mg/kg or 126 mg/kg and 100 mg α-tocopheryl acetate was added per 1 kg feed. Basal feeds of both experiments differed only in vitamin E supplements. In experiment 2 only 1/5 of the tocopheryl acetate amount from experiment 1 (20 mg/kg) was added. The dark meat of broilers from experiment 1 had a lower content of intramuscular and subcutaneous fat, lower amount of saturated fatty acids and higher amount of polyunsaturated fatty acids. The summary content of subcutaneous and intramuscular fat decreased as a result of copper supplementation, but the difference was not significant as it was in our preceding experiment (Skřivan *et al*., 2002) where the supplement of 126 mg copper/kg reduced intramuscular fat, skin with fat from breasts and legs and insignificantly reduced abdominal fat.

Lauridsen *et al*. (2000) demonstrated that a supplement of 200 mg vitamin E/kg feed for pigs inhibited the formation of free radicals and a supplement of 175 mg Cu was co-active although the interaction was not significant. Changes in the pattern of fatty acids can be an indicator of prooxidative or antioxidative effects. E.g. the effects of copper, meat cooking or other factors can be evaluated in this way. It is necessary to take into account the level of vitamin E supplementation because vitamin E affects the ratios of fatty acids by decreasing SFA and increasing PUFA (Harris *et al*., 1992). The same effect was also observed in our experiments when the results of two separate experiments were compared carefully. Mainly n-3 PUFA are susceptible to peroxidation. The relatively low dietary copper content administered to group 3 in experiment 1, 35 mg/kg, reduced ($P < 0.01$) EPA and DPA (Table 3). On the contrary, 200 mg Cu/kg and 50 mg vitamin E/kg feed with 6% rapeseed oil did not influence EPA and DHA in breast muscle lipids nor in abdominal fat of broilers; it was related to a higher supply of MUFA by 1/2 (Skřivan *et al*., 2000). The increase in monounsaturated fatty acids after copper supplements in experiment 1 improved the cardioprotective composition of fat because MUFA reduce LDL-cholesterol significantly (Williams *et al*., 1999). The nutritive copper allowance for broilers is usually given in the range of 6–9 mg/kg feed. It meets the requirement for copper as a microelement, provides for its involvement in antioxidative enzymes and the resultant effect will be antioxidative. The content of 13 mg Cu/kg was determined analytically in the basal feed. If 35 mg Cu/kg had prooxidative effects in our experiment as it was deduced from the changes in the composition of polyunsaturated fatty acids, a hypothesis can be correct that the change from antioxidant to prooxidant occurred somewhere between 13–35 mg Cu

Table 5. Changes of fatty acids of raw, stewed and roasted leg meat from broilers (% of total methyl esters present)

| Meat | 1. Raw | 2. Stewed | 3. Roasted | Significance | | | SEE |
|---|---|---|---|---|---|---|---|
| | | | | 1:2 | 1:3 | 2:3 | |
| C 14:0 | 0.59 | 0.51 | 0.52 | * | ns | ns | 0.064 |
| C 15:0 | 0.10 | 0.09 | 0.10 | ns | ns | ns | 0.352 |
| C 16:0 | 20.40 | 19.98 | 20.09 | ns | ns | ns | 0.209 |
| C 17:0 | 0.21 | 0.13 | 0.13 | ns | ns | ns | 0.017 |
| C 18:0 | 5.31 | 5.60 | 5.65 | ns | ns | ns | 0.063 |
| C 20:0 | 0.08 | 0.10 | 0.10 | * | ns | ns | 0.002 |
| C 14:1 | 0.17 | 0.14 | 0.15 | ns | ns | ns | 0.005 |
| C 16:1 | 5.30 | 4.77 | 4.71 | ns | * | ns | 0.098 |
| C 18:1 | 44.74 | 46.14 | 45.53 | ns | ns | ns | 0.313 |
| C 20:1 | 0.53 | 0.60 | 0.60 | *** | *** | ns | 0.009 |
| C 22:1 | 0.04 | 0.04 | 0.03 | ns | ns | ns | 0.015 |
| C 18:2 n-6 | 16.64 | 16.90 | 17.28 | ns | ns | ns | 0.258 |
| C 18:3 n-6 | 0.17 | 0.28 | 0.24 | *** | *** | ns | 0.011 |
| C 20:2 n-6 | 0.17 | 0.07 | 0.06 | *** | *** | ns | 0.011 |
| C 22:2 n-6 | 0.06 | 0.05 | 0.05 | ns | ns | ns | 0.004 |
| C 20:3 n-6 | 0.03 | 0.01 | 0.01 | * | * | ns | 0.003 |
| C 20:4 n-6 | 0.51 | 0.40 | 0.40 | ns | ns | ns | 0.022 |
| C 22:4 n-6 | 0.11 | 0.09 | 0.09 | ns | ns | ns | 0.006 |
| C 18:3 n-3 | 3.51 | 3.33 | 3.48 | ns | ns | ns | 0.065 |
| C 20:3 n-3 | 0.05 | 0.04 | 0.04 | ns | ns | ns | 0.002 |
| C 20:5 n-3 | 0.16 | 0.10 | 0.10 | ns | ns | ns | 0.034 |
| C 22:5 n-3 | 0.22 | 0.18 | 0.18 | ns | ns | ns | 0.009 |
| C 22:6 n-3 | 0.32 | 0.20 | 0.19 | * | * | ns | 0.019 |
| Others | 0.59 | 0.28 | 0.29 | | | | |
| SFA | 26.69 | 26.41 | 26.58 | ns | ns | ns | 0.257 |
| MUFA | 50.78 | 51.69 | 51.02 | ns | ns | ns | 0.296 |
| PUFA | 21.94 | 21.62 | 22.12 | ns | ns | ns | 0.332 |
| PUFA n-6 | 17.68 | 17.78 | 18.13 | ns | ns | ns | 0.270 |
| PUFA n-3 | 4.26 | 3.84 | 3.99 | ns | ns | ns | 0.079 |
| PUFA : SFA | 0.82 | 0.82 | 0.83 | ns | ns | ns | 0.018 |
| PUFA n-6 : PUFA n-3 | 4.15 | 4.63 | 4.54 | ** | ns | ns | 0.055 |

SEE = standard error of estimation; ns = not significant; SFA = the sum of saturated fatty acids; MUFA = the sum of monounsaturated fatty acids; PUFA = the sum of polyunsaturated fatty acids

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

in the given conditions. But when Lauridsen *et al*. (2000) applied a several times higher copper dose with twice higher supplementation of vitamin E, they reported a trend of inhibition of free radical formation as a result of copper effect in pigs. Copper did not influence cholesterol concentration in blood plasma nor did it affect the fatty acid profile in liver fat significantly (Lauridsen *et al*., 1999). The indication of antioxidative effects of vitamin E and copper is evidenced in the same paper. Different results can be caused by a double dose of vitamin E. In our experiments all tested copper doses reduced cholesterol: 126 mg Cu by 22%, 35 mg by 6% and 126 mg in combination with 35 mg copper in relation to broiler age by 7%. A cholesterol reduction in chicken meat after higher copper doses was already described (Bakalli *et al*., 1996), and the results of our experiment document that copper starts reducing cholesterol at a much lower amount. The permitted copper content per 1 kg feed for all species of farm animals until slaughter weight is 35 mg (EU Directive No. 91/248).

Meat cooking in experiment 1 did not influence the content of any fatty acid. In experiment 2 more changes in the numerical values of fatty acids were caused by stewing compared to roasting. Among the valuable fatty acids n-3 long-chain acids (EPA, DPA and DHA) were influenced by both cooking procedures to the largest extent. It coincides with copper effects. Docosapentaenoic acid was reduced to about 60% of the original value. On the other hand, a decrease in myristic acid by 14% in stewed meat and 12% in roasted meat was positive. An increase on monoenoic fatty acids was also positive. Dal Bosco *et al*. (2001) reported a similar development for rabbit mean even though the differences were insignificant. Some n-6 fatty acids increased after cooking surprisingly; finally, it resulted in the wider ratio of n-6 : n-3 PUFA. Neither cooking nor stewing and roasting affected cholesterol content where the differences were not significant. Echarte *et al*. (2001) considered the temperature of 120°C as limiting for cholesterol oxidation. Therefore roasting is supposed to increase oxidation products of cholesterol; it will be verified by further research.

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## ABSTRAKT

### Vliv vitaminu E a mědi v krmných směsích na profil mastných kyselin a obsah cholesterolu v syrovém a vařeném mase brojlerových kuřat

Byl sledován vliv krmné směsi se 4 % řepkového oleje, 35 mg nebo 126 mg mědi a přídavkem 100 mg vitaminu E/kg na profil mastných kyselin a obsah cholesterolu v syrovém a vařeném mase z pánevních končetin brojlerových kuřat. Zdrojem přidávané mědi byl $CuSO_4.5H_2O$. Profil mastných kyselin a obsah cholesterolu byl dále stanoven v syrovém, dušeném a pečeném mase kuřat krmených stejnou základní krmnou směsí, ale pouze s doplňkem 20 mg vitaminu E. Výsledky ukázaly, že 126 i 35 mg Cu/kg signifikantně zvýšilo obsah kyseliny olejové. Celkový podíl mononenasycených mastných kyselin byl 51 a 52 % ze všech mastných kyselin. Poměrně nízký obsah mědi, 35 mg/kg krmné směsi s převahou sóji, pšenice a kukuřice a s doplňkem 100 mg/kg vitaminu E snížil ($P < 0,01$) zastoupení EPA o 17 %, DPA o 16 % a DHA o 11 a 15 % ($P < 0,05$). Všechny testované dávky mědi redukovaly cholesterol: 126 mg Cu o 22 %, 35 mg o 6 % a 126 mg v kombinaci s 35 g Cu dle věku kuřat o 7 % ($P < 0,05$). Žádná z tepelných úprav masa cholesterol neovlivnila. Vaření masa v pokusu 1 nezměnilo zastoupení mastných kyselin. Interakce měď × vaření byla nevýznamná pro všechny mastné kyseliny i cholesterol. Dušením masa v pokusu 2 došlo ke změnám v číselných hodnotách mastných kyselin proti pečení. Z n-3 mastných kyselin s dlouhým řetězcem klesla dokosahexanová kyselina na 60 % původní hodnoty ($P < 0,05$). Na druhé straně byl příznivý pokles kyseliny myristové o 14 % u dušeného masa a o 12 % u masa pečeného ($P < 0,05$).

**Klíčová slova**: brojlerová kuřata; síran měďnatý; stehenní svalstvo; tepelné úpravy; mastné kyseliny; cholesterol

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