FEEDING EFFECT OF THE ADDITION OF LINOLEIC ACID ON MEAT QUALITY OF CHICKENS

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Abstract


The aim of this study was to analyse the influence of linoleic acid which was added in the broiler chickens feed mixtures in relation to chemical composition of meat, content of fatty acids and composition of blood serum. There were compared the characteristics of two groups of ROSS 308 chickens in the experiment (the experimental group with 5% addition of linoleic acid and the control group). The protein content of breast was significantly lower (P ≤ 0.05) in the experimental group than in the control group. There were found significant differences (P ≤ 0.05) in the protein content between sexes. There was found statistically significant (P ≤ 0.01) higher fat content in the breast of experimental group in comparison with control group. Statistically significant differences (P ≤ 0.05) were found in fat from the thigh meat of experimental group than the control group. The analysis of the chemical composition showed higher content of fat in the breast (1.9 g.100 g−1) than in the thigh (11.66 g.100 g−1) of chickens which were fed with the addition of linoleic acid to feed mixture. This resulted in lower share of the other components. The addition of linoleic acid in the chickens feed mixture showed significantly higher proportion of polyunsaturated fatty acids to saturated fatty acids (0.76). Analysis of blood serum showed higher concentration of chlorides (P ≤ 0.01) in the group with the addition of linoleic acid than the control group. Proportion of monounsaturated fatty acids was 47.06% in the experimental group and significantly higher one (53.77%) was found in the control group.

Keywords: linoleic acid, ROSS 308, chemical composition, fatty acids, blood serum

INTRODUCTION

Fat content and fatty acids composition of meat have major importance for consumers due to meat quality and nutritional value (Wood et al., 2004). Saturated fatty acids (SFA) and trans fatty acids have been recognized by the international dietary authorities as primary targets for diet reduction (WHO, 2003). Marked reductions in these specific nutrients as well as an increase in the polyunsaturated fatty acids (PUFA) content, especially n-3 and n-6 PUFA, may have a noticeable knock-on effect on public health improvement (British Department of Health, 1994). Ruminant meat provides a valuable amount of PUFA, namely n-3 fatty acids, for the human diet (Scollan et al., 2001). In contrast, it is well known that the low
PUFA/SFA and high n-6/n-3 ratios of some meats contribute to the imbalance in the fatty acid intake of consumers (Wood et al., 2004). There has been an increased interest in food containing high amount of polyunsaturated fatty acids (PUFAs) because PUFA are considered as functional ingredients to prevent coronary heart disease and other chronic diseases (Krauss et al., 2001; Russo, 2009). Linoleic acid (LA; C18:2n-6) in one of the essential fatty acids and the primary precursor of all n-6 PUFAs (Russo, 2009). It is converted to arachidonic acid (AA; C20:4n-6) in animal tissues (Smith, 2008) and (Russo, 2009). It is converted to arachidonic acid (AA; C20:4n-6) in animal tissues (Smith, 2008) and it has been shown to possess anti-inflammatory effects (Zhao et al., 2005). Previous study reported that the high levels of dietary LA suppressed lymphocyte proliferation in rats (Yaqoob et al., 1994). So several studies had been conducted to increase the content in PUFAs in chickens meat and eggs by using dietary fat sources such as natural oil containing PUFAs and LA (Crespo and Esteve-Garcia, 2001; Kim et al., 2005) according to this reason. However, PUFAs are prone to oxidation since they have been the first targets for free radical strike at initiating peroxidation (Scislowski et al., 2005). The oxidation products lead to deterioration of food quality such as flavour, colour, texture and nutritional value, and they can be responsible for tissue and organ damage (Michal et al., 2006; Priscilla and Prince, 2009). Conjugated linoleic acid (CLA) is a group of geometric and positional isomers of LA (18:2 n-6) with conjugated double bonds. Previous studies have demonstrated that CLA as a dietary supplement has a potential ability to improve some meat quality traits (such as enhancing intramuscular fat content, shear force and CLA incorporation into tissues, and changing fatty acid composition) in pigs (Jiang et al., 2010; Sun et al., 2004; Wiegang et al., 2001), chickens (Szymczyk et al., 2001) and fish (Berge et al., 2004; Valente et al., 2007). Dietary inclusion of CLA can also promote the deposition of CLA isomers in meat (Bandarra et al., 2006; Jiang et al., 2010; Szymczyk et al., 2001). CLA has been reported to possess health benefits, such as anti-obesity (Gauli et al., 2005), anti-tumour (Kim et al., 2005) and others. Consequently, these CLA-rich products are healthy foods for consumers. There is considerable interest including CLAs in animal feeds in the expectation that they may improve production efficiency and meat quality, because CLAs are incorporated into meat, provide value-added “healthful” meat products for human consumption. Studies on growing pigs (Dugan et al., 1997; Thiel-Cooper et al., 2001) and rats (Chin et al., 1994; Ostrowska et al., 1999) indicate that CLA dietary supplementation can improve feed efficiency, growth and body composition. More recent studies indicate that dietary CLAs may enhance whole-body lean tissue deposition and protein accretion thereby decreasing carcass fat content in pigs (Dugan et al., 1997) and in mice (Park et al., 1997). Ahn et al. (1999) reported that the yolks of hard-boiled eggs from hens fed dietary CLA were rubbery and elastic. In pork, CLA feeding improved the marbling of loin, but with no significant changes in sensory characteristics. Dugan et al. (1999) and Du et al. (2001) reported that meat patties made from chickens fed CLA had improved oxidative stability. However, there is no report about the effect of CLA on the quality of further processed meat products. CLAs have several mechanisms of action. They directly affect the enzymes of lipid metabolism (Pariza et al., 2001; Park et al., 1997) giving rise to changes in fat deposition and fatty acid composition (Bee, 2001; Corino et al., 2002). CLAs also affect adipogenesis by inhibiting the proliferation of fibroblasts and their differentiation into adipocytes (Brodie et al., 1999). Maroufyan et al. (2012) found out 3 n-6/n-3 PUFA ratios in chicken meat from 5.5 to 1.5. Meluzzel et al. (2009) found out the content of intramuscular fat in the breast from 1.06–1.08% and in the thigh from 2.99 to 3.48% and content of PUFAs from 35.3 to 37.5% in the breast muscle and from 32.2 to 35.1% in the thigh muscle. Chicken meat had a proportion of saturated 36.4% and polyunsaturated fatty acids 21.3%. Long chain omega-3 polyunsaturated fatty acids (PUFA) eicosapentaenoic and docosahexaenoic were observed only in dark chicken meat 23 mg/100g (Carnevale de Almeida et al., 2006). Haščík et al. (2011) found out fat content from 0.98 to 1.50% in the chicken breast muscle and in the range from 9.4 to 11.37%. Foltyn et al. (2013) found out fat content in the breast muscles 1.63% at the carcass weight 1840g. Protein content varied in the breast muscle from 22.79 to 23.09% and in the thigh muscle from 18.99 to 19.25% (Haščík et al., 2011).

The aim of the experiment was to analyse the influence of linoleic acid added in the broiler feed mixtures on the chemical composition of meat, content of fatty acids and composition of blood serum.

**MATERIAL AND METHODS**

The one day old broilers (ROSS 308) were divided into two groups:
- **K** – control group (n = 100).
- **P** – experimental group (n = 100).

Chickens were fed ad libitum standard feed mixtures for broilers. Feed mixture HYD 01 was given from 1 day to 14 day to both groups. Feed mixture HYD 01/HYD 02 (3:2) was given from 1 day to 14 day to both groups. Feed mixture HYD 01/HYD 02 (3:2) was given from 15 day to 21 day to control group and 5% linoleic acid was added in the feed mixture for the experimental group.

Feed mixture HYD 02 with a 5% addition of linoleic acid was given to the experimental group from 22 day to the end of the fattening period. Linoleic acid was applied by spraying to ensure a homogeneously mixed. The chickens were slaughtered after 42 days of feeding and lasting for 12 hours and subsequently there were performed detailed carcass dissection.
Chemical Composition

The basic chemical composition of meat (water, protein, fat) was determined using the device INFRATEC Meat Analyser 1265 (TECATOR, Sweden), which works on the principle of reflection near infrared radiation (NIR) from chemical bonds typical of protein and fat. The samples were homogenized using a blender before analysing:

- muscle without skin and subcutaneous fat were homogenized for analysis of breast,
- femoral muscle with skin and subcutaneous fat were homogenized for analysis of thigh.

Extraction of Lipids

Meat from the breast (without the skin and fat) was homogenized and there was taken 5 g of samples. Lipid extraction of the sample was performed using petroleum ether as an extraction agent. The homogenized sample of meat was partly dried over anhydrous sodium sulfate and extracted in 250 ml of petroleum ether for 2 hours in a laboratory shaker. Then the sample was filtered over anhydrous sodium sulfate and evaporated to constant volume on a rotary shaker.

Preparation of Methyl Esters of Fatty Acids

Fatty acid methyl esters were prepared by a modified method according to Juarez et al. (2008). The sample of meat was diluted with 10 ml of petroleum ether. Then there was put 2 ml of transesterification reagent (1.4 M KOH in methanol:benzene 60:40, v/v). The sample was stayed to stand for 20 minutes at room temperature in a laboratory shaker. Then the sample was filtered over anhydrous sodium sulfate and evaporated to constant volume on a rotary shaker.

Separation of Fatty Acid Methyl Esters

Esters were analysed by the chromatographic capillary column RTX 225 (inner diameter 0.53 mm, length 30 m, stationary phase thickness 1 μm) with helium which was a carrier gas (head pressure of 90 kPa at a flow rate of 18 cm³.min⁻¹). The temperature of the oven increased from the original 95 °C to 200 °C by the rate 8 °C.min⁻¹ and then it was held at this temperature for 19 minutes. Injector and detector temperatures were set at 240 °C respectively 250 °C. ChemStation software (Agilent Technologies, USA) was used for determination of fatty acid methyl esters. The peaks were identified by comparing their retention times with the retention times of a mixture of standard fatty acid methyl esters (Supelpco) and metylester arachidonic acid (SigmaAldrich). The proportion of individual fatty acids of total fatty acids were calculated by automatically integrator of the area of each peak.

Analysis of Blood Serum

Blood was obtained at slaughter of chickens. Blood serum was obtained by centrifugation of clotted blood at 3000 rpm.min⁻¹ for 30 min. Mineral profile of blood serum (chlorides – Cl, calcium – Ca, potassium – K, sodium – Na [mmol.dm⁻³]) was determined by semi-automatic analyzer EasyLyte (Medica, Bedford, USA). Biochemical indicators of blood serum (Glucose [mmol.dm⁻³], cholesterol [mmol.dm⁻³]), total proteins – CB [g.dm⁻³] and triacylglycerols [mmol.dm⁻³] were determined by semi-automatic analyzer RX Monza (Randox Laboratories Ltd., United Kingdom) using commercial kits DiaSys (Diagnostic Systems GmbH, Germany).

Statistical Analyses

The results were statistically processed by the program SAS (basic statistics, ANOVA).

RESULTS AND DISCUSSION

The basic chemical analyses are shown in Tab. I. Water content in meat from the thigh was 69.45 g.100 g⁻¹ (70.15 g.100 g⁻¹ male and female 68.59 g.100 g⁻¹) in the experimental group and 70.23 g.100 g⁻¹ (70.95 g.100 g⁻¹ male and female 69.34 g.100 g⁻¹) in the control group. There was not a significant difference between experimental group with the addition of linoleic acid (74.81 g.100 g⁻¹–74.85 g.100 g⁻¹ male and 74.76 g.100 g⁻¹ female) and the control group (74.93 g.100 g⁻¹–74.96 g.100 g⁻¹ male and 74.89 g.100 g⁻¹ female) in water content in breast meat. Breast protein content was significantly lower (P ≤ 0.05) in the experimental group (22.30 g.100 g⁻¹) in comparison with the control group (22.61 g.100 g⁻¹). Significant differences (P ≤ 0.05) were founded between sexes in the breast protein content. The breast female protein content was 22.48 g.100 g⁻¹ in experimental the female group and 22.77 g.100 g⁻¹ in the control group. The differences were not significant in protein content in breast meat between (among) the males experimental group (22.15 g.100 g⁻¹) and the control group (22.47 g.100 g⁻¹). Protein content of thigh meat was significant lower (P > 0.05) in the experimental group (17.90 g.100 g⁻¹ male and female) than in the control group (18.16 g.100 g⁻¹ male, 18.23 g.100 g⁻¹ female). There was statistically significant differences (P ≤ 0.01) between content of fat in the breast of experimental group (1.90 g.100 g⁻¹) and breast meat of the control group (1.47 g.100 g⁻¹). The higher proportion of fat in the meat from the thigh in experimental group was 11.66 g.100 g⁻¹ in comparison with the control group (10.58 g.100 g⁻¹). Crespo and Esteve-Garcia (2001) found out a slightly higher fat content in the breast and thigh muscles of chickens ROSS 308 hybrid with addition of linoleic acid in food. We found out similar results. There were higher fat content of meat from the thigh (11.66 g.100 g⁻¹) than in breast (1.90 g.100 g⁻¹). The average fat content of male breast
meat was 2.0 g.100 g⁻¹ in the experimental group and 1.56 g.100 g⁻¹ in the control group. The average fat content in the female breast was 1.77 g.100 g⁻¹ in the experimental group and 1.34 g.100 g⁻¹ in the control group. The average fat content of male thigh meat was 10.95 g.100 g⁻¹ in experimental group and 9.88 g.100 g⁻¹ in control group. The fat content in the female thigh meat in experimental group was 12.51 g.100 g⁻¹, and in control group was 11.42 g.100 g⁻¹.

Content of saturated, monounsaturated and polyunsaturated fatty acids is shown in Tab. II. Overall, the proportion of saturated fatty acids was 30.33% in the experimental group, 32.33% in the control group. Proportion of monounsaturated fatty acids was 47.06% in the experimental group and 53.77% in the control group. The proportion of monosaturated fatty acids was significantly higher (P ≤ 0.01) in the control group than in the experimental group. We acquired similar results as Smink et al. (2010). Smink et al. (2010) found out
The addition of linoleic acid in meat of broilers which were fed with linoleic acid, which reduced proportion of monounsaturated fatty acids and increased polyunsaturated acids. The content of polyunsaturated fatty acids in meat from the thigh was significantly higher in the experimental group than in the control group. Zelenka et al. (2008) found out the increases of the proportion of polyunsaturated fatty acids in the breast and thigh muscle with increasing addition of the linseed oil (1–7%).

The addition of linoleic acid in feed mixture caused an increase of PUFA in male from 13.80% (control group) to 22.34% (experimental group) and in female from 14.03% (control group) to 23.45% (experimental group). The differences between groups in both sexes were statistically significant (P ≤ 0.001).

### Table III: Biochemical indicators of blood serum of chickens fed the addition of linoleic acid to the compound feed (experimental group) or without the addition of linoleic acid to feed (control group)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sex</th>
<th>Experimental group x ± s</th>
<th>Control group x ± s</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein [g.dm⁻³]</td>
<td>♂♀</td>
<td>28.92 ± 1.92</td>
<td>31.26 ± 3.44</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>28.04 ± 1.77</td>
<td>31.14 ± 4.25</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>30.01 ± 1.57</td>
<td>31.39 ± 2.35</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose [mmol.dm⁻³]</td>
<td>♂♀</td>
<td>12.02 ± 0.61</td>
<td>12.21 ± 1.06</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>12.15 ± 0.64</td>
<td>12.46 ± 1.07</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>11.86 ± 0.57</td>
<td>11.9 ± 1.02</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol [mmol.dm⁻³]</td>
<td>♂♀</td>
<td>3.85 ± 0.37</td>
<td>4.02 ± 0.44</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>3.91 ± 0.34</td>
<td>3.7 ± 0.28</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>0.70 ± 0.11</td>
<td>0.65 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Triacylglycerols [mmol.dm⁻³]</td>
<td>♂♀</td>
<td>0.66 ± 0.08</td>
<td>0.62 ± 0.08</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>0.74 ± 0.14</td>
<td>0.68 ± 0.11</td>
<td>NS</td>
</tr>
</tbody>
</table>

P > 0.05 NS, P ≤ 0.05 *

### Table IV: Mineral profile of blood serum of chickens fed the addition of linoleic acid to the compound feed (experimental group) or without the addition of linoleic acid to feed (control group)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sex</th>
<th>Experimental group x ± s</th>
<th>Control group x ± s</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium [mmol.dm⁻³]</td>
<td>♂♀</td>
<td>2.44 ± 0.34</td>
<td>2.50 ± 0.43</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>2.57 ± 0.33</td>
<td>2.37 ± 0.35</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>2.27 ± 0.29</td>
<td>2.66 ± 0.48</td>
<td>NS</td>
</tr>
<tr>
<td>Sodium [mmol.dm⁻³]</td>
<td>♂♀</td>
<td>146.74 ± 1.68</td>
<td>147.69 ± 1.78</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>146.31 ± 1.79</td>
<td>147.72 ± 2.04</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>147.26 ± 1.47</td>
<td>147.66 ± 1.52</td>
<td>NS</td>
</tr>
<tr>
<td>Potassium [mmol.dm⁻³]</td>
<td>♂♀</td>
<td>5.29 ± 0.27</td>
<td>5.30 ± 0.43</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>5.23 ± 0.29</td>
<td>5.25 ± 0.33</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>5.36 ± 0.24</td>
<td>5.36 ± 0.54</td>
<td>NS</td>
</tr>
<tr>
<td>Chlorides [mmol.dm⁻³]</td>
<td>♂♀</td>
<td>114.04 ± 1.61</td>
<td>111.92 ± 3.05</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>113.83 ± 1.33</td>
<td>111.20 ± 3.78</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>114.30 ± 1.95</td>
<td>112.80 ± 1.63</td>
<td>NS</td>
</tr>
</tbody>
</table>

P > 0.05 NS, P ≤ 0.05 *, P ≤ 0.01 **
in control group (Tab. III), Crespo and Esteve-Garcia (2003) compared to our results found out the significant reduction (P ≤ 0.05) of cholesterol in chickens serum which were fed with the addition of sunflower oil with a high proportion of linoleic acid compared to chickens fed with no added oil.

There were not found any significant differences between sexes in the experimental group and the control group in total proteins, total lipids, glucose and cholesterol. Concentrations of calcium, sodium, potassium and chloride in the blood serum of chickens in experimental and control groups were evaluated as a part of the mineral profile (Tab. IV). There were no significant differences in concentrations of calcium, sodium and potassium between these groups of chickens. Calcium concentration ranged from 2.27 mmol.dm⁻³ in the experimental group (female) to 2.66 mmol.dm⁻³ in the control group (female). The average concentration of sodium was 146.31 mmol.dm⁻³ in the experimental group (male) and 147.72 mmol.dm⁻³ in control group (male). The concentration of potassium in the female blood serum was the same (5.36 mmol.dm⁻³) as in the experimental and the control groups. The concentration of potassium was 5.23 mmol.dm⁻³ in the male blood serum in the experimental group and 5.25 mmol.dm⁻³ in the control group. Higher concentration of chlorides (P ≤ 0.01) was detected in the serum of chickens with the addition of linoleic acid (114.04 mmol.dm⁻³) in comparison with the control group (111.92 mmol.dm⁻³).

The concentration of chloride in the blood of chickens fed with the addition of linoleic acid was higher in comparison with control group, which may be associated with a higher requirement for the concentration of HCl in the stomach with a higher intake of lipids and better management body with chloride ions. The concentration of sodium, potassium and calcium was similar in both groups. Our findings are consistent with the results Čupka et al. (2010), which showed no changes in the concentration of sodium and potassium in the blood serum of chickens.

CONCLUSION
This work evaluated the impact of addition 5% linoleic acid to feed mixture, compared with the control group without addition of linoleic acid on the chemical composition of the chickens meat and representation of fatty acids in the meat. Analysis of the chemical composition showed a higher content of fat in the breast (1.9g.100g⁻¹) and the thigh parts (11.66g.100g⁻¹) of chickens fed with the addition of linoleic acid to feed mixture. This led to a lower proportion of the other components. The addition of linoleic acid in feed mixture significantly caused an increase of PUFA in male from 13.80% (control group) to 22.34% (experimental group) and in female from 14.03% (control group) to 23.45% (experimental group). The addition of linoleic acid in chickens feed mixture influenced significantly higher proportion of polyunsaturated fatty acids to saturated fatty acids (0.76). Proportion of mono unsaturated fatty acids was 47.06% in the experimental group and it was significantly higher (53.77%) in the control group.

There was not found a positive impact of a higher proportion of polyunsaturated fatty acids in feed for chickens cholesterol (3.88 mmol.dm⁻³ in both groups) and triglycerides (0.70 mmol.dm⁻³ in the experimental group and 0.65 mmol.dm⁻³ in the control group) in blood serum of chickens fed with the addition of linoleic acid.

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