**SHORT COMMUNICATION**

**Blood lipids and fatty acid composition of abdominal fat in castrated and intact male common pheasant (Colchicus colchicus)**

Tomislav Mašek,1 Krešimir Severin,2 Josip Kos,3 Zdravko Janicki,4 Natalija Filipović, Lidija Kozačinski,5 Željka Cvrtila,6 Petar Džaja7

1Zavod za prehranu i dijketiku životinja, Sveučilište u Zagrebu, Croatia
2Zavod za sudsko i upravno veterinarstvo, Sveučilište u Zagrebu, Croatia
3Klinika za kirurgiju, ortopediju i oftalmologiju, Sveučilište u Zagrebu, Croatia
4Zavod za biologiju, patologiju i uzgoj divljači, Sveučilište u Zagrebu, Croatia
5Zavod za fiziologiju i radiobiologiju, Sveučilište u Zagrebu, Croatia
6Zavod za higijenu i tehnologiju animalnih namirnica, Sveučilište u Zagrebu, Croatia

**Introduction**

Removal of the testes and the resultant androgen deficiency reduces the male sex instincts and changes their behaviour and metabolism (Andrew and Jones, 1992). Castrated chickens and pheasants have more abdominal and subcutaneous fat but also more intramuscular fat in both the light and dark meat (Severin et al., 2007; Chen et al., 2000; Tor et al., 2002). According to Hsieh et al. (2001) lipid accumulation is attributed to increased hepatic lipogenesis capability and increased blood lipid concentrations. Several authors found that capons exhibit altered proportions of lipoproteins and thus have alterations in their lipoprotein transport mechanisms (Hsieh et al., 2001; Chen et al., 2005).

Interest in the study of fatty acids is mainly aimed at understanding their role in affecting human and animal health. Especially polyunsaturated fatty acids have been shown to be of nutritional importance for humans and animals (Horrocks and Yeo, 1999). In several animal species, it has been shown, that castration and testosterone deficiency can influence the fatty acid composition of muscle and adipose tissue (Werdi Pratiwi et al., 2006; Höberg et al., 2004; Cinci et al., 2000).

The influence of male sex hormones on lipid metabolism is well established in man and several animal species. Nevertheless, only few publications investigated that influence in pheasants (Nagra et al., 1965; Sarra et al., 1985; Severin et al., 2007). We have therefore attempted to establish the influence of castration on plasma lipids and lipoproteins and additionally on the fatty acid profile of abdominal fat in this species.

**Materials and methods**

Thirty seven-week-old pheasants were obtained from a certified commercial pheasantry. During the experiment birds were kept in an aviary and fed ad libitum standard pheasant finisher. The chemical composition, ingredients and fatty acid composition of the diet is provided in Table 1.

After an adjustment period of 7 days, half of the pheasants were castrated and the other half underwent sham surgery. Castration was performed as described by Severin et al. (2007). The absence of testicular regeneration in the castrated pheasants was determined on live birds by visual assessment and confirmed later after slaughtering. Four castrated pheasants that showed testicular regeneration were later excluded from the trial. Birds were reared until the age of 24 weeks in the nearby experimental station.

Blood samples were collected early in the morning from the brachial vein following 12 hours of feed and water withdrawal. Blood samples were taken into a sterile blood tube without additives (BD Vacutainer, Plymouth, UK) and immediately cooled and centrifuged within 1 hour after sampling. The sera were frozen at -20°C for a maximum of 30 days until they were assayed. Blood serum tryglicerides, cholesterol and glucose concentrations were assayed by an automatic analyser (SABA-18, Analysyer Medical System, Roma, Italy) by using commercial kits of reagents (Herbos Dijagnostika d.o.o., Sisak, Croatia). Lipoprotein fractions were determined by electrophoresis in tapes of gelled cellulose-acetate (Cellogel®, MALTA Chemetron, Milano, Italy). Interrelation and absolute concentration of lipoprotein fractions were determined by Global-scan densitometer (MALTA Chemetron, Milano, Italy).

From ten birds per group, abdominal fat was collected to evaluate the fatty acid composition. All the samples were stored at -40°C until the lipid analysis. The fat from the abdominal fat was directly saponified and methylated and the fatty acid composition determined by gas chromatography. Fatty acid methyl esters were methylated with NaOH in dried methanol...
(60°C, 30 min) and extracted with hexane prior to gas chromatography. All samples were analysed in duplicates. The methyl esters were separated using a 30 m × 0.25 mm SGE capillary column, connected to a Shimadzu Gas Chromatograph fitted with a flame ionization detector. High purity hydrogen was used as the carrier gas.

All statistical analyses were performed using the SAS (1991) general linear model procedure. Statistical significance of the differences was determined by the t-test.

**Results**

Table 2 presents the effects of castration on blood lipids and lipoprotein proportions. Plasma levels of triglycerides and high density lipoproteins (HDL) were significantly higher (P≤0.05) in castrated pheasants. Values for cholesterol tended to be higher in castrated pheasants. Lipoproteins (HDL) were significantly higher in castrated pheasants compared to intact pheasants. Compared to the intact pheasants, the fatty acid content of abdominal fat from the castrated pheasants contained higher values for saturated fatty acids (SFA) and lower values for unsaturated fatty acids (UFA), unsaturated to saturated fatty acids ratio (UFA/SFA) and polyunsaturated to saturated fatty acids ratio (PUFA/SFA).

| Biochemical indicators | Intact, g/L | Castrated, g/L | Significance |
|------------------------|------------|---------------|-------------|
| Total lipids           | 9.54±2.12  | 10.01±2.17    | ns          |
| Triglycerides, mmol/L  | 1.38±0.35  | 1.71±0.46     | **          |
| Cholesterol, mmol/L    | 4.12±0.70  | 4.57±0.78     | *           |

Lipoproteins

| Lipoproteins | Intact, % | Castrated, % | Significance |
|--------------|-----------|--------------|-------------|
| VLDL %       | 31.92±6.67| 27.02±4.41   | ns          |
| HDL %        | 47.25±6.31| 54.13±4.67   | **          |
| LDL %        | 20.83±5.18| 18.85±3.85   | ns          |
| Glucose, mmol/L | 22.22±9.11| 20.52±6.12   | ns          |

**Discussion**

Our trial shows that castration induces changes in the lipid metabolism. When a value differs between normal and castrated pheasants, the effect is reported to be due to testosterone. In general, serum lipid values for pheasants were similar to those reported by other authors (Mašek et al., 2001). An increase in triglyceride values

**Table 1. Analyzed fatty acid composition, ingredients and chemical composition of experimental diet.°**

| Fatty acid | % of total fatty acids | % of experimental diet |
|------------|------------------------|------------------------|
| C14:0      | 1.39±0.33              | 49.5                   |
| C16:0      | 20.30±1.92             | 15.5                   |
| C18:0      | 10.23±1.08             | 25.0                   |
| C20:0      | 0.23±0.02              | 5.0                    |
| C22:0      | 0.09±0.01              | 5.0                    |
| C24:0      | 0.03±0.01              | 5.0                    |
| C16:1      | 1.50±0.29              | 5.0                    |
| C18:1      | 32.11±0.56             | 5.0                    |
| C20:1      | 0.60±0.09              | 5.0                    |
| C18:2      | 23.34±1.27             | 5.0                    |
| C18:3      | 0.40±0.08              | 5.0                    |

**Table 3. Fatty acid profile (% of total fatty acids) of abdominal fat from castrated and intact pheasants.°**

| Saturated fatty acids | Intact, n=10 | Castrated, n=10 | Significance |
|-----------------------|--------------|-----------------|-------------|
| C12:0                 | 0.20±0.09    | 0.60±0.06       | ns          |
| C14:0                 | 0.85±0.13    | 1.10±0.48       | ns          |
| C16:0                 | 25.25±1.86   | 27.33±2.95      | ns          |
| C18:0                 | 8.85±0.81    | 9.40±1.07       | ns          |
| C20:0                 | 0.05±0.02    | 0.13±0.05       | *           |
| C22:0                 | 0.25±0.06    | 0.15±0.05       | ns          |
| C24:0                 | 0.45±0.02    | 1.03±0.23       | ns          |

| Monounsaturated fatty acids | Intact, n=10 | Castrated, n=10 | Significance |
|----------------------------|--------------|-----------------|-------------|
| C16:1                      | 6.50±0.54    | 6.88±1.28       | ns          |
| C18:1                      | 42.58±8.64   | 40.33±1.85      | **          |
| C20:1                      | 0.30±0.11    | 0.45±0.18       | ns          |

| Polyunsaturated fatty acids | Intact, n=10 | Castrated, n=10 | Significance |
|-----------------------------|--------------|-----------------|-------------|
| C18:2                       | 14.05±1.17   | 12.65±1.29      | ns          |
| C18:3                       | 0.40±0.08    | 0.35±0.10       | ns          |
| ∑SFA                       | 36.00±0.78   | 39.73±2.73      | *           |
| ∑UFA                       | 63.83±8.83   | 60.65±2.71      | **          |
| ∑MUFA                     | 49.36±0.75   | 47.65±3.17      | ns          |
| ∑PUFA                     | 14.45±1.17   | 13.00±1.37      | ns          |
| ∑UFASFA                    | 1.77±0.06    | 1.54±0.17       | **          |
| ∑PUFASFA                   | 0.40±0.04    | 0.33±0.04       | ns          |

°Values represent means±standard deviation; ns, not significant; *P≤0.05; **P≤0.01.
after castration was also observed by Hsieh et al. (2001) in meat type chicken. Chen et al. (2005) found no significant increase in serum triglyceride values after castration, although triglyceride values were 19% higher in castrated chicken. In other animals, like rats, triglyceride values were not influenced by castration (Patsch et al., 1980; Haug et al., 1984) or were even decreased (Takeuchi et al., 1986). Similar to our results serum cholesterol values were not significantly different in castrated chicken (Chen et al., 2005) nevertheless, serum cholesterol values in our trial tended to be higher in castrated pheasants. On the contrary, serum cholesterol values significantly increased after castration in rats (Takeuchi et al., 1986; Haug et al., 1984). Although there is an obvious influence of castration on serum triglyceride and cholesterol values, the described differences between our trial and data from literature are probably due to the use of different species and because the pheasants in our trial were fattened for a longer period after castration.

In our investigation, castration and the resultant elimination of the male sex hormones had a significant effect on lipoprotein profile. The effects of sex hormones on lipoproteins have been extensively investigated in humans because men have a greater risk of coronary heart disease than women, and this sex differential has been attributed partly to sex differences in the circulating lipoprotein levels (Godsland et al., 1987). The HDL-cholesterol lowering effect of testosterone is indicated by the decrease of HDL-cholesterol in male puberty and in hypogonadal men who substitute testosterone, as well as by the rise of HDL-cholesterol in individuals with suppressed testosterone (Eckardstein, 1998). The effect of testosterone on lipid metabolism has also been observed in animals. Similar to our results, Takeuchi et al. (1986) found a significant increase in serum HDL in castrated rats. These changes in serum lipids and lipoproteins could be reversed by the administration of testosterone (Takeuchi et al., 1986; Haug et al., 1984). A similar increase in HDL was also observed in castrated chicken by Chen et al. (2005), but they also found higher LDL. Chen et al. (2005) noticed that castration increases the protein percentage and decreases the free fatty acid composition in those fed linseed oil, (Ferrini et al., 2008). Compared to the results of Ferrini et al. (2008) in chicken broilers, pheasants had lower UF/SA ratio. Castration caused significant changes in the abdominal fatty acid profile in pheasants. According to Cinci et al. (2000) sex hormones have important effect on fatty acid metabolism in rats by influence on Δ 9 and Δ 6 desaturase and Δ 5 desaturase-elongase. The results of fatty acid composition of abdominal fat in castrated pheasants, in the present study, appear to be different from the results reported for other castrated animals (Werdi Pratiwi et al., 2006; Högberg et al., 2004), nevertheless, the results between trials are highly variable and therefore difficult to compare. Högberg et al. (2004) stated that differences in fatty acid composition between sexes can be considered as negligible and that the main influence on fatty acid composition is still the dietary fatty acid composition fed to the animals.

Conclusions

We may conclude that castration has important effects on the triglyceride and lipoprotein values in male common pheasants. The fatty acid composition of the abdominal fat from pheasants was only slightly modified by castration and the implications of observed differences for human nutrition can be considereed as negligible. The main influence on fatty acid composition will still be the dietary fatty acid composition fed to the animals. Therefore future studies should primarily include dietary manipulations of fatty acid profile in pheasants.

References

Andrew, R.J., Jones, R.B., 1992. Increased distractibility in capons: an adult parallel to androgen- induced effects in the domestic chick. Behav. Process. 26:201-210.

Chen, K.L., Chi, W.T., Chiou, P.W.S., 2001. Fatty acid composition of phospholipids, triglycerides and cholesterol in serum of castrated and estradiol treated rats. Life Sci. 66:1647-1654.

Crespo, N., Esteve-Garcia, E., 2001. Dietary fatty acid profile modifies abdominal fat deposition in broiler chickens. Poultry Sci. 80:71-78.

Eckardstein, A., 1998. Androgens, cardiovascular risk factors and atherosclerosis. In: E. Nieschlag and H.M. Behre (eds.) Testosterone: action, deficiency, substitution. 2nd ed. Springer-Verlag, Berlin, Germany, pp 229-258.

Ferrini, G., Baucells, M.D., Esteve-Garcia, E., Barroeta, A.C., 2008. Dietary polyunsaturated fat reduces skin fat as well as abdominal fat in broiler chickens. Poultry Sci. 87:528-535.

Godsland, I.F., Wynn, V., Crook, D., Miller, N.E., 1997. Sex, plasma lipoproteins, and atherosclerosis: prevailing assumptions and outstanding questions. Am. Heart J. 114:1467-1503.

Haug, A., Hostmark, A.T., Spydevold, O., 1984. Plasma lipoprotein responses to castration and androgen substitution in rats. Metabolism 33:465-470.

Högberg, A., Pickova, J., Stern, S., Lundström, K., Bylund, A.C., 2004. Fatty acid composition and tocopherol concentrations in muscle of entire male, castrated male and female pigs, reared in an indoor or outdoor housing system. Meat Sci. 68:659-665.

Horrocks, L.A., Yeo, Y.K., 1999. Health benefits of docosahexaenoic acid (DHA). Pharma-col. Res. 40:211-225.

Hsieh, C.Y., Chen, K.L., Chiou, P.W.S., 2001. The lipoprotein composition and structure of capon and incomplete caponize in Taiwan country chicken. J. Chin. Soc. Anim. Sci. 30:229 (abstr.).

Mašek, T., Severin, K., Horvátek, D., Janicki, Z., Konjević, D., Slavica, A., Mikuleč, Ž., 2007. Serum parameters of intensively reared common pheasant (Phasianus colchicus) during fattening. Arch. Geflügelkd. 71:135-138.

Nagra, C.L., Breitenbach, R.P., Meyer, R.K., 1965. Relation of castration to fat stores in male pheasants. Ecology 46:571-575.

Patsch, W., Kim, K., Wiest, W., Schonfeld, G., 1980. Effects of Sex Hormones on Rat Lipoproteins. Endocrinology 107:1085-1094.

Sarra, C., Boccignone, M., Damasio, L., 1985.
The effect of age, sex, and anatomical location on the fatty-acid composition of pheasant meat. Poultry Sci. 64:1090-1097.

SAS, 1991. User’s Guide Statistics. Version 6. SAS Institute, Inc., Cary, NC, USA.

Severin, K., Mašek, T., Horvatek, D., Konjević, D., Janicki, Z., Cvrtila, Ž., Kozačinski, L., Hadžiosmanović, M., Barić-Rafaj, R., 2007. The effects of castration on the growth parameters, carcass yield and meat chemical composition of intensively reared Common Pheasant (Phasianus colchicus colchicus L.). Ital. J. Anim. Sci. 6:213-219.

Smink, W., Gerrits, W.J.J., Hovenier, R., Geelen, M.J.H., Lobee, H.W.J., Verstegen, M.W.A., Beynen, A.C., 2008. Fatty acid digestion and deposition in broiler chickens fed diets containing either native or randomized palm oil. Poultry Sci. 87:506-513.

Strakova, E., Vitula, F., Suchy, P., Vecerek, V., Skaloud, J., 2001. Cholesterol concentration in yolks and blood plasma in five species of game birds. Arch. Tierzucht. 44:339-343.

Takeuchi, N., Go, S., Murase, M., Nomura, Y., Takase, H., Uchida, K, 1986. Effects of castration and testosterone administration on serum lipoproteins and their apoproteins in male spontaneously hypertensive rat. Endocrinology 118:1787-1794.

Tor, M., Estany, J., Villalba, D., Molina, E., Cubilo, D., 2002. Comparison of carcass composition by parts and tissues between cocks and capons. Anim. Res. 51:421-431.

Werdi Pratiwi, N.M., Murray, P.J., Taylor D.G., Zhang, D., 2006. Comparison of breed, slaughter weight and castration on fatty acid profiles in the longissimus thoracic muscle from male Boer and Australian feral goats. Small Ruminant Res. 6:94-100.