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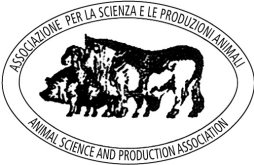
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Effects of selection for yolk cholesterol on growth and meat quality in Japanese quail (*Coturnix coturnix japonica*)

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ABSTRACT

Descendants (♀♀) of S18 generation of Japanese quails (*Coturnix coturnix japonica*), selected for low (line 11, n=15) or for high (line 12, n=6) yolk cholesterol content were used for this experiment. The high (12) and low (11) lines were developed from a control egg type (line13) wild color plumage stock 07, from Poultry Breeding Station in Ivanka at Danube (Slovakia) that was maintained without conscious selection. The results of selected lines were compared to this one from unselected lines, egg type (line 13, n=15) and meat type (line 8, n=15), to study productive performance (growth and carcass weight) as well as meat quality traits (intramuscular collagen properties, fatty and cholesterol content). The birds were grown up to 35 days of age. Quails were fed *ad libitum* with commercial diets according to their ages. Animals had free access to water during the experiment. The body weight of the quails was controlled every week (from week 1 to 5). At the slaughter (at avg. weight of 104.0, 107.7, 110.6 g vs 209.3 g for lines 11, 12, 13 vs 8, respectively; $P<0.05$) the hot carcass weight was recorded and dressing percentage was calculated. *Pectoralis superficialis* muscles were removed from the carcasses to assess intramuscular collagen (IMC) properties (collagen and crosslink concentrations), intramuscular lipid and cholesterol contents. Growth and slaughter performance, intramuscular fat percentage and IMC properties of *Pectoralis superficialis* were not different between the birds selected for low (line 11) and high (line 12) yolk cholesterol content and were also found to be similar between the quails of egg type and those selected for yolk cholesterol content (lines 11 and 12). Compared to the birds of egg type line and those selected for yolk cholesterol content, the quails of meat type had higher growth and carcass traits ($P<0.05$), while meat quality traits were found to be similar. The meat of the quails selected for low cholesterol in the egg yolk (line 11) had lower ($P<0.05$) cholesterol (-35.9%) than that of the birds characterized by high cholesterol in the egg yolk (line 12). In conclusion, divergent selection for yolk cholesterol content did not significantly influence growth and slaughter traits, intramuscular fat and IMC properties of Japanese quail, while it affected the amount of cholesterol in meat. The quails of meat type showed the highest growth and slaughter traits.

Key words: Japanese quail, Genetic selection, Growth, Meat quality.

RIASSUNTO

EFFETTO DELLA SELEZIONE PER IL CONTENUTO DI COLESTEROLO NEL TUORLO D'UOVO SULL'ACCRESIMENTO E QUALITÀ DELLA CARNE DI QUAGLIE GIAPPONESI (*COTURNIX COTURNIX JAPONICA*)

Questo studio ha inteso valutare le performance produttive e la qualità della carne (proprietà del collagene intramuscolare, contenuto di grasso e colesterolo) di quaglie Giapponesi (*Coturnix coturnix japonica*) selezionate da quaglie tipo uovo (linea 13, con piumaggio di colore naturale, stock 07) presso la Poultry Breeding Station in Ivanka at Danube (Slovacchia), per 18 generazioni (♀♀), per basso (linea 11, n=15) o alto (linea 12, n=6) contenuto di colesterolo del tuorlo d'uovo. Le quaglie tipo uovo (linea 13, n=15), mantenute in purezza, sono state impiegate come controllo insieme alle quaglie tipo carne (linea 8, n=15). Gli uccelli sono stati allevati a terra in gabbie collettive sino al 35° giorno d'età ed alimentati ad libitum con diete commerciali, somministrate in funzione dell'età. Durante la sperimentazione, gli animali hanno avuto libero accesso all'acqua di bevanda. Ogni settimana (dalla 1ª alla 5ª settimana di vita) è stato controllato il peso corporeo. Alla macellazione (Peso vivo medio: 104,0-107,7 e 110,6 vs 209,3 g per le linee 11, 12 e 13 vs 8, rispettivamente; $P < 0,05$) è stato rilevato il peso della carcassa a caldo e calcolata la resa. Dalle carcasse è stato rimosso il muscolo *Pectoralis superficialis* per determinare le proprietà del collagene (quantità totale e legami crociati) ed il contenuto di grasso e colesterolo. Le performance di crescita e di macellazione non furono significativamente diverse tra le linee 11 e 12, selezionate per basso e alto contenuto di colesterolo del tuorlo, e tra le quaglie della linea uovo e quelle selezionate per il contenuto di colesterolo (linea 11 e 12). La linea carne ha mostrato migliori accrescimenti e performance di macellazione più elevate sia rispetto alla linea uovo che alle linee selezionate per alto e basso contenuto di colesterolo nel tuorlo ($P < 0,05$). La carne delle quaglie selezionate per basso contenuto di colesterolo ha evidenziato una minore ($P < 0,05$) quantità di colesterolo (-35,9%) rispetto a quella degli animali della linea 12, caratterizzata da un più alto contenuto di colesterolo. Non sono emerse differenze apprezzabili tra i gruppi messi a confronto per la percentuale di grasso intramuscolare, nonché per la sintesi del collagene intramuscolare, la sua maturità e la concentrazione dei legami crociati di idrossilisipiridinolina. In conclusione, la selezione divergente per il contenuto di colesterolo del tuorlo d'uovo non ha significativamente influenzato l'accrescimento, le performance di macellazione, il grasso intramuscolare e le proprietà del collagene intramuscolare di quaglie Giapponesi, mentre ha avuto un effetto significativo sul contenuto di colesterolo nella carne. Le quaglie della linea carne hanno mostrato le migliori performance.

Parole chiave: Quaglia giapponese, Selezione genetica, Accrescimento, Qualità carne.

Introduction

Cholesterol has a large number of functions important for cell viability and steroid biology, and as a component of yolk lipids, represents the primary nutrient source for the chick embryo (Speake *et al.*, 1998). Egg yolk is also a rich source of cholesterol in the human diet. However, the high consumption of it may increase the risk of coronary heart disease (Weggemans *et al.*, 2001). Several studies have been conducted to investigate the effects of nutritional manipulation,

housing and breeding on the egg yolk components, including the cholesterol contents (for review see Hartmann and Wilhelmson, 2001).

Line and breed differences in yolk cholesterol have been shown in a number of studies on different avian species: chickens (Sainz *et al.*, 1983; Campo, 1995), Japanese quail (Baumgartner and Simeonovová, 1992; Baumgartner *et al.*, 2008), geese (Speake *et al.*, 1999), ducks (Speake *et al.*, 2002) and pheasant (Speake *et al.*, 1999) and support a genetic basis for yolk cholesterol content.

The variation among genetically different populations for egg yolk cholesterol concentration was important, and range, for example from 1620-1831 mg/100 g yolk to 2046-2052 mg/100 g yolk, for light and high body weight lines of Japanese quail, respectively (Baumgartner and Simeonovová, 1992). However, only a few reports have studied the effect of selection on cholesterol concentration. Marks and Washburn (1977) suggested that selection for yolk cholesterol is effective only in the upward direction. Ansah *et al.* (1985) found a significant decrease in egg weight in selected generations of Leghorn-type chickens in the low yolk cholesterol line in comparison with unselected control but no changes in yolk weight. The basic results of long term selection for low yolk cholesterol content and its influence on development and relationship with other egg quality traits during nineteen selected generations were presented by Baumgartner *et al.* (2008). The authors concluded that selection on low yolk cholesterol content is associated with correlated response in other egg quality traits. There were, among others, highly significant positive correlations between cholesterol content in yolk and cholesterol content in the edible part of egg, and significant negative correlations between yolk cholesterol content and egg weight, yolk weight and weight of the edible part of egg. Washburn and Marks (1977) studied the correlated changes in egg production, hatchability, egg weight, age at sexual maturation and body weight of lines selected for divergence in yolk cholesterol content in two different chicken populations (meat type and egg type). Body weights of the high and low yolk cholesterol lines of the meat type, but not of egg type populations, were significantly different, as a result of selection.

According to available information, no research work has been conducted yet to stu-

dy the effect of divergent selection on yolk cholesterol content on the growth and meat quality traits. This study was aimed to determine the consequences of Japanese quail selection for a low or a high yolk cholesterol content on body and carcass weight, and on indicators of some meat quality traits (intramuscular collagen properties, fatty and cholesterol content). The selected lines were compared to a control line, an unselected line from the same egg type population, and to a line of meat type quails.

Material and methods

Animals

Animal handling followed the recommendations of European Union directive 86/609/EEC concerning animal care.

Descendants ($\sigma\sigma$) of S18 generation of Japanese quails (*Coturnix coturnix japonica*), selected for low (line 11, n=15) or for high (line 12, n=6) yolk cholesterol content were used for this experiment. The high (12) and low (11) lines were developed from a control egg type (line 13) wild colour plumage stock 07, from Poultry Breeding Station in Ivanka at Danube (Slovakia) that was maintained without conscious selection. In this experiment the results of selected lines were compared to the one from unselected lines: egg type (line 13, n=15), and meat type (line 8, n=15). The lowering of yolk cholesterol content in line 11 was 313 mg/100 g yolk, and the increase in yolk cholesterol content in line 12 was 116 mg/100 g yolk, i.e. -17.25 %, and +6.39% in comparison to parental line 13, respectively (Maciuszonek *et al.*, 2006).

The quail-chicks were wing-banded and placed in the conventional poultry house of the Experimental Station at Gorzryn of the University of Life Sciences in Poznan. The birds were grown on a deep litter floor up to 35 days of age in collective cages under continuous lighting. Quails were fed *ad libitum*

with commercial diets (Table 1) according to the age. Animals had free access to water during the experiment. To calculate average weekly weight gain, quails were individually weighed (in the morning after an overnight fast) every week (from 1 to 5).

At the end of the experiment at 35 day of age, all birds were slaughtered (after a fasting period of 12h) and hot carcass wei-

ght was recorded and dressing percentage was calculated. The left *Pectoralis superficialis* muscle was removed, frozen in liquid nitrogen and stored for the intramuscular lipids content analyses. The right *Pectoralis superficialis* muscle was removed after 24h at 2 to 4°C, vacuum packaged, and stored frozen (-40°C) until intramuscular collagen (IMC) and cholesterol analyses.

Table 1. Composition and nutritional value of the diets.

| | Period | | | |
|-------------------------------|---------|----------|-----------|--------|
| | 1-7 day | 8-28 day | 29-35 day | |
| Components (%): | | | | |
| Maize | 37.0 | 54.2 | 61.6 | |
| Soybean meal | 41.5 | 31.9 | 16.8 | |
| Rapeseed meal | - | - | 8.0 | |
| Fish meal | 10.0 | 8.0 | 5.0 | |
| Milk powder, skimmed | 3.0 | 2.0 | 2.0 | |
| Alfalfa dehydrated | - | - | 4.0 | |
| Rapeseed oil | 6.2 | 1.8 | 0.3 | |
| Calcium phosphorus | 0.5 | 0.4 | 0.8 | |
| L-lysine | - | - | 0.2 | |
| DL-methionine | 0.5 | 0.4 | - | |
| NaCl | 0.3 | 0.3 | 0.3 | |
| Premix IB-1-2 | 1.0 | 1.0 | - | |
| Premix IB-3 | - | - | 1.0 | |
| Calculated nutritional value: | | | | |
| Metabolisable energy | kcal/kg | 3001.7 | 2898.3 | 2797.9 |
| Metabolisable energy | MJ/kg | 12.57 | 12.14 | 11.72 |
| Crude protein | % | 28.0 | 24.0 | 20.0 |
| Crude fibre | " | 3.84 | 3.70 | 4.76 |
| Lysine | " | 1.48 | 1.21 | 1.00 |
| Methionine | " | 0.60 | 0.53 | 0.43 |
| Ca | " | 1.33 | 1.13 | 1.04 |
| Available P | " | 0.56 | 0.46 | 0.41 |
| Na | " | 0.27 | 0.24 | 0.21 |

Measurement of intramuscular fat

The intramuscular fatty tissue content in *Pectoralis superficialis* was determined by a histochemical method. The samples were cut in cryostat into 10 µm thick sections and stained by oil red method (Dubowitz and Brooke, 1973) to differentiate intramuscular fatty tissue content. MultiScan v.14.02 Image Analysis System was used to estimate the fatty tissue content. For each sample twenty-five successive fields, from an area of 2.68 mm², were analysed in order to define the percentage of fatty tissue in the muscles.

Measurement of muscle cholesterol

Cholesterol was extracted using the method of Maraschiello *et al.* (1996) and then quantified by HPLC. A Kontron HPLC (Kontron Instruments, Milan, Italy) model 535, equipped with a C18 reverse-phase column (250x4.6 mm x5 µm) (Phenomenex, Torrance, CA), was used. The mobile phase was acetonitrile/2-propanol (55: 45 v/v) at a flow rate of 1.2 mL/min. The detection wavelength was 210 nm and retention time was 13.89 min.

Collagen analysis

Muscle samples were thawed and lyophilized for 48h, weighed, and hydrolyzed in Duran tubes in 6 N HCl at 110°C for 18 to 20h (Etherington and Sims, 1981) for determination of hydroxyproline (Woessner, 1961) and crosslinking. All analyses were carried out in duplicate. IMC concentration was calculated, assuming that collagen weighed 7.25 times the measured hydroxyproline weight (Eastoe and Leach, 1958) and expressed as µg hydroxyproline/mg of lyophilized tissue. The concentration of hydroxylysylpyridinoline (HLP), the principal non-reducible crosslink of muscle collagen (McCormick, 1999), was determined using a modification (Maiorano *et al.*, 1999) of the HPLC procedure developed by Eyre *et*

al. (1984); it was expressed as moles of HLP per mole of collagen and also as µg HLP/mg of lyophilized tissue.

Statistical analyses

Data were evaluated by ANOVA using GLM procedure (SPSS, 2006). Differences among groups were determined by contrasts.

Results and discussion

Growth and slaughter traits are shown in Table 2. Neither quail growth nor slaughter performance was significantly affected by divergent selection for yolk cholesterol content. Growth and slaughter performance were also found to be similar between the quails of egg type and those selected for yolk cholesterol content (lines 11 and 12). As expected, the quails of meat type had higher ($P<0.05$) growth, slaughter weight, carcass weight, and dressing percentage than those of the egg type and selected for yolk cholesterol content (lines 11 and 12). Washburn and Marks (1977) reported that the meat-type of the Athens-Canadian population selected for low or high yolk cholesterol showed, after four generation, significant differences in growth performance between the two selected lines. In fact, the line selected for low yolk cholesterol weighed 80% of the line selected for high yolk cholesterol levels. However, this effect was reported only in the meat type chickens, but not in the egg type populations. Our selected lines 11 and 12 confirm the observation of Washburn and Marks (1977) that there is no effect of yolk cholesterol selection on the body weight in egg type lines. Our findings on the comparison of growth performance between line 13 and line 8 are comparable to the results of Boon *et al.* (2000) and Caron *et al.* (1990). Boon and co-workers reported significant differences in body weight gain between fast growing Japanese quails bred for meat

Table 2. Least square means and standard error for growth and slaughter performances.

| Group | | Line 11 | Line 12 | Line 13 | Line 8 |
|--------------------|---|--------------------------|--------------------------|--------------------------|--------------------------|
| AWG (7-14 d) | g | 19.7 ± 2.1 ^a | 20.8 ± 2.5 ^a | 23.5 ± 2.2 ^a | 48.1 ± 2.6 ^b |
| AWG (14-21 d) | " | 30.8 ± 2.4 ^a | 28.2 ± 3.2 ^a | 27.8 ± 2.9 ^a | 51.9 ± 4.4 ^b |
| AWG (21-28 d) | " | 24.0 ± 2.0 ^a | 20.5 ± 4.7 ^a | 26.0 ± 2.3 ^a | 44.6 ± 4.7 ^b |
| AWG (28-35 d) | " | 10.7 ± 2.6 ^a | 19.0 ± 2.0 ^a | 14.1 ± 3.1 ^a | 33.1 ± 5.8 |
| AWG (trial) | " | 84.7 ± 2.3 ^a | 88.5 ± 1.2 ^a | 91.3 ± 1.9 ^a | 177.7 ± 3.0 ^b |
| Slaughter weight | " | 104.0 ± 2.2 ^a | 107.7 ± 1.4 ^a | 110.6 ± 2.0 ^a | 209.3 ± 3.0 ^b |
| Hot carcass weight | " | 60.3 ± 1.7 ^a | 62.0 ± 1.1 ^a | 64.7 ± 1.3 ^a | 129.1 ± 2.9 ^b |
| Dressing | % | 58.3 ± 2.0 ^a | 57.7 ± 1.5 ^a | 58.7 ± 1.3 ^a | 61.8 ± 1.5 ^b |

AWG: Average weekly gain.

^{a, b}Means in the same row with different superscripts differ ($P < 0.05$).

production (broilers) and normal growing ones bred for egg production (layers): broilers grew faster than layers. Caron and co-workers found a lighter increase in carcass yield with an increase in mean body weight of Japanese quails.

Results on intramuscular fat and cholesterol for *Pectoralis superficialis* muscle are presented in Table 3. Intramuscular fat percentage did not differ significantly among experimental groups. Comparable intramuscular fat percentage values were found in breast muscle of Pharaoh female (ranging from 1.47 to 2.15%) and male (ranging from 2.12 to 3.32%) quails slaughtered at 6 weeks (Gardzielewska *et al.*, 2005).

Cholesterol muscle content did not significantly differ among the birds of line 8 and line 13, and those lines *versus* line 11 and line 12. The meat of the quails selected for low cholesterol in the egg yolk (line 11) had lower ($P < 0.05$) cholesterol (-35.9%) than that of the birds characterized by high cholesterol in the egg yolk (line 12). Harris *et al.* (2003) reported no differences in cholesterol accretion for muscle or adipose tissue when pigs selected for high or low serum cholesterol

were fed either high fat, high-cholesterol or low-fat, low-cholesterol diets. We were not able to find information in literature on the cholesterol content of the meat from quails but the values detected in the present study for the all the studied samples were quite low and ranged from 27.83 to 43.38 mg/100 g.

Meat and meat products are considerable sources of cholesterol in the diet (Valsta *et al.*, 2005). The cholesterol is a potential cause of cardiovascular disease, particularly associated with high dietary levels of fat (Werdi Pratiwi *et al.*, 2006). The amount of cholesterol in meat and meat products depends on numerous factors, but in general it is less than 75 mg/100 g, except in the case of some edible offal (heart, kidney, brains, etc.) where the concentrations are much higher (Chizzolini *et al.*, 1999; National Public Health Institute, 2001). Avian meat is one of the main meat products consumed by humans. It is low in cholesterol (broiler *Pectoralis*, 47.41 mg/100 g muscle, see Chizzolini *et al.*, 1999) and thus is considered to be healthier than other meat products, especially the red meat of mammalian origin, i.e. beef 66 mg/100 g, pork 65 mg/100 g and

Table 3. Least square means and standard error for chemical composition (fresh basis), and intramuscular collagen properties from *Pectoralis superficialis* muscle.

| Group | | Line 11 | Line 12 | Line 13 | Line 8 |
|-------------------|----------------|---------------------------|---------------------------|----------------------------|----------------------------|
| Intramuscular fat | % | 2.00 ± 0.32 | 1.76 ± 0.27 | 2.54 ± 0.40 | 1.48 ± 0.26 |
| Cholesterol | mg/100g | 27.83 ± 2.18 ^a | 43.38 ± 9.84 ^b | 36.72 ± 5.01 ^{ab} | 33.27 ± 3.53 ^{ab} |
| IMC | µg/mg* | 17.91 ± 0.81 | 17.67 ± 0.61 | 17.25 ± 0.78 | 18.90 ± 0.56 |
| HLP | " | 3.74 ± 0.77 | 4.02 ± 0.28 | 4.12 ± 0.67 | 4.18 ± 0.69 |
| HLP | mol/mol of IMC | 0.141 ± 0.021 | 0.159 ± 0.002 | 0.175 ± 0.040 | 0.154 ± 0.026 |

IMC: Intramuscular collagen; HLP: Hydroxylysylpyridinoline.

*of lyophilized muscular tissue.

^{a, b}Means in the same row with different superscripts differ ($P < 0.05$).

lamb 50 mg/100 (for a review, see Chizzolini *et al.*, 1999). However, the cholesterol content of *Pectoralis superficialis* muscle in our quails is similar to the cholesterol content in breast chicken muscle found by Komprda *et al.* (1999) in 42-day-old hybrid ROSS 208 and by Al-Najdawi and Abdullah (2002) in the handboned meat of "spent" Leghorn chickens.

IMC properties for *Pectoralis superficialis* muscle are presented in Table 3. Collagen and muscle HLP concentrations (µg/mg) and the degree of collagen maturation, expressed as HLP crosslink (mol HLP/mol collagen), did not differ significantly among experimental groups. IMC and intramuscular fat are important parameters to the meat industry and may impact meat toughness and meat quality (Ouali, 1992; Karunaratne *et al.*, 2005) of different domestic animals including birds (Baeza *et al.*, 1998). Although connective tissue is a major determinant of meat texture and tenderness (Bosselmann *et al.*, 1995; McCormick, 1999), there are no studies on IMC in quails. In addition, experiments involving divergent selection effect of domestic birds on intramuscular collagen properties have not previously been studied. Our findings contrast with a number of re-

ports on the effect of genotype and growth rate in lambs (Heinze *et al.*, 1986; Filetti *et al.*, 2002; Martínez-Cerezo *et al.*, 2005), beef (Boccard *et al.*, 1979; Campo *et al.*, 2000), pigs (Lebret *et al.*, 2001; Maiorano *et al.*, 2003) and rabbits (Pascual and Pla, 2008) on collagen properties. The literature documents collagen growth rate-dependent shifts in muscle collagen amount and/or crosslinking (Aberle *et al.*, 1981; Harper, 1999; Maiorano *et al.*, 2001, 2007). During rapid growth, newly synthesized collagen dilutes older collagen and is less crosslinked than the pre-existing collagen (Etherington, 1987), with a positive effect on meat tenderness (McCormick, 1994; Bosselmann *et al.*, 1995). In fact, the crosslinks are largely responsible for the tensile strength attributed to collagen (Velleman, 2002). However, the role of collagen on meat tenderness not only depends on the crosslinks but also on amount of collagen. McCormick (1999) suggests that mature crosslinks and collagen concentration have an additive effect on the toughening of meat. The collagen content in *Pectoralis superficialis* muscle in quail is lower (-30%) than the values observed in pectoral muscle in 6-week-old control fowl (Leghorn chicken) by Velleman *et al.* (1996). Moreo-

ver, of particular interest in the present study are the overall low values for HLP in the *Pectoralis superficialis* muscle; these values, ranging from 0.141 to 0.175 mol HLP/mol collagen, are nearly three-fold less than those observed in pectoral muscle of 6-week-old control fowl (Leghorn chicken) (Velleman *et al.*, 1996). It is known that collagen synthesis and maturation differ between species, muscles, animal age, growth rate and management practices (McCormick, 1994; Bosselmann *et al.*, 1995; Purslow, 2005).

Conclusions

The performed study showed that divergent selection for yolk cholesterol content did not significantly influence growth and slaughter traits, and intramuscular fat and IMC properties of Japanese quail, while it had greater impact on the amount of cholesterol in meat; in fact, the meat of the quails

selected for low cholesterol in the egg yolk contained lower cholesterol (-35.9%) than that of the birds characterized by high cholesterol in the egg yolk. Therefore, the quails selected for low yolk cholesterol content could be proposed in order to reduce cholesterol in the meat, with advantages from the human nutritional point of view. The quails of meat type had the highest growth and slaughter performance. Growth and slaughter performance, as well as meat quality were found to be similar between the quails of egg type and those selected for yolk cholesterol content. Further research is warranted to increase knowledge regarding the effect of divergent selection on performance and meat quality of quail.

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