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Polymorphism of prolactin gene and its association with growth and some biometrical traits in ducks

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ABSTRACT

The main aim of the study was to estimate the polymorphism of prolactin (PRL) gene and its relation with some morphological traits (body weight – BW, length of trunk with neck – LTN, length of trunk – LT, chest girth – CG, length of breast bone – LBB, length of shank – LS) of Muscovy, Pekin and Mulard ducks. A secondary objective of this study was to evaluate the effect of age, origin on ducks’ growth performances. Three genotypes at locus PRL/XbaI and one genotype at locus PRL/PstI were found. The results showed that Pekin ducks with the PRL/TT genotype in selected terms of evaluation characterised by higher ($p<0.05$) LS and LBB values than those with the PRL/TG genotype. In Mulard ducks, PRL/XbaI polymorphism had an effect ($p<0.05$) on BW and LS in birds aged 10 and 12 weeks (wk). The effect of age, origin on the growth traits of ducks was evaluated as well. All growth traits examined significantly increased with age. There was a significant ($p<0.01$) effect of the ducks’ origin. Until the 7 wk of age, the Muscovies were lighter and had lower ($p<0.01$) values of LTN, LT, CG, LBB, LS than Pekin and Mulard ducks. The results confirm that there were significant associations between interaction of considered factors and estimated traits.

Introduction

Duck production for meat products is a growing food industry on a global scale. Between 2000 and 2013, in terms of the numbers of ducks slaughtered worldwide, the total went up from 1969 million to 2886 million (ThePoultrySite 2015). In Europe, France is by far the largest producer of duck meat, accounting for more than half of the total EU production (0.5 million tonnes) (Eurostat 2015). The main species used to produce duck meat are Pekin duck (\textit{Anas platyrhynchos domestica}), Muscovy duck (\textit{Cairina Moschata}) and cross-breeds of Muscovy drakes with Pekin ducks (called Mulards) (Baéza 2006). It has been reported that the modern domestic White Pekin duck perform better than the modern broiler chicken in terms of weight gain and feed efficiency to the same live weight due to genetic improvement (Adzitey & Adzitey 2011). In Europe, Pekin ducks are often substituted for Muscovy ducks, very popular in farms due to very good adaptation to rearing conditions. Muscovy ducks are characterised by lower fatness and higher meatiness than Pekin ducks. While, hybrids of Muscovy and Pekin ducks, so-called Mulards, are distinguished by good meatiness and low fatness of carcasses (Wawro et al. 2004).

Across vertebrate species, body size is correlated with physiological and life history characteristics, such as metabolic rate, age of maturity and longevity (Stearns 1984; Gaillard et al. 1989; Speakman 2005). Moreover, the biometric traits are reliable predictors of carcass composition (Assan 2013). Body dimensions showed a higher positive and significant correlation with carcass components in guinea fowl (Ogah 2011), similar findings were reported in Muscovy ducks (Wilkiewicz-Wawro & Szypulewska 1999) and in broiler chickens (Kleczek et al. 2006). Available literature (Zerehdaran et al. 2004) has shown that some single nucleotide polymorphisms (SNP) of genes indeed affect growth traits and body measurements...
significantly. On the other hand, there is a necessity to search other genetic markers potentially useful in selection for growth traits. Among genes which can be examined in order to evaluate the relation with growth traits is prolactin gene (PRL). The expressed product of this gene is the prolactin hormone, which is a multifunctional protein hormone involved in the control of a wide variety of physiological processes in vertebrates, including osmoregulation, reproduction, immune responses (Clapp et al. 1994), water and electrolyte balance, endocrinology (Bole-Feytrot et al. 1998). In avian species, prolactin is a crucial hormone in induction and maintenance of incubation behaviour and regulation of the follicular development (Wang et al. 2011). Furthermore, it has been observed that the prolactin hormone is involved in the formation of the egg (Hazelwood 1983). However, this ‘hormone of maternity’ has a diverse repertoire of functions not only in reproductive traits of birds. A large number of the reported effects of prolactin are related with growth and development. Many of these are seen in lower vertebrates, but more recent data confirm that cellular proliferation is also one of the important functions of prolactin in mammals (Bole-Feytrot et al. 1998).

Meanwhile, PRL gene is one of the growth hormone gene family and is synthesised mainly in the anterior pituitary of all vertebrates (Wang et al. 2011). The PRL gene has been cloned already in a variety of avian species (e.g. chicken, turkey, quail, duck, pigeon) (Liu et al. 2008). The duck PRL gene is 10 kb in size and consists of 5 exons and 4 introns, encoding 229 amino acids. Duck PRL was found to have 92.0%, 91.7% and 91.4% sequence identity at the cDNA level compared to PRL of chicken, turkey and quail, respectively. The mature duck PRL has an overall similarity with a comparable region of chicken (95.5%), turkey (92.5%) and quail (95.5%) PRL (Kansaku et al. 2005). Moreover, since the avian PRL gene was cloned and sequenced, most of the research concentrates on identifying new polymorphic sites in this gene. So far, the results of study carried out on chicken showed significant association between SNP in exon 5 and egg number and between SNP in exon 2 and body weight (BW) at hatch and age of sexual maturity (Rashidi et al. 2012).

The main objective of our study was detection of PRL/XbaI and PRL/PstI gene polymorphism, evaluation of the impact of these polymorphic sites on growth and some body measurements in Muscovy, Pekin and Mulard ducks. A secondary objective of this study was to evaluate, the effect of age, origin of ducks at 3, 5 and 7 weeks (wk) of age on their growth performances.

Materials and methods

Animals

Animal handling followed the recommendation of the Ethical Committee in Bydgoszcz, Poland (no. 27/2012).

The study was carried out at the Mochełek Experiment Station in the Kuyavian-Pomeranian Province (Poland). The experimental materials were composed of 53 Muscovy ducks (27 males, 26 females), 48 Pekin ducks AF51 (30 males, 18 females) and 45 STE Mulards (23 males, 22 females). Birds in presented study were not related. The Muscovy duck descended from parents imported from Grimaud Frères Sélection from France, whereas the AF51 ducks are two-strain crosses created in Poland (A55 males × F11 females). STE Mulards (male Muscovy and female Pekin hybrid) descended from parents imported from French company SAS Breheret.

Ducks were housed in pens according to sex and origin in a confined building on deep litter. During the trial, birds were fed ad libitum commercial complete diets (Cargill, Poland) according to their age: a starter diet (from 0 to 3 wk of age) containing 20.0% crude protein and 2800 kcal of metabolisable energy (ME) and a grower diet (from 4 to 12 wk of age) containing 18.5% crude protein and 2920 kcal of ME. Birds had also free access to water. Moreover, each duck was fitted with a wing band, to enable the individual parameters to be monitored.

BW and some biometrical characters were measured on each animal in the morning before they were fed. Drakes and ducks were weighed and taped-measured individually in selected weeks of growth. The males and females of the Pekin ducks AF51 were evaluated for growth traits at 3, 5 and 7 wk of age, the Muscovy females – at 3, 5, 7 and 10 wk of age, the Muscovy males and Mulards (males and females) – at 3, 5, 7, 10 and 12 wk of age. Dates assessment of ducks growth characteristics were related: to the date of termination of the starter diet (changing the supply of nutrients from the 22nd day), to the date of completion of most intensive growth phase of ducks (3-wk Pekin, 5-wk Muscovy duck and Mulards) and slaughter maturity (7-wk Pekin, 10-wk female Muscovy duck, 12-wk male Muscovy duck, male and female Mulards). Ducks were tape-measured to an accuracy of 0.1 cm. The following body measurements were taken: length of trunk with neck (LTN) – between the first cervical vertebra and base of tail, length of trunk (LT) – between shoulder joint and base of tail, chest girth (CG) – behind the wings, through the anterior border of the breast–bone crest and the central thoracic vertebra, length of breast bone (LBB) – from the anterior to the posterior edge,
length of shank (LS) – between the hock joint and bottom posterior area of first toe at its base.

Genetic variants detection and polymorphism analyses

Genetic tests were conducted on genomic DNA isolated from peripheral blood drawn from wing vein. From all animals blood samples were drawn into test tubes containing K-EDTA which then were kept at −25 °C until required. Genomic DNA was isolated from aliquots of 15 μL of blood samples using Gene Jet Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific, Waltham, MA) according to manufacturer’s instructions. The quality and amount of DNA was measured using a NanoDrop® 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Genomic DNA of each animal was stored at −20°C until subjected to allelic discrimination assays.

Prolactin genotypes were determined using the PCR-RFLP method according to Wang et al. (2011). Intron 1 of the PRL gene was amplified with the following primers: forward: 5′-AAATTCCTCTCACTATTACA-3′, reverse: 5′-GATGCGAGACAAGTTTACC-3′. Exon 5 of the PRL gene was amplified with the following primers: forward: 5′-GGACAACTAAAGAAAAAAGA-3′, reverse: 5′-CAATGAAAAGTGGCAAAGCAA-3′. The PCR was performed in 15 μL total volume using 50–100 ng of genomic DNA, 1× Dream Taq PCR buffer (included MgCl2), 0.2 U Dream Taq polymerase, 0.5 μM of each primer, 25μM of dNTPs (Thermo Fisher Scientific, Waltham, MA). Thermal cycling began with an initial cycle of 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 53 °C for 30 s and 72 °C for 30 s, and concluded with a final extension at 72 °C for 5 min, and hold at 4 °C (Wang et al. 2011).

The PCR product corresponding to exon 5 of PRL gene, containing 400 base pairs was digested with 5U of PstI restriction enzyme (Thermo Fisher Scientific, Waltham, MA) and PCR product that corresponds to intron 1, consisting of 417 base pairs, was digested with 5U of XbaI restriction enzyme (Thermo Fisher Scientific, Waltham, MA) and PCR product that corresponds to exon 5 of this gene with PstI enzyme differentiated one allele (PRLT). There is little information in the literature regarding the allele and genotypic frequencies of RFLPs in PRL gene due to XbaI and PstI restrictionase. Since only one genotype PRL/PstI was detected in our research, tables do not contain data concerning allelic and genotype frequency and association of this genotype with growth traits in the evaluated ducks. The allelic and genotypic frequencies of PRL/XbaI polymorphic sites in the different duck populations are shown in Table 1.

In the present study, the PRLT allele was detected in all examined groups of ducks, whereas the PRLG allele did not occur at all in the Muscovy ducks. The usage of XbaI restriction enzyme in studied bird populations enabled the identification of three genotypes: PRL/GG – 417 bp, PRL/TG – 417, 354, 63 bp, PRL/TT – 354, 63 bp (Figures 1 and 2). The allelic distribution for the PRL/XbaI marker in the three populations followed a different pattern.
In the group of Muscovy ducks only one allele (PRLG) and one genotype (PRL/ GG) were observed. In Pekin ducks AF51, the observed allelic frequencies for allele PRLT was 0.604 and for allele PRLG – 0.394. Genotypic frequencies for PRL/TT genotype was 0.208, for PRL/TG genotype – 0.792. In Pekin ducks genotype PRL/GG was not detected. The obtained frequencies identified with the XbaI enzyme are similar to those found by Wang et al. (2011) in Chinese native duck breeds. The inverse trend between estimated frequencies noted in population of Mulard ducks for allele PRLG – 0.589 and for allele PRLT – 0.411. Wang et al. (2011) obtained allelic frequencies at a similar level for generation F2 of studied ducks, where allele PRLG was in dominance. In the present study, it has been observed that the most frequent genotype in Pekin and Mulard ducks was PRL/TG (0.792 vs. 0.822). The analysis of \( \chi^2 \) test showed that in the PRL/XbaI and PRL/PstI locus the distribution of genotypes in those populations was not in Hardy–Weinberg equilibrium (\( p < 0.01 \)).

### Association of PRL gene polymorphism with growth traits

To study the association of the examined PRL gene polymorphism with growth traits we analysed the relationship between BW and some body measurements with identified genotypes in Pekin ducks (PRL/TG, PRL/ TT) and Mulards (PRL/GG, PRL/TT). Since only one

| Genotypes/frequencies (%) | TT | TG | GG | Allelic frequencies | Hardy–Weinberg equilibrium | p Value |
|---------------------------|----|----|----|---------------------|-----------------------------|---------|
| Ducks origin              |    |    |    |                     |                             |         |
| Muscovy                   | 0  | 0  | 0 (0.000) | 0  | 0  | 0 (0.000) | 27 | 26 | 53 (1.000) | 0.000 | 1.000 | – |
| Pekin                     | 8  | 2  | 10 (0.208) | 22 | 16 | 38 (0.792) | 0  | 0  | 0 (0.000) | 0.604 | 0.394 | <0.01 |
| Mulard                    | 0  | 0  | 0 (0.000) | 21 | 16 | 37 (0.822) | 2  | 6  | 8 (0.178) | 0.411 | 0.589 | <0.01 |

Table 1. Genotype and allele frequencies of PRL/XbaI gene in Muscovy, Pekin and Mulard ducks.

Table 2. Association of PRL gene polymorphism with body weight and some body measurements of Pekin ducks at 3, 5 and 7 weeks (wk) of age.

| Age | Trait | PRL/TG | PRL/TT | SEM | p Value |
|-----|-------|--------|--------|-----|---------|
| 3 wk | BW (g) | 1098.16 | 1138.00 | 14.81 | ns |
|     | LTN (cm) | 31.84 | 31.75 | 0.21 | ns |
|     | LT (cm) | 20.57 | 20.70 | 0.15 | ns |
|     | CG (cm) | 25.13 | 25.10 | 0.27 | ns |
|     | LBB (cm) | 5.79 | 5.65 | 0.68 | ns |
|     | LS (cm) | 5.45 | 5.75 | 0.67 | <0.05 |
| 5 wk | BW (g) | 2036.84 | 2100.00 | 25.99 | ns |
|     | LTN (cm) | 37.05 | 37.75 | 0.29 | ns |
|     | LT (cm) | 25.63 | 26.05 | 0.23 | ns |
|     | CG (cm) | 32.95 | 32.85 | 0.25 | ns |
|     | LBB (cm) | 11.67 | 12.00 | 0.10 | ns |
|     | LS (cm) | 6.26 | 6.35 | 0.05 | ns |
| 7 wk | BW (g) | 2513.16 | 2615.00 | 34.01 | ns |
|     | LTN (cm) | 40.55 | 41.15 | 0.29 | ns |
|     | LT (cm) | 26.97 | 26.80 | 0.21 | ns |
|     | CG (cm) | 36.28 | 36.45 | 0.28 | ns |
|     | LBB (cm) | 14.83 | 17.30 | 0.14 | <0.05 |
|     | LS (cm) | 6.33 | 6.40 | 0.05 | ns |

*aBW: body weight; LTN: length of trunk with neck; LT: length of trunk; CG: chest girth; LBB: length of breast bone; LS: length of shank; ns: not significant. p > 0.05.*
genotype PRL/XbaI was detected in our research in Muscovy ducks, tables do not contain data concerning association of this genotype with growth traits in the evaluated ducks. The results of the statistical analysis of associations between the PRL/XbaI polymorphism and growth traits in Pekin duck at 3, 5 and 7 wk of age are summarised in Table 2. It was observed that in all above-mentioned weeks of growth, the Pekin ducks with the PRL/TG genotype were (p > 0.05) similar to the Pekin ducks with PRL/TT genotype at locus PRL/XbaI for most body parameters evaluated. The exception was LS value in birds aged 3 wk and LBB value in Pekin ducks aged 7 wk; values of those traits were higher in Pekin ducks with the PRL/TT genotype (p < 0.05). Effects of PRL gene polymorphism on BW and body-measurements of Mulard ducks at 3, 5, 7, 10 and 12 wk of age are presented in Table 3. There were no significant (p > 0.05) differences between Mulard ducks with the PRL/GG genotype and those with the PRL/TG genotype in BW and all studied body measurements recorded at 3, 5 and 7 wk. The study also revealed no significant (p > 0.05) differences for LTN, LT, CG and LBB values between both Mulard duck populations with detected genotypes at 10 and 12 wk. However, the comparison of the Mulard ducks aged 10 and 12 wk with the PRL/GG genotype and those with the PRL/TG genotype, demonstrated that the latter were heavier (p < 0.05) and had significantly higher (p < 0.05) value of LS.

The present report is the first one about the effect of PRL gene on BW and body measurements in poultry. Hence, the verification of our results with those in previous literature is hampered. Until now, the influence of polymorphism of the above-mentioned gene was investigated among other in fish. He et al. (2012) observed significant association of Asian seabass PRL gene with growth traits.

Growth performance

Effects of main factors, age (3, 5 and 7 wk), ducks origin (Muscovy, Pekin AF51 and Mulard), and their interactions on BW and body-measurements are presented in Table 4. As expected, most of parameters studied increased (p < 0.01) with age, from 3 to 5 wk and from 5 to 7 wk. No difference (p > 0.05) was observed in LS value between 5 and 7 wk of age. These results confirm the findings reported by previous studies.

Table 3. Association of PRL gene polymorphism with body weight and some biometrical traits of Mulard ducks at 3, 5, 7, 10 and 12 weeks (wk) of age.

| Genotype  | Age  | Trait* | PRL/GG | PRL/TG | SEM | p Value |
|-----------|------|--------|--------|--------|-----|---------|
| 3 wk      | BW (g) | 997.50 a | 1024.86 a | 14.14 a | ns  |
|           | LTN (cm) | 30.44 a | 30.69 a | 0.25 a | ns  |
|           | LT (cm) | 20.81 a | 21.20 a | 0.26 a | ns  |
|           | CG (cm) | 23.94 a | 23.80 a | 0.20 a | ns  |
|           | LBB (cm) | 5.69 a | 5.77 a | 0.07 a | ns  |
|           | LS (cm) | 5.81 a | 5.74 a | 0.06 a | ns  |
| 5 wk      | BW (g) | 2068.75 a | 2095.95 a | 29.99 a | ns  |
|           | LTN (cm) | 36.44 a | 37.11 a | 0.31 a | ns  |
|           | LT (cm) | 26.94 a | 27.00 a | 0.30 a | ns  |
|           | CG (cm) | 32.75 a | 33.00 a | 0.21 a | ns  |
|           | LBB (cm) | 11.38 a | 11.27 a | 0.80 ns | |
|           | LS (cm) | 6.44 a | 6.32 a | 0.07 a | ns  |
| 7 wk      | BW (g) | 2556.20 a | 2589.19 a | 40.11 a | ns  |
|           | LTN (cm) | 36.44 a | 37.11 a | 0.31 a | ns  |
|           | LT (cm) | 27.06 a | 27.00 a | 0.23 a | ns  |
|           | CG (cm) | 32.75 a | 33.00 a | 0.21 a | ns  |
|           | LBB (cm) | 11.38 a | 11.27 a | 0.80 ns | |
|           | LS (cm) | 6.44 a | 6.32 a | 0.07 a | ns  |
| 10 wk     | BW (g) | 2724.32 a | 3068.75 a | 57.10 a | <0.05 |
|           | LTN (cm) | 40.80 a | 41.84 a | 0.30 ns | |
|           | LT (cm) | 28.25 a | 28.28 a | 0.30 a | ns  |
|           | CG (cm) | 39.13 a | 39.23 a | 0.33 a | ns  |
|           | LBB (cm) | 17.88 a | 17.94 a | 0.13 ns | |
|           | LS (cm) | 6.56 a | 6.51 a | 0.07 ns | |
| 12 wk     | BW (g) | 2943.75 a | 3127.03 a | 59.64 a | <0.05 |
|           | LTN (cm) | 40.80 a | 41.84 a | 0.30 ns | |
|           | LT (cm) | 29.38 a | 29.47 a | 0.27 ns | |
|           | CG (cm) | 39.08 a | 39.23 a | 0.33 a | ns  |
|           | LBB (cm) | 18.18 a | 18.31 a | 0.13 ns | |
|           | LS (cm) | 6.57 a | 6.88 a | 0.05 a | <0.05 |

*BW: body weight; LTN: length of trunk with neck; LT: length of trunk; CG: chest girth; LBB: length of breast bone; LS: length of shank.

Table 4. Effect of age, origin on body weight and some biometrical traits of ducks at 3, 5 and 7 weeks (wk) of age.

| Trait*          | BW (g) | LTN (cm) | LT (cm) | CG (cm) | LBB (cm) | LS (cm) |
|-----------------|--------|----------|---------|---------|----------|---------|
| Age             |        |          |         |         |          |         |
| 3 wk            | 920.95 a | 28.43 a | 18.61 a | 22.86 a | 5.37 a   | 5.37 a  |
| 5 wk            | 1859.25 a | 34.74 a | 24.35 a | 31.02 a | 10.87 b  | 6.36 b  |
| 7 wk            | 2448.1 a | 39.26 a | 25.90 c | 35.45 c | 14.93 c  | 6.47 b  |
| Origin          |        |          |         |         |          |         |
| Muscovy         | 1450.84 a | 29.65 a | 19.50 a | 26.79 a | 9.11 a   | 6.00 a  |
| Pekin           | 1883.89 a | 36.32 b | 24.30 b | 31.39 b | 11.09 b  | 6.01 a  |
| Mulard          | 1895.29 a | 36.48 b | 25.06 c | 31.15 b | 10.97 b  | 6.20 b  |
| SEM             | 7.31    | 0.08     | 0.07    | 0.09    | 0.03     | 0.02    |
| p Value         |        |          |         |         |          |         |
| A               | <0.01   | <0.01    | <0.01   | <0.01   | <0.01    | <0.01   |
| O               | <0.01   | <0.01    | <0.01   | <0.01   | <0.01    | <0.01   |
| A*O             | <0.01   | <0.01    | <0.01   | <0.01   | <0.01    | <0.01   |

*BW: body weight; LTN: length of trunk with neck; LT: length of trunk; CG: chest girth; LBB: length of breast bone; LS: length of shank.

Values within a row followed by different letter differ significantly (p < 0.01).
indicated that marked differences exist between ducks observed for all evaluated traits. Those interactions between age and LS values, which were higher \( p < 0.01 \) in Mulard ducks. The result of the present study confirms the consistent association of Pekin and Mulard ducks, except for LT traits between Pekin and Mulard ducks, except for LT and LS values, which were higher \( p < 0.01 \) in Mulard ducks. The result of the present study confirms the previous observations that duck origin influenced BW and body dimensions (Solomon et al. 2006; Bernacki et al. 2008; Kokoszyński & Bernacki 2011). Additionally, interactions between age \( \times \) origin \( p < 0.01 \) were observed for all evaluated traits. Those interactions indicated that marked differences exist between ducks with different origin in different age.

**Conclusions**

The results of presented study conducted on Muscovy, Pekin and Mulard ducks confirmed previous results on the effect of age and ducks origin. In the studied ducks sample one allele \( (PRL^C) \) and one genotype \( (PRL/CC) \) at locus \( PRL/PstI \) were found. The usage of \( XbaI \) restriction enzyme enabled the identification of two alleles \( (PRL^G \) and \( PRL^T \) and three genotypes \( (PRL/GG, PRL/TG \) and \( PRL/TT \)). \( PRL/XbaI \) locus was found polymorphic in Pekin and Mulard duck populations, while monomorphic in Muscovy duck group. Thus, the result of this research would be useful as a control for genetic equilibrium in Muscovy ducks. Moreover, the results of this study with regard to \( PRL/XbaI \) genotype showed association of this polymorphic site with some morphological traits, in Pekin ducks on values of LBB and LS, in Mulard ducks on BW and LS. Due to the fact that the distribution of genotypes in the studied groups of duck was not in Hardy–Weinberg equilibrium, further analysis should be extended to a large and more homogeneous population of ducks to confirm the observed associations. Moreover, it is necessary to conduct a survival assessment and post-slaughter analysis of ducks to establish any possible links with polymorphic variants in the \( PRL \) gene present in duck. Nevertheless, these data could serve as a basis for further insight into this avian gene.

**Disclosure statement**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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