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Duck, meat quality, microsatellite markers.

Successful Identification of Duck Genome Region Determining Desirable Uniformity of Meat Performance Traits

ABSTRACT

The objective of this study was to identify genome regions determining duck meat performance traits with possible small variation. In total, 368 crossbred ducks of F2 generation obtained from two parental lines: Pekin-type ducks of Polish origin (A55) and Pekin-type ducks of French origin (GL-30) were recorded. The following seven traits were analyzed: body weight, breast muscle weight, leg muscle weight, water holding capacity in the breast and leg muscles, and color lightness L* of the breast and leg muscles. All birds (including parental and F1 generations) were genotyped (29 microsatellite markers). Means and coefficients of variation (CV) were calculated for 28 full-sibs (four sires by six dams and one sire by four dams). Number of progeny per full-sib group ranged from 7 to 17. The multivariate cluster analysis using grouping by k-means algorithm was used on transformed data. The multivariate cluster analysis gave two clusters: first group with 10 full-sibs and second one with 18 families. Differences among half-sibs in the CV of the recorded traits were determined. It should be noted that one out of five sire groups showed statistically significant differences from the other ones. Moreover, the CVs in this group were smaller. The analysis of microsatellite markers indicated three alleles from three loci were present only in the "superior" sire group. The obtained results indicate a promising opportunity of effective selection for improving carcass technological quality using molecular markers.

INTRODUCTION

Ducks play an important role in the world poultry market. Their number in the structure of commercially-slaughtered poultry has increased, including in Poland. From the perspective of industrial meat processing, the uniformity of the carcass and its parts is desirable. Hence, breeders' efforts have mainly been focused on the improvement of carcass and meat quality traits and their uniformity. Pekin ducks are successfully used for intensive production of duck meat all over the world. For many years, their selection mainly aimed at increasing carcass weight and meat yield, and decreasing fat content (Pingel, 2011; Xu *et al.*, 2011).

For a long time, the market gave preference to whole carcasses without giblets. Duck parts are currently a growing poultry market segment because consumers are willing to pay more for fresh or frozen breast fillets and hind- or forequarters rather than buying cheaper whole carcasses. Raw meat preparations are generally bought by consumers based on overall appearance, with special consideration of color and drip loss (Resurreccion, 2003; Makala & Olkiewicz, 2004; Nowak & Trziszka, 2010).

Research to date on fattening ducks (Pekin, Muscovy, and their crosses) has shown that many characteristics of their slaughter value and



meat quality are related to species, breed, selection, and sex (Farhat *et al.*, 2000; Baeza *et al.*, 2002; Wołoszyn *et al.*, 2011). When subjected to sensory evaluation by a panel of experts, breed (Pekin, Muscovy and Rouen) was shown (Omojola, 2007) to influence dressing percentage and meat color, whereas breed and sex did not affect texture and overall sensory acceptability of the meat. In a study on four breed/varieties of selected vs. non-selected Pekin-type ducks (Witkiewicz *et al.*, 2006), significant ($p \leq 0.05$) differences in body weight, proportion of breast muscle, collagen content, and mono- and polyunsaturated fatty acids (MUFA and PUFA) content of muscle *pectoralis superficialis*. Similar results for the effect of Pekin duck strains on breast muscle weight were obtained by Smith *et al.* (2015), who concluded that breed had little effect on drip loss and on lightness (L^*) and redness (a^*) values of the breast muscles. In a study characterizing meat traits and meat quality of Pekin-type ducks strains A-44 and A-55, selected in Poland, the meat of A-55 ducks was found to have higher culinary value (Mazanowski *et al.*, 2003; Mazanowski & Książkiewicz, 2004; Adamski *et al.*, 2005).

The present study is a continuation of earlier works by Mucha *et al.* (2014a, 2014b) and Moliński *et al.* (2015) with the same experimental material. It should be mentioned that the parental lines A-55 (Pekin-type ducks of Polish origin) and GL-30 (Pekin-type ducks of French origin) are conventionally used in Poland for production of commercial hybrids (A-55 x F-11, A-55 x P-55, A-55 x P-44 and GL-50 x GL-30) intended for intensive rearing of meat ducks (Wencek *et al.*, 2015).

The objective of this study was to identify genome regions determining desirable duck meat performance traits characterized additionally by a small variation. The analyzed set of traits included maximization of body weight as well as leg and breast muscle weights with acceptable color lightness L^* , and breast and leg muscle water holding capacity.

MATERIAL AND METHODS

Birds

The experimental procedures were approved by the Local Ethical Commission for Animal Experiments in Poznań (Poland) by resolution 60/2009.

The slaughter value of Pekin-type ducks was evaluated using 368 hybrids (F2 generation) of known origin. As already mentioned, two parental lines: A-55 (Pekin-type ducks of Polish origin) and GL-30 (Pekin-type ducks of French origin) were used in the experiment. More details on the crossbreeding

experiment are given in the article of Mucha *et al.* (2014b). All birds of each generation were kept under the same environmental and feeding conditions.

After 11 weeks of rearing, and following 12 hours of feed (but not water) withdrawal, birds were slaughtered and subjected to post-slaughter processing for the next three days. The procedures were consistent with the standard industry practice. Immediately before slaughter, the birds were individually weighed on an AXIS B15S electronic balance with ± 5 g accuracy.

Methods for assessment of slaughter value

The hybrid Pekin ducks were evaluated for body weight (BW) at 11 weeks, and the following breast and leg muscles parameters: weight (BMW and LMW, respectively), water holding capacity (WHCBM and WHCLM, respectively), and color lightness L^* (LB and LL, respectively). During dissection (Ziołocki & Doruchowski, 1989), the carcasses were divided into breast muscles (*m. pectoralis superficialis* and *m. pectoralis profundus*) and leg (thigh and shank) muscles. The carcasses and individual muscle parts were weighed on an electronic balance (WPT 5C, RADWAG, Poland) with ± 0.2 g accuracy. Color lightness (L^*) was measured on the left side muscles.

Meat lightness (L^*) was measured 24 h after slaughter, immediately after the breast muscle was removed from the breast bone and ribs and turned inside out. This ensured that the slices of both breast muscles were uniform. Leg muscles were ground. Instrumental color measurement (CIE $L^*a^*b^*$ system; CIE, 1986) was performed using a trichromatic colorimeter (Chroma Meter C580D65, Minolta, Japan) with illuminant, 10° observer, 8-mm aperture, and calibrated using a white plate: $L^* - 99.18$, $a^* - 0.07$, $b^* - 0.05$. In this system, L^* stands for lightness, which is a space vector. The color of the homogeneous product (slice of breast muscles) was measured once at three sites, and of the non-homogeneous product (ground leg meat) three times on each of the three sites. Color results are given as the mean value of the measurements made in the different muscle groups.

Ground BM and LM were analyzed for water holding capacity (WHC BM and WHC LM) 48 h postmortem by the Grau and Hamm method (Grau & Hamm, 1952) as modified by Pohja & Niinivaara (1957).

Molecular analysis

The DNA of 401 individuals (from P, F1, and F2 generations) was extracted from the blood using standard methods. The primers for the amplification of microsatellite sequences were chosen based on the



literature (Huang *et al.*, 2006). Finally, 29 polymorphic microsatellite markers were further analyzed. The detailed description of the molecular methods applied in the present study is given in Mucha *et al.* (2014b). The molecular identification of the sex of the individuals was determined using the method given by Clinton *et al.* (2001).

Statistical analysis

In the first step, the standardization of the observations was performed. Briefly, the mean and standard deviation of each trait were calculated, after which the mean was subtracted from the observed values, and the result was divided by the standard deviation.

Multivariate cluster analysis was applied to the transformed data (McQuenn, 1967), grouping by k-means algorithm. Two clusters of families were obtained. The results obtained from the progenies of five sires were subjected to analysis of variance and compared by least significant differences.

Next, the coefficients of variation (CV) of the analyzed traits in the half-sibs were calculated and compared by the χ^2 test of Miller & Feltz (1997).

In the last step, all sire markers were analyzed. The purpose was to find the loci characteristic for the indicated sire, i.e., loci occurring only in its specific genotype.

Table 1 – Means and coefficients of variation of the analyzed traits according to sire.

Sire	BW (g)	BMW (g)	LMW (g)	WHCBM (%)	WHCLM (%)	LB	LL
101	3123.3±11.6	339.4±15.6	236.6±14.5	33.0±11.6	33.1±10.9	44.7±8.2	51.1±6.0
107	3152.7±8.3	347.7±13.4	242.1±11.5	33.9±10.8	32.9±10.4	45.3±7.6	50.6±5.6
109	3023.8±8.6	319.4±11.6	233.5±11.6	33.0±10.3	33.3±10.3	43.8±9.4	51.0±4.9
113	3038.9±9.3	317.3±11.6	234.8±11.9	33.5±11.0	33.8±10.7	43.6±8.6	50.9±6.5
116	3281.8±6.1	350.1±11.1	250.1±10.4	32.8±10.0	33.1±9.6	45.0±8.4	52.3±4.7
p(χ^2)	0.000	0.017	0.056	0.753	0.820	0.575	0.015

BW – body weight, BMW - breast muscle weight, LMW - leg muscle weight, WHCBM - water holding capacity of the breast muscle, WHCLM - water holding capacity of the leg muscle, LB - color lightness L* of the breast muscle, LL - color lightness L* of the leg muscle.

RESULTS

The multivariate cluster analysis, grouping by k-means, gave two clusters: the first one with 10 families and the second one with 18 families. The first cluster contains five families of sire 116. Only one of its families is in the other cluster. Moreover, the first cluster groups families with higher values of important traits, such as BW, BMW, and LMW (Figure 1). These results indicate that sire 116 was the most interesting.

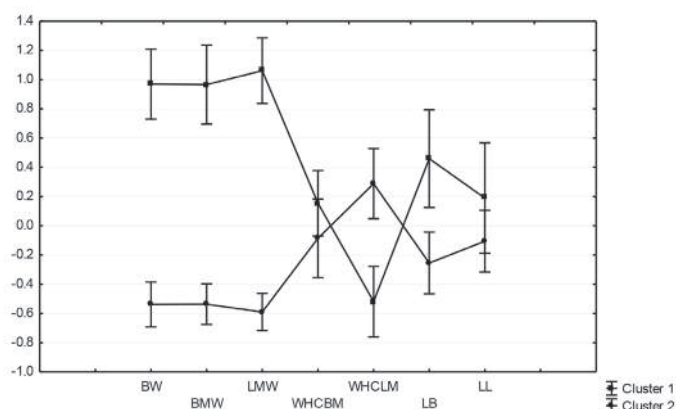


Figure 1 – Means and standard errors for the standardized variables

Note on symbols: BW – body weight, BMW - breast muscle weight, LMW - leg muscle weight, WHCBM - water holding capacity in breast muscles, WHCLM - water holding capacity in leg muscles, LB - colour lightness L* of breast muscles, LL - colour lightness L* of leg muscles.

Table 1 gives the means and coefficients of variation of the analyzed traits obtained for each sire.

By the analysis of variance, the general hypothesis of the equality of the means of the five sire progenies was rejected for five out of the seven analyzed traits. No differences were stated only for BHCBM and WHCLM. The probabilities of post-hoc tests are given in Table 2. Again, sire 116 was different from the others in most cases.

The χ^2 test of Miller and Feltz was applied to verify if there were any significant CV differences between the male and female progeny of each sire. In 35 comparisons, the hypotheses were rejected only in five cases at 0.05 significance level and only one at 0.01 level. This allowed us to neglect the sex in the further analysis.

The coefficients of variation (CV) of the seven analyzed traits given in Table 1 show that except for one trait, namely LB, those obtained for the progenies of sire 116 are smaller compared with the those of the other sires. This again indicates that sire 116 produced the most balanced progeny. The last row in Table 1 contains probabilities of χ^2 statistics used for the verification of the hypothesis about the equality of CVs. The general hypotheses were rejected in three cases: for BW, BMW, and LL.



Table 2 – Probability for tests of coefficients of variation differences among sires for the evaluated traits.

Trait	Sire			
	107	109	113	116
BW	101	0.607	0.295	0.421
	107	0.152	0.222	0.174
	109		0.803	0.004
	113			0.007
BMW	101	0.424	0.186	0.103
	107	0.054	0.029	0.978
	109		0.740	0.031
	113			0.015
LMW	101	0.452	0.806	0.887
	107	0.333	0.381	0.367
	109		0.917	0.044
	113			0.054
WHCBM	101	0.346	0.904	0.867
	107	0.295	0.426	0.194
	109		0.774	0.769
	113			0.562
WHCLM	101	0.613	0.762	0.169
	107	0.440	0.088	0.588
	109		0.277	0.793
	113			0.181
LB	101	0.467	0.076	0.115
	107	0.025	0.038	0.660
	109		0.828	0.039
	113			0.061
LL	101	0.509	0.936	0.755
	107	0.465	0.702	0.004
	109		0.695	0.012
	113			0.005

BW – body weight, BMW - breast muscle weight, LMW - leg muscle weight, WHCBM - water holding capacity of the breast muscle, WHCLM - water holding capacity of the leg muscle, LB - color lightness L* of the breast muscle, LL - color lightness L* of the leg muscle.

Probabilities of significant differences among coefficients of variability of full-sibs are in bold.

Considering these results, the molecular analysis (Table 3) was focused on sire 116. Microsatellite markers which were formed by loci that did not occur in the genotypes of other sires were searched. The analysis of all sire markers identified three markers with loci occurring only

in the 116 male genotype. These are: SM007, CAUD024 and CAUD069, and may indicate sites responsible for uniform progenies for the analyzed traits.

Table 3 – Sire genotypes at the three most differentiated microsatellite markers

Sire	SMO07	SMO07	CAUD024	CAUD024	CAUD069	CAUD069
101	186	186	274	278	174	182
107	186	186	278	282	174	174
109	186	186	278	278	174	182
113	186	186	282	286	202	242
116	184	190	262	270	186	198



DISCUSSION

In contrast with other livestock and poultry species, the whole duck genome has not been fully analyzed yet. Therefore, the analysis of the association between *Anas platyrhynchos* the genome and phenotypes is still limited. However, over the last decades a number of studies on the associations of single loci with performance traits of ducks (Huang *et al.*, 2005, 2006, 2008; Maak *et al.*, 2000; Wu *et al.*, 2008; Gong *et al.*, 2014; Mucha *et al.*, 2014b), using different methodological approaches, have been published. Generally, they focus on single measures per individual. Thus, the homogeneity of single traits and their clusters are omitted. Final meat production effectiveness can be perceived as a function of many traits, such as body weight, carcass weight including most important components (breast and leg muscle weights), as well as water holding capacity and color lightness of the mentioned muscles. As already mentioned, some duck genome regions determining some performance traits have been identified. Gong *et al.* (2014) found significant effects of three polymorphisms within the MSTN gene on body weight of duck in consecutive weeks of age (from 5 to 11 weeks), whereas no effects have been estimated for younger birds. Furthermore, Zhao *et al.* (2015) detected significant associations between the VLDLR gene (for four diplotypes) and body weight at 10 weeks in Gaoyou domestic duck breed. Also, considerable effects of these traits were estimated by Huang *et al.* (2007a, 2007b). In addition, polymorphisms of the mitochondrial coding gene were also associated with 42-d-old body weight and breast muscle weight in Pekin ducks, but no effect on leg muscle weight has been reported. Some authors found important genome regions determining meat quality traits. Zhang *et al.* (2010) reported significant effects of the ApoVLDL-II gene on water holding capacity. However, no influence of these polymorphisms on meat color was estimated in the above paper. Zhang *et al.* (2015) obtained no significant effects of nine polymorphisms of the LXR α gene on water holding capacity in White Muscovy ducks.

As already stated, the population examined here was analyzed in previous studies by Mucha *et al.* (2014b), who did not find any significant effects of genome regions on the traits included in the present study. The detection of important duck genome regions was performed using classical methodology with construction of a linkage map and estimation of parameters based on a linear unitrait genetic additive model.

The above-mentioned results clearly confirm that body weight and its components have a complex genetic background. On the other hand, it is well known that estimates of single locus effects vary across populations and their genetic structures and size as well as applied methodologies. The effectiveness of statistical inference is considerably determined by the above factors.

From a practical point of view, the breeding goal should be focused on the most important traits. Unfortunately, undesirable dependences between some animal characteristics do not allow to contrast clusters of populations with good and poor traits. Fortunately, a previous study by Molinski *et al.* (2015) indicated a possibility of successful clustering of the population into two alternative groups. It was an optimistic signal for the present investigation.

The main goal of this study was to investigate some variants of microsatellite loci connected with desirable traits in the context of their level and uniformity. The "discrimination ability" of a given file is mainly determined by the number of variables. Hence, the number of traits included in this study is limited. Seven basic traits were considered in this analysis, although this research project covered 36 traits describing different aspects of meat production in ducks. From an economic point of view, both means of given traits and their uniformity seem to be very important. To our knowledge no reports are available in the literature on duck performance traits.

As already mentioned, the next stage of the analysis was to find the genotypes of sires influencing both desirable level and uniformity of the traits recorded. Applicability of genetic markers in association studies is strongly determined by their polymorphism. Polymorphism Information Content coefficients for these microsatellite loci are as follows (Mucha *et al.* 2014b): 0.32 (SMO07), 0.84 (CAUD069) and 0.85 (CAUD024). They are obviously positively correlated with the number of alleles and heterozygosity parameters. Averages and variability coefficients of 28 full-sib groups distributed across five sires were analyzed. Even though the number of sires seems to be limited, specific genotypes of one sire were determined. It should be noted that the least polymorphic locus (SMO07) was found by Huang *et al.* (2007a) as a flanking marker for breast muscle weight. Whereas Molinski *et al.* (2015) reported that, out of the six studied, the CAUD024 and CAUD069 loci were most important for clustering the present population according to some carcass traits.



The duck hybrids evaluated here were characterized by excellent meat performance traits (Mucha *et al.* 2014a). The private sire alleles in three of the identified loci seem to be a promising element in further genetic improvement programs. Although the crossbreeding scheme was designed for the detection of quantitative trait loci, it can also be perceived as a suggestion for breeding practice.

CONCLUSIONS

The analysis of the microsatellite markers indicated three alleles from three loci that were present only in the “superior” sire group. The obtained results provide a promising opportunity of effective selection for enhancing carcass technological quality using molecular markers.

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