Quantitative Trait Loci (QTL) Associated With Total Body Fat In Chicken Carcass

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Introduction

Considerable genetic progress for several traits has been achieved by broiler breeding companies over the years, but differences in body weight, growth rate and abdominal fat weight among broiler lines still exist due to specific company selection practices (Emmerson (1997)). Modern commercial broilers show rapid growth and enhanced deposition of nutrients in body tissues, mainly as protein and fat. The relationship between protein and fat deposition in the body can be influenced by genetics and nutrition. Genetics, however, contributes about 85 to 90% of the differences observed in carcass and parts yield (Havenstein *et al.* (2003)). Studies have reported QTLs for abdominal fat, intramuscular fat in the breast muscle and skin fat weight (Ikeobi *et al.* (2002), Jennen *et al.* (2005), Lagarrigue *et al.* (2006), Park *et al.* (2006), Nones *et al.* (2006), Zhou *et al.* (2006) and Campos *et al.* (2009)). However, no QTLs had been previously reported for fat (ether extract) content in chicken carcasses. Trying to better understand the genetic architecture controlling fat distribution in chicken carcass, we aimed to identify genomic regions associated with total fat content in the carcass.

Material and methods

Experimental population. The F_2 chicken resource population used to map QTL was developed by crossing a broiler line (TT) with a layer line (CC). Seven TT males and seven CC females were crossed (1:1) to produce F1 chickens (TC). Seven F_1 TC males were each mated to three unrelated F_1 TC females, resulting in 21 full-sib families with about 98 F2 chicks each. A total of 2,063 F_2 offspring from 17 hatches were reared as broilers and slaughtered at 42 days of age. Of these, up to 649 F_2 chickens were genotyped and used to map QTL. The chickens received feed and water *ad libitum*. Commercial broiler diets containing 3,150 kcal/kg and 21% crude protein (1 to 21 days), 3,200 kcal/kg and 20% crude protein (22 to 35 days), and 3,200 kcal/kg and 18.5% crude protein (36 to 41 days) were provided. Blood samples were collected at slaughter for DNA analyses.

Body fat analysis. After slaughter, eviscerated carcasses were stored at -20ºC. Whole eviscerated carcasses including skin, abdominal fat and bones (except for the right tibia), and

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without feathers, head and shank were ground frozen, homogenized, and returned to -20ºC until the analyses were performed. Fat and water contents were measured by near-infrared reflectance spectroscopy (NIRS) in a 250g-sample of the carcass (ground and homogenized) using a cell type Coarse in a NIR System 6500 (Silver Spring, MD, USA). Calibration curves for the NIRS measurements were constructed based on chemical analyses of 100 carcasses from different chicken lines and crosses as described by Klein *et al.* (2003). The ether extract and water contents were determined in accordance with standards of the Association of Official Analytical Chemists (AOAC 1995). Klein *et al.* (2003) compared laboratorial results and NIRS estimations from 19 carcasses and found coefficients of determination equal to 0.96 for ether extract and 0.95 for water content in the carcass. In the present study, fat (ether extract) content was estimated as percentage of the sample's weight (250g of ground and homogenized carcass). Then, the carcass fat content (in grams) was obtained by multiplying percentage of fat in the sample by body weight at 42 days. To avoid a possible undesirable effect of carcass water content, the fat content was also expressed on a dry matter basis (%), by dividing sample fat content by carcass dry matter (%) and multiplying by 100 (Table 1).

Genotyping. DNA was extracted from 5 μL of blood with the DNAzol Reagent® (Invitrogen). Genotyping was carried out using a *MegaBACE* automated sequencer (GE HealthCare), and fragment size analysis was performed with the Genetic Profiler software (GE HealthCare). A total of 127 microsatellite markers plus a single nucleotide polymorphism in the leptin receptor gene (*LEPR*) were genotyped (Campos *et al.* (2009)). Markers were distributed in 22 chromosomes, covering 2,630 cM which corresponds to approximately 63% of the chicken genome. In the present study, up to 649 F_2 chickens (up to 7 full-sib families) were genotyped and used to map QTL.

QTL mapping analysis. Interval mapping using regression methods in the QTL Express software (http://qtl.cap.ed.ac.uk/; Seaton *et al.* (2002)) was applied to the line-cross analysis model, according to Haley *et al.* (1994), assuming that different QTL alleles were fixed in the founder lines. The fixed effects of sex, hatch, and family were included in the model for QTL mapping. Significance thresholds were calculated using a permutation test (Churchill and Doerge (1994)). A total of 10,000 permutations were computed to determine suggestive linkage, and 5% and 1% genome-wide significance levels. Confidence intervals for QTL positions were estimated with bootstrapping as presented by Visscher *et al.* (1996).

Results and discussion

Descriptive statistics for fat and water contents in the carcasses of F_2 individuals evaluated in this study are listed in Table 1. As expected, the largest proportion of the carcass is water, which varies according to body weight (data not shown). Dry matter represents mainly protein, fat and ash. In this study we mapped QTL for carcass fat content which showed large variability in this resource population (Table 1).

A total of three significant and five suggestive QTLs were mapped for fat content (ether extract) in the carcasses (Table 2). All QTLs presented additive effects. Individual QTLs accounted for 1.57 to 3.76% of the phenotypic variance of the traits (Table 2). QTLs mapped

for fat on a dry matter basis explained altogether 11.03% of the phenotypic variance of the trait. No QTL was mapped for water content in the carcasses.

Table 1: Descriptive analysis for the traits evaluated in this study (n = 1,926)

Variable	Mean	SD	Minimum	Maximum
Fat /dry matter (%)	40.77	4.66	24.38	59.21
Fat (g)	155.51	41.55	41.82	306.66
Dry Matter (%)	37.44	2.57	27.36	49.90
Water (%)	62.56	2.57	50.10	72.64

Table 2: Quantitative trait loci in the F_2 model.

Trait	GGA (cM)	CI	Flaking Markers	F-value	a	SE	%PV
Fat /DM (%)	1 (90)	NE	*MCW0297 – LEI0146*	11.6†	-0.77	0.23	1.93
Fat (g)	1 (91)	NE	*LEI0146 – LEI0174*	11.8†	-4.09	1.19	1.96
Fat (g)	1 (219)	NE	*LEI0160 – ADL0148*	9.7†	4.19	1.35	1.57
Fat /DM (%)	1 (392)	NE	*MCW0020 – ROS0025*	9.1†	-0.80	0.27	1.90
Fat /DM (%)	15 (43)	24-61	*MCW0231 – MCW0080*	13.7*	1.22	0.33	3.61
Fat (g)	15 (46)	0-61	*MCW0231 – MCW0080*	14.2*	6.40	1.70	3.76
Fat /DM (%)	27 (100)	0-109	*MCW0300 – MCW0328*	13.6*	-1.05	0.28	3.59
Fat (g)	27 (100)	26-108	*MCW0300 – MCW0328*	9.6†	-4.53	1.46	2.47

∗ genome-wide 5% significance; † suggestive linkage. DM = Dry matter, CI = Confidence Interval, NE = CI not estimable, a = additive effect. %PV = [(MSR- MSF)/MSR] · 100. Where %PV = percentage of phenotypic variance, MSR = residual mean square in the reduced model, MSF = residual mean square in the full model.

Four suggestive QTLs for fat content in the carcass were mapped on GGA1. A QTL for fat content on a dry matter basis was mapped between markers *MCW0297* and *LEI0146,* and a second QTL for fat content in grams was mapped in the adjacent interval just one cM apart, suggesting that these might be the same QTL. A third suggestive QTL was mapped between *LEI0160* and *ADL0148*. These QTLs were mapped in similar regions to those identified as associated with abdominal fat weight on GGA1 (between *LEI0146 – LEI0174* and *ADL0148-MCW0036*) using the same population (Nones *et al.* (2006)). These results are supported by the high genetic correlation between abdominal fat and total carcass lipid from 0.6 to 0.9 (Chambers, 1990). The fourth suggestive QTL for fat content on GGA1 was mapped between *MCW0020* and *ROS0025*. This region was not associated with abdominal fat in the same population (Nones *et al.* (2006)), suggesting that this QTL might be associated with fat deposition in other parts of the carcass, such as skin and intramuscular fat.

QTLs for fat in grams and on a dry matter basis were mapped on GGA15 in the same interval (*MCW0231-MCW0080*) just a few cM apart suggesting being the same QTL. No QTL associated with abdominal fat was mapped by Campos *et al.* (2009) on GGA15 and no QTL for body weight was mapped on GGA15 by Ambo *et al.* (2009), both studies using the same population. These results suggest that this region might be associated with fat deposition in tissues other than abdominal fat. Jennen *et al.* (2004) and Ikeobi *et al.* (2002) mapped QTLs for abdominal fat on GGA15 in adjacent interval mapped here using different populations.
QTLs for fat content on a dry matter basis and in grams were mapped to the same position of GGA27 suggesting being the same QTL. Jennen *et al.* (2005) mapped QTLs for abdominal fat and intramuscular fat on GGA27. Campos *et al.* (2009) mapped QTL for abdominal fat and a

suggestive QTL for triglycerides concentration in the blood in the same interval of GGA27 in the same population. Ambo *et al.* (2009) mapped QTL for body weight in the same interval of GGA27. These results suggest that this region might be involved in overall growth rather than just in fat deposition per se. The QTLs mapped here for carcass fat content along with the results previously reported by Ambo *et al.* (2009) for body weight and Campos *et al.* (2009) for abdominal fat and blood triglycerides provide some clues on genomic regions controlling carcass fat content and possible differential fat deposition, which need to be further investigated. Multi-trait analyses and fine mapping should be conducted to search for evidences of closely linked QTLs or QTLs affecting multiple traits in these regions of interest.

Conclusion

QTLs for total fat carcass content (ether extract) were mapped on GGA1, 15 and 27. Some of the QTLs mapped here matched regions previously mapped to abdominal fat. New regions not associated with abdominal fat weight were also mapped, which indicates interesting regions for further studies to better understand the regulation of fat deposition in chickens. The results described here also reinforce the need for fine mapping and multi-trait analyses to help unravel the genetic control underlying fat content in chicken carcasses.

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