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GENOME-WIDE SCAN FOR COMMON VARIANTS ASSOCIATED WITH INTRAMUSCULAR FAT AND MOISTURE CONTENT IN RAINBOW TROUT

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ABSTRACT

Background

Genetic improvement of fillet quality attributes is a priority of the aquaculture industry. Muscle composition impacts quality attributes such as flavor, appearance, texture, and juiciness. Fat and moisture make up about ~80% of the tissue weight. The genetic architecture underlying the fat and moisture content of the muscle is still to be fully explored in fish. A 50K gene transcribed SNP chip was used for genotyping 789 fish with available phenotypic data for fat and moisture content. Genotyped fish were obtained from two consecutive generations produced in the National Center for Cool and Cold Water Aquaculture (NCCCWA) growth-selective breeding program. Estimates of SNP effects from weighted single-step GBLUP (WssGBLUP) were used to perform genome-wide association (GWA) analysis to identify quantitative trait loci (QTL) associated with the studied traits.

Results

Using genomic sliding windows of 50 adjacent SNPs, 137 and 178 SNPs were identified as associated with fat and moisture content, respectively. Chromosomes 19 and 29 harbored the highest number of SNPs explaining at least 2% of the genetic variation in fat and moisture content. A total of 61 common SNPs on chromosomes 19 and 29 affected the aforementioned traits; this association suggests common mechanisms underlying intramuscular fat and moisture content. Additionally, based on single-marker GWA analyses, 8 and 24 SNPs were identified in association with fat and moisture content, respectively.

Conclusion

SNP-harboring genes were primarily involved in lipid metabolism, cytoskeleton remodeling, and protein turnover. This work provides putative SNP markers that could be prioritized and used for genomic selection in breeding programs.

Keywords: fat content, moisture, GWAS, single-step, QTL.

BACKGROUND

Fish are excellent source of protein with lower content of total fat, saturated fat, and cholesterol and higher omega-3 fatty acids compared to other animals. These characteristics make fish fillets an ideal source of nutrition according to a consensus dietary studies and recommendation [1]. Thus, fillet quality traits have economic importance to the aquaculture industry [2], and consumer attitude towards fish is influenced by fillet quality attributes [3]. For profitable aquaculture production, there is a need for fish fillets with optimum nutritional values and consistent organoleptic qualities. Rainbow trout fish fillet contains ~4-18% by weight fat. Rainbow trout cultured at Clear Springs Foods Inc. (Buhl, ID, USA), the largest producer in the U.S., contains fat content of 12-13% [4]. Variations in fat content can result in positive and negative impacts on fillet quality [4, 5]. Both the quantity and quality of intramuscular lipid impact fillet juiciness, flavor, color, texture, and shelf-life [5-8]. Selection on fat content can enhance fillet color and texture [9], feed conversion ratio (FCR), and protein-retention efficiency [10]. However, accumulating excessive lipids in the muscle makes fillet processing difficult and reduces fillet firmness [11-13]. In addition, high levels of polyunsaturated fatty acids makes the fillet more prone to lipid oxidation, which contributes to the development of rancid flavor and changes in color and nutritional value [14]. Therefore, management of fat content in fish could be used to minimize the variation in eating quality and yield a product of predictable quality [6]. The aquaculture industry usually controls the fat content of fillets by adjusting lipid content in the diet [15-17]. However, there are limitations in using the dietary lipids approach without deteriorating fillet quality due to lipid oxidation and diminishing profitability due to increased feed cost and accumulation of fat in the viscera instead of the muscle. Also, a widely adopted culturing triploid in rainbow trout can prevents loss of fillet quality associated with fat mobilization and protein catabolism during sexual maturation [9, 18].

Fish fillet is a highly perishable food, at least partially, due to high moisture content (60-70%) which results in off-flavors and faster flesh spoilage because water facilitates enzymatic activity and bacterial growth [19, 20]. Low-temperature storage is used to control water activity. However, slow enzymatic reactions can still support microbial growth at low temperatures [21]. Previous studies showed a high correlation between fat and moisture content [22]. In mammals, the intramuscular fat content exhibits a significant negative correlation with moisture content [23, 24]. In fish, this correlation depends greatly on the energetic demands associated with various physiological conditions [18, 25, 26]. An antagonistic biological relationship between traits may hinder their simultaneous improvement, which could lead to unwanted changes in fillet quality [27, 28]. Therefore, an optimal balance among important economic traits needs to be established to enhance product quality and industry returns [2]. Knowledge of the heritability and genetic

architecture of each trait provides information necessary in developing appropriate multi-trait selection programs.

Selective breeding can be used to enhance phenotypic traits of interest. A two-way program of selection on muscle fat content was initiated in rainbow trout to produce lean and fat lines where the fat percentage increased by ~15 to 31% in the fat line depending on the diet [9, 29]. These lines were used as a model to study the effect of muscle fat content on fillet quality [9]. Separately, five generations of family-based selection on body weight of rainbow trout were performed at the USDA NCCCWA [30]. In the third-generation (year class (YC) 2010), fish fillet fat content showed a moderate correlation with whole body weight (coefficient of determination R^2 value of 0.50) [31]. Therefore, selection for bodyweight yielded heavier fish with more fat in the muscle. Similarly, gilthead seabream exhibited a 0.1% increase in muscular fat content concomitant with a 0.08% decline in moisture content per increment of ten grams in weight [32]. Muscle fat and moisture content showed moderate heritability in fish, including rainbow trout, implying the existence of genetic variance in a rainbow trout population selected for an enhanced rate of growth [33], thus making genetic responses to selection possible. However, in salmonids, the genetic architecture of fat and moisture content has not been fully explored in a genome-wide scale [34]. Understanding the genetic basis of the phenotypic traits in question and development of fish strains of improved genetic gain will enhance efficiency of breeding programs, aquaculture industry profitability, and consumer satisfaction.

Genome-wide association (GWA) studies can identify large-effect variants responsible for phenotypic variations, which can be prioritized in genomic selection. A few GWA studies have been conducted on aquaculture species to identify quantitative genomic loci (QTL) responsible for the genetic variability in body weight [35], fillet quality [35, 36], and disease resistance [37]. In fish, a few GWA studies were performed on Atlantic salmon [11, 34] and common carp [38] to identify QTL associated with muscle fat content. In Atlantic salmon, few significant SNPs associated with muscle fat content were identified using a ~5K and 57K SNP panels [11, 34]. In common carp, a high-density, 250K SNP array revealed eight SNPs related to muscle fat content; however, none of the SNPs surpassed the genome-wide significance level [38]. The two studies did not identify QTL explaining a large proportion of the genetic variance in fat content in fish. To the best of our knowledge, no GWA studies have been performed in rainbow trout to identify SNP markers associated with genetic variance for fat and moisture content.

A 50K transcribed SNP-chip, suitable for GWA analyses, has been recently developed in our laboratory. The array has been used to identify large-effect QTL responsible for genetic variance in fillet yield, firmness, protein content, and body weight gain using the same fish population used in this study [36, 39, 40]. The current study aimed to identify

QTL associated with the additive genetic variance in fillet fat and moisture content for the same rainbow trout population.

RESULTS AND DISCUSSION

Muscle fat and moisture contents are interrelated attributes that affect the organoleptic quality and nutritional value of muscle foods [8, 38, 41, 42]. In fish, high-fat content may influence fillet processing and reduce the firmness leading to fillet downgrading [11]; moreover, it significantly impacts texture, juiciness, and flavor [5-7]. In mammals, increased marbling scores are positively related to beef tenderness, accounting for ~9% of the shear force variation [43]. The inability to retain moisture during postmortem storage, in both fish and mammals, is associated with a high drip loss and, in turn, reduces the industry profitability by influencing processing yield and palatability [44, 45]. In the pork industry, drip loss results in up to 10% product losses affecting profitability at wholesale and retail levels [44]. Similarly, 1.5 to 5% of drip losses were reported in salmon [46, 47]. Muscle quality traits in rainbow trout are complex and controlled by many genes (i.e., polygenic in nature) [31, 39]. Increased knowledge of the genetic basis of muscle quality traits will facilitate to advance the commercial breeding in salmonids. GWA studies are powerful tools to identify genetic variants associated with complex traits [36, 39, 40]. However, no GWA studies were previously conducted to dissect the genetic architecture of fillet fat and moisture contents in rainbow trout. The SNP-based heritability for fat and moisture content was 0.39 and 0.51, respectively, suggesting existence of adequate genetic variability in the NCCCWA fish population to allow genetic improvement through selective breeding. A higher rate of genetic gain is obtained when genomic information is used [48].

In this study, we used genomic windows of 50 SNPs of a 50K SNP chip to perform GWA analyses, in addition to the single-marker analysis approach, to identify genomic regions associated with the traits. Given that the 50 K, SNP chip contains SNPs of potential association with intramuscular fat content, all fish used to build the SNP-chip were excluded from the GWA analysis in the present study.

The fish population used for the current GWA analyses had an average muscle fat content of 9.2 ± 1.91 (%) and moisture content of 69.93 ± 1.75 (%). Variations in fat and moisture content are shown in Figure (1). Previous studies reported a significant correlation between changes in fat and moisture content in fish [26, 32]. Consistently, our data showed a significant negative correlation between fat and moisture content (R = -0.88; p-value = 6.3E-262). Further, fat content was regressed on moisture content using phenotypic data from separate year classes to make sure the size and age differences

between fish from YC 2010 and YC 2012 are not influencing the correlation between the traits. Interestingly, the negative correlation between fat and moisture content (R = -0.88) was maintained in fish from the two consecutive generations.

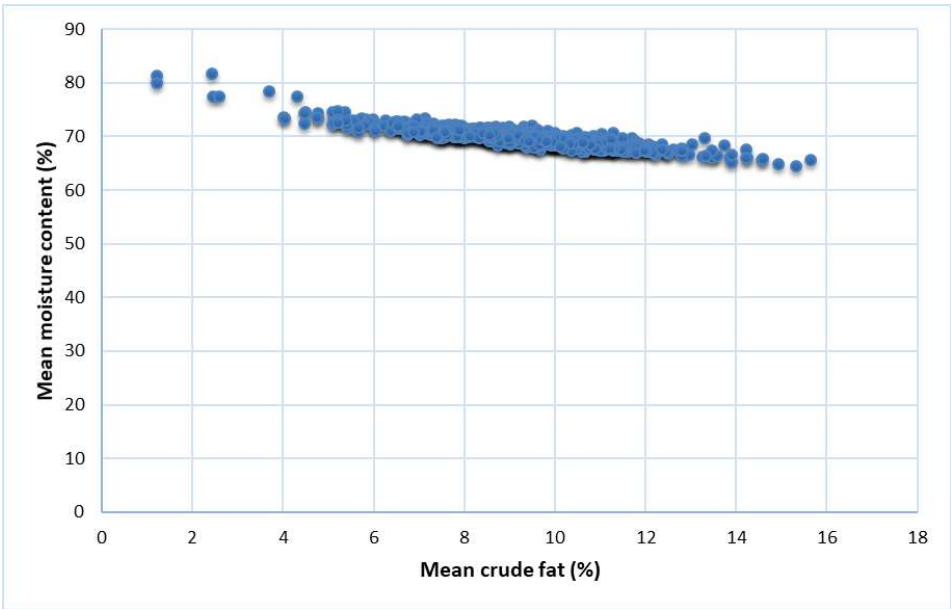

Figure 1. Reverse relationship between intramuscular fat and moisture content in fish used for GWA analyses.

QTL affecting muscle fat and moisture content using WssGBLUP

All 35,322 SNPs (70.6%) that passed QC were used in the WssGBLUP analysis. A complete list of proportions of additive genetic variance for fat content explained by all genomic windows is provided in Table S1. Of them, a total of 137 genomic sliding windows explaining at least 2% (arbitrary value) of the additive genetic variance for fat content are listed in Table S2. Most of the SNP sliding windows (n = 124; ~91%) were located within 62 protein-coding genes. Genomic loci affecting the additive variance for fat content were clustered in 5 chromosomes (1, 4, 5, 19, and 29) (Figure 2).

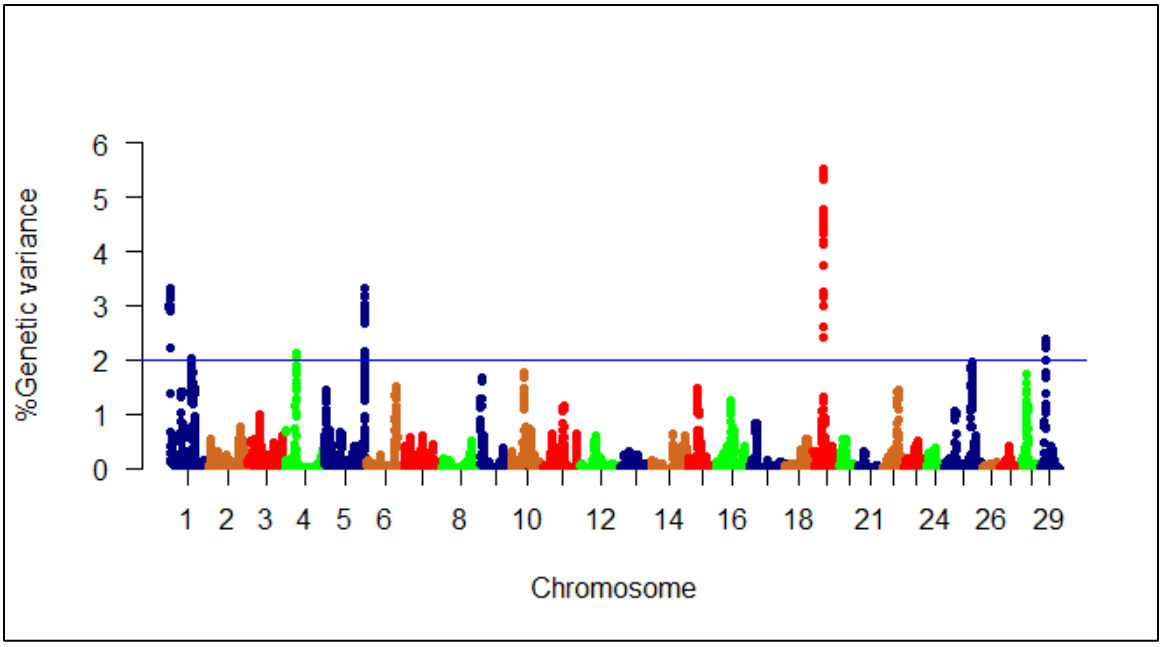

Figure 2. Manhattan plot showing association between 50 SNP-genomic sliding windows and muscle fat content. Chromosome 19 showed the highest peaks with genomic loci explaining up to 5.51% of the additive genetic variance. The basal blue line represents 2% of the genetic variance explained by the sliding windows.

Chromosome 19 harbored the highest number (n = 50) and the most significant peaks affecting fat content (up to 5.51%) (Table S2, Figure 2). Many of the SNPs were located within the CDS of the SNP-harboring genes (n = 58) as well as their 3'UTR (n = 55). In order to understand the biological significance of the QTL associated with fat content, we annotated the SNP-harboring genes and searched their functions in the literature (described below).

Similarly, a complete list of the proportions of additive genetic variance for moisture content explained by all windows identified in this study is provided in Table S3. A total of 178 genomic sliding windows revealing at least 2% of the additive genetic variance for moisture content are listed in Table S4. Most of the SNP sliding windows (n = 165; ~93%) were located within 86 genes coding for proteins. Genomic loci affecting the additive variance for moisture content were clustered on 5 chromosomes (5, 14, 19, 25, and 29) (Figure 3). Chromosome 29 harbored the highest number (n = 48), whereas the most significant peaks affecting moisture content (up to 4.46%) were identified on chromosome 19 (Table S4, Figure 3). Many of the SNPs were located within CDS of the SNP-harboring genes (n = 68) as well as their 3'UTR (n = 72).

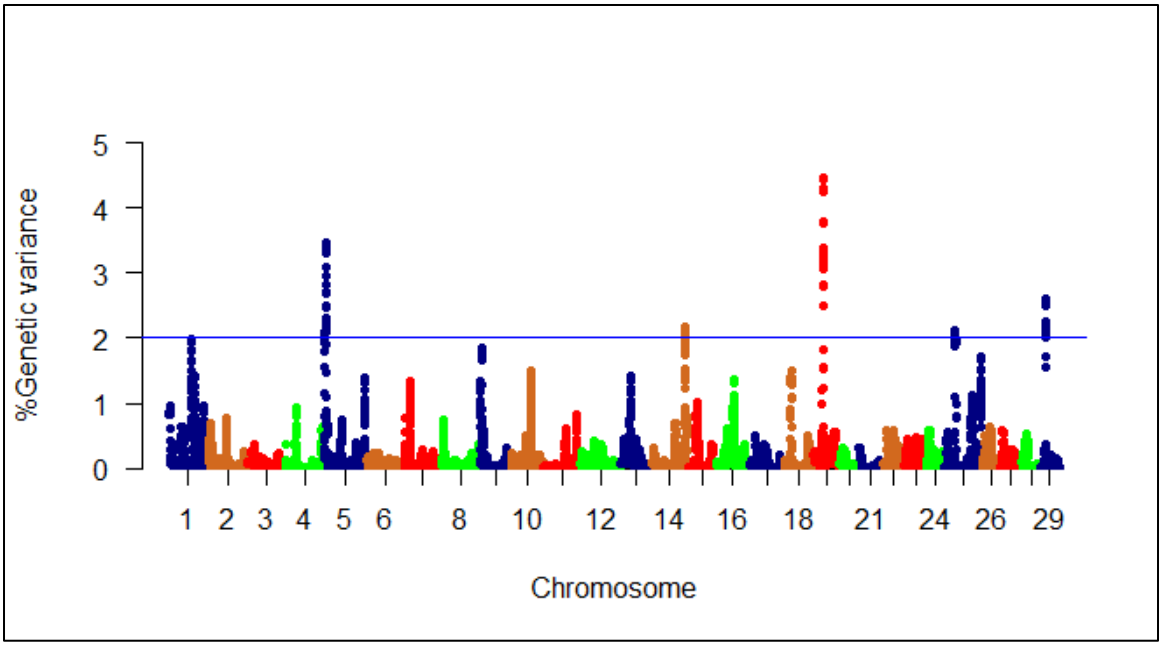

Figure 3. Manhattan plot showing association between 50 SNP-genomic sliding windows and muscle moisture content. Chromosome 19 showed the highest peaks with genomic loci explaining up to 4.46% of the additive genetic variance. The basal blue line represents 2% of the genetic variance explained by the sliding windows.

Common genes affecting muscle fat and moisture content

As shown above, a negative linear relationship has been established between fat and moisture content in this selectively bred rainbow trout population (YC 2010 and YC 2012), suggesting a common mechanism underlying the genetic variation in the two traits. This negative correlation was consistent with other studies in fish and mammals [23, 24, 26, 32]. In rainbow trout, the correlation between fat and moisture content depends on the physiological status of the fish. For instance, gravid fish approaching spawning and maintained on a high plane of nutrition showed reduced intramuscular fat with a concurrent increase in moisture, shear force, and protein content [26]. On the other hand, fat content was not affected during spawning, while moisture content increased [25]. This was explained by a selective mobilization of either fat or protein during sexual maturation. Depleted macromolecules were replenished by water [25, 26]. In addition to sexual maturation, season, feeding, starvation, temperature, salinity, and selection for WBW, were found to affect the fat/moisture balance and impact the product quality. For instance, increased fat content due to fast growth of salmon in the summer was accompanied by a high drip loss [49]. The drip loss negatively impacted the sensory attributes and developed unpleasant odors [49]. Exposure of juvenile salmon to a high salinity stimulated lipid depletion that was partially explained by increased depot lipase activity [50]. Channel catfish fed supplemental diets deposited fat concomitant with loss of moisture. Starved

channel catfish at 8.9ºC mobilized muscular fat to supply energy for metabolic process, whereas both fat and protein were mobilized at 21.1ºC; in either case, moisture content increased [51]. Selection for WBW in rainbow trout [30] and gilthead seabream [32] led to high muscular fat content associated with a decline in moisture content (in particular, the more loosely bound water).

The current WssGBLUP identified common SNPs affecting the additive genetic variance for fat and moisture content on chromosomes 19 and 29 (Tables S2 & S4). The majority of the common SNPs (n = 47) were located on chromosome 19. Thirty-two SNPs, out of 47, involved in lipid metabolism were identified in 16 protein-coding genes on chromosome 19 (Table 1). Briefly, cathepsin B had a single 3'UTR SNP. Cathepsin B regulates very-low-density lipoprotein (VLDL) secretion and free fatty acid uptake in response to oleic acid exposure in mice [52]. Thioredoxin-related transmembrane protein 1-like (TMX1) had three SNPs. Loss of TMX increases lipid peroxidation in TMX(−/−) mice, which, in turn, enhances oxidative stress [53]. Guanine nucleotide-binding protein GI/GS/GO gamma-2 subunit (GNG2) had a single 3'UTR SNP. GNG2 expression is positively correlated with adipocyte size [54]. SNPs in genes encoding beta-taxilin and Alpha-L-fucosidase 2 (FUCA2) were covering windows explaining the highest proportion of the additive genetic variation for fat and moisture content. Adipose tissue of obesity susceptible and resistant rats differentially expressed beta-taxilin under a high-fat diet [55]. FUCA2 is a glycolipid processing enzyme [56]. Two SNPs in F-box only protein 30 (FBXO30) and the microtubule-binding protein ensconsin were ranked next to beta-taxilin and FUCA2. An SNP in FBXO30 was located in a genomic region, explaining 4.95% of the additive genetic variance for polyunsaturated fatty acids in cattle [57]. Knockdown of microtubule-binding or -associated proteins led to changes in fat accumulation during adipogenesis [58]. Dihydropyrimidinase-related protein 5-like (CRMP5) had a single synonymous SNP. CRMP5 has GO terms belong to lipid metabolic processes [59]. Five SNPs were identified in a gene encoding trifunctional enzyme subunit alpha, mitochondrial (HADHA). This gene is involved in fatty acid beta-oxidation [59]. mRNA decay activator protein ZFP36L1 had three SNPs in the 3'UTR. Knockdown of mammalian ZFP36L1 led to downregulation of ERK activation and inhibition of adipogenesis [60]. A single 3'UTR SNP was identified in ELM2 and SANT domain-containing protein 1 (ELMSAN1). Epigenome-wide association analysis showed DNA methylation changes in ELMSAN1 were associated with body mass index (a key measure of adiposity) [61]. Prostaglandin reductase 2 (PTGR2) had two nonsynonymous SNPs. This enzyme catalyzes reduction of the conjugated α,β-unsaturated double bond of 15-keto-PGE2 in an NADPH-dependent manner, which is a critical step in inhibition of PPARγ-mediated adipocyte differentiation [62]. Spectrin beta chain, erythrocytic (SPTB) gene had two SNPs. The SPTB interacts with phospholipids in natural [63] and model membrane systems [64] and has a role in controlling the fluidity of the inner lipid leaflet of the cell membrane (reviewed in [65]).

275

276 Table 1. SNP markers in genomic sliding windows explaining at least 2% of the genetic
277 variance for fat and moisture content and involved in lipid metabolism. A color gradient
278 on the left indicates differences in additive genetic variance explained by windows
279 containing the representative SNP marker (green is the highest and red is the lowest).
280 SNPs are sorted according to their chromosome positions.

281

Var%_Fat	Var%_Moisture	CHR	Position	Strand	Gene ID	Function	Gene annotation	Region/effect
3.25	3.16	19	23140902	+	LOC110497560	Lipid metabolism	cathepsin B-like	3'UTR
4.19	3.77	19	23257105	+	LOC110497565	Lipid metabolism	thioredoxin-related transmembrane protein 1-like	CDS/nonsyn
4.15	3.77	19	23259435	+	LOC110497565	Lipid metabolism	thioredoxin-related transmembrane protein 1-like	3'UTR
4.15	3.78	19	23260053	+	LOC110497565	Lipid metabolism	thioredoxin-related transmembrane protein 1-like	3'UTR
4.14	3.78	19	23443626	+	gbg2	Lipid metabolism	Guanine nucleotide-binding protein GI/GS/GO gamma-2 subunit	3'UTR
5.50	4.45	19	23495709	-	LOC110497567	Lipid metabolism	beta-taxilin-like	CDS/syn
5.51	4.46	19	23496538	-	LOC110497567	Lipid metabolism	beta-taxilin-like	CDS/syn
5.51	4.46	19	23527643	-	fuca2	Lipid metabolism	alpha-L-fucosidase 2	3'UTR
5.33	4.28	19	23528291	-	fuca2	Lipid metabolism	alpha-L-fucosidase 2	3'UTR
5.42	4.31	19	23699374	-	LOC110497583	Lipid metabolism	F-box only protein 30-like	3'UTR
5.32	4.26	19	24247835	-	LOC110497599	Lipid metabolism	ensconsin-like	3'UTR
4.66	3.39	19	24329620	-	LOC110497602	Lipid metabolism	dihydropyrimidinase-related protein 5-like	CDS/syn
4.55	3.22	19	24502571	-	LOC110497612	Lipid metabolism	trifunctional enzyme subunit alpha, mitochondrial-like	3'UTR
4.56	3.23	19	24503147	-	LOC110497612	Lipid metabolism	trifunctional enzyme subunit alpha, mitochondrial-like	3'UTR
4.56	3.24	19	24509666	-	LOC110497612	Lipid metabolism	trifunctional enzyme subunit alpha, mitochondrial-like	CDS/nonsyn
4.67	3.33	19	24512704	-	LOC110497612	Lipid metabolism	trifunctional enzyme subunit alpha, mitochondrial-like	CDS/nonsyn
4.63	3.28	19	24512932	-	LOC110497612	Lipid metabolism	trifunctional enzyme subunit alpha, mitochondrial-like	CDS/syn
4.47	3.20	19	25037839	+	LOC110497620	Lipid metabolism	mRNA decay activator protein ZFP36L1-like	3'UTR
4.33	3.19	19	25039247	+	LOC110497620	Lipid metabolism	mRNA decay activator protein ZFP36L1-like	3'UTR
4.33	3.18	19	25039632	+	LOC110497620	Lipid metabolism	mRNA decay activator protein ZFP36L1-like	3'UTR
3.74	3.07	19	25366465	-	LOC110498698	Lipid metabolism	ELM2 and SANT domain-containing protein 1-like	3'UTR
3.74	3.07	19	25409419	+	ptgr2	Lipid metabolism	prostaglandin reductase 2	CDS/nonsyn
3.74	3.07	19	25414599	+	ptgr2	Lipid metabolism	prostaglandin reductase 2	CDS/nonsyn
3.74	3.07	19	25563733	+	LOC110497630	Lipid metabolism	spectrin beta chain, erythrocytic-like	CDS/nonsyn
3.74	3.07	19	25581001	+	LOC110497630	Lipid metabolism	spectrin beta chain, erythrocytic-like	CDS/syn
2.24	2.54	29	10494611	+	LOC110509620	Lipid metabolism	short-chain specific acyl-CoA dehydrogenase, mitochondrial-like	3'UTR
2.39	2.61	29	10714305	+	LOC110509628	Lipid metabolism	arrestin domain-containing protein 3-like	mRNA
2.39	2.62	29	10776686	+	LOC110509321	Lipid metabolism	myocyte-specific enhancer factor 2C-like	5'UTR
2.23	2.59	29	10798819	+	LOC110509321	Lipid metabolism	myocyte-specific enhancer factor 2C-like	mRNA
2.24	2.59	29	10800769	+	LOC110509321	Lipid metabolism	myocyte-specific enhancer factor 2C-like	CDS/syn
2.22	2.59	29	10801091	+	LOC110509321	Lipid metabolism	myocyte-specific enhancer factor 2C-like	CDS/syn
2.01	2.53	29	10801136	+	LOC110509321	Lipid metabolism	myocyte-specific enhancer factor 2C-like	CDS/syn

282

283 Chromosome 29 had 14 SNPs in genomic windows explaining at least 2% of the
284 additive genetic variance for intramuscular fat and moisture content (Tables S2 & S4). Of
285 them, seven SNPs were involved in lipid metabolism (Table 1). A single SNP was
286 identified in a gene encoding short-chain specific acyl-CoA dehydrogenase, mitochondrial
287 (ACADS). This enzyme has a role in fatty acid beta-oxidation [66]. An intronic SNP was
288 identified in a gene coding for arrestin domain-containing protein. The latter has GO terms
289 belonging to fat pad and skin development and regulates the body mass [67]. Myocyte
290 enhancer factor 2c (MEF2C) had the highest number of SNPs (n = 5) on chromosome 29.

291 MEF2C is a transcription factor involved in skeletal muscle differentiation; however, it has
292 been reported as a constituent of a mechanism that programs gene expression involved in
293 development of brown adipocytes [68]. MEF2A and MEF2D isoforms exhibited *in vivo*
294 differential expression in mammalian striated muscle and white adipose tissue of insulin-
295 deficient diabetic mice [69]. To our knowledge, the role of MEF2C in white adipose tissue
296 remains uncertain.

297 In addition, twelve SNPs in genes involved in transmembrane transport and
298 cytoskeleton remodeling were identified in common QTL affecting additive variance for
299 fat and moisture content (Table 2). The majority of these SNPs were identified on
300 chromosome 19 (n = 11). Three synonymous SNPs were identified in a gene encoding
301 intersectin-2 (ITSN2). This protein is necessary for the clathrin-mediated endocytosis and
302 actin cytoskeleton remodeling [70]. Six SNPs were identified in 3 genes involved in
303 vesicle-mediated transport (i.e., exocytosis); dnaJ homolog subfamily C member 5B,
304 visinin-like protein 1, and syntaxin-binding protein 5. The actin cytoskeleton remodeling
305 controls each step of exocytosis [71]. Three SNPs were identified in microtubule-
306 associated protein RP/EB family member 3 (MAPRE3) and centrin-3. MAPRE3 and
307 centrin-3 control the dynamics of the microtubule cytoskeleton [59, 72].

308 Overall, the analysis revealed that most of the loci in association with both fat and
309 moisture content are involved in lipid metabolic process. Therefore, our results suggest
310 that variation in moisture content is likely to be driven by changes in fat content in an
311 antagonistic fashion. This notion was previously suggested as depletion of
312 macromolecules under catalytic muscle conditions is likely forming voids for water
313 accumulation [28].

314 Table 2. SNP markers in genomic sliding windows explaining at least 2% of the genetic
315 variance for fat and moisture content and involvement in transmembrane transport and
316 cytoskeleton regulation. A color gradient on the left indicates differences in additive
317 genetic variance explained by windows containing the representative SNP marker (green
318 is the highest and red is the lowest). SNPs are sorted according to their chromosome
319 positions.

320

Var%_Fat	Var%_Moisture	CHR	Position	Strand	Gene ID	Function	Gene annotation	Region/effect
2.60	2.81	19	23060380	-	LOC110497555	Endocytosis & cytoskeleton	intersectin-2	CDS/syn
2.59	2.80	19	23062680	-	LOC110497555	Endocytosis & cytoskeleton	intersectin-2	CDS/syn
3.17	3.11	19	23082360	-	LOC110497555	Endocytosis & cytoskeleton	intersectin-2	CDS/syn
5.33	4.28	19	23561806	-	LOC110497574	Exocytosis	dnaJ homolog subfamily C member 5B	3'UTR
5.33	4.29	19	23621300	-	LOC110497579	Exocytosis	visinin-like protein 1	3'UTR
5.34	4.31	19	23627722	-	LOC110497579	Exocytosis	visinin-like protein 1	5'UTR
5.42	4.31	19	23627740	-	LOC110497579	Exocytosis	visinin-like protein 1	5'UTR
5.32	4.26	19	23838038	+	LOC110497586	Exocytosis	syntaxin-binding protein 5	CDS/syn
5.33	4.26	19	23851302	+	LOC110497586	Exocytosis	syntaxin-binding protein 5	3'UTR
5.34	4.30	19	24316246	-	LOC110497601	Microtubule cytoskeleton	microtubule-associated protein RP/EB family member 3	3'UTR
4.54	3.34	19	24317314	-	LOC110497601	Microtubule cytoskeleton	microtubule-associated protein RP/EB family member 3	3'UTR
2.39	2.61	29	10746517	+	LOC110509629	Microtubule binding	centrin-3	CDS/nonsyn

321

322 ***Unique genes affecting the additive genetic variance for moisture***

323 The actin cytoskeleton interacts with the cell membrane to control water transport [73].
324 Expression of genes involved in the cytoskeletal organization has previously shown a
325 positive correlation with the drip loss [74]. In the current study, thirty-five variants in
326 genes (n = 14) involved in cytoskeleton remodeling were identified, affecting the additive
327 variance for moisture content in rainbow trout (Table 3). Briefly, bone morphogenetic
328 protein receptor type-2 (BMPR2) had a single synonymous SNP. BMPR2 is known to
329 interact with the cytoskeleton, and BMPR2 mutant mice exhibited cytoskeletal defects
330 [75]. A single SNP was identified in a gene encoding muscle associated receptor tyrosine
331 kinase (MUSK). Activation of MUSK in myotubes regulates the reorganization of the
332 actin cytoskeleton [76]. Two SNPs were identified in THAP domain containing 1
333 (THAP1), which has a role in regulation of the mitotic cell cycle [77]. The gene encoding
334 asparaginyl-tRNA synthetase (NARS) had 3 SNPs in windows explaining the highest
335 additive variance (up to 3.46%; Table 3). Mutations in NARS leads to cell cycle arrest in
336 the S phase [78]. The actin cytoskeleton undergoes dramatic changes during the cell cycle
337 [79]. Ten SNPs were identified in three genes coding for cyclin-I (CCNI), cyclin-G1
338 (CCNG1), and cyclin-G2 (CCNG2). Cyclins function as regulators of the cell cycle and
339 actin cytoskeleton dynamics (reviewed in [80]). The serine/threonine-protein, phosphatase
340 2A (PP2A), had a 3'UTR SNP. This phosphatase is associated with microtubule
341 stabilization, where it binds and dephosphorylates the microtubule-associated proteins
342 [81]. Annexin A6 (ANXA6) had two synonymous SNPs. ANXA6 contributes to
343 membrane and cytoskeleton organization in a Ca^{2+}-dependent manner [82]. Tubulin beta-
344 4B chain (TUBB4B) had four synonymous SNPs within 1Kb of chromosome 25.
345 TUBB4B is a critical component of microtubules [59]. Five SNPs, clustered in ~2Kb, were
346 identified in a gene coding for mid1-interacting protein 1 (MID1IP1). This protein
347 enhances fatty acid biosynthesis [83] and stabilizes microtubule organization [59]. Two
348 SNPs were identified in a gene encoding tubulin-specific chaperone A (TBCE). TBCE is

349 a tubulin-folding protein required for proper microtubule cytoskeleton organization [84].
350 Additionally, mutations in TBCE drive muscle atrophy [84]. Proteinase-activated receptor
351 1 (PAR1) and PAR2 had four SNPs. PAR-mediated, RhoA activation is vital for
352 cytoskeletal reorganization [85].

353 Table 3. SNP markers in genomic sliding windows explaining at least 2% of the genetic
354 variance for moisture content and involved in cell cycle and cytoskeleton regulation. A
355 color gradient on the left indicates differences in additive genetic variance explained by
356 windows containing the representative SNP marker (green is the highest and red is the
357 lowest). SNPs are sorted according to their chromosome positions.

358

Var%	CHR	Position	Strand	Gene ID	Function	Gene annotation	Region/effect
3.42	5	1581801	+	LOC110524928	Cytoskeleton	bone morphogenetic protein receptor type-2	CDS/syn
3.43	5	2416345	+	musk	Cytoskeleton	muscle associated receptor tyrosine kinase	3'UTR
3.39	5	3076608	-	thap1	Cell cycle	THAP domain containing 1	3'UTR
3.39	5	3077900	-	thap1	Cell cycle	THAP domain containing 1	CDS/syn
3.34	5	3948426	+	nars	Cell cycle	asparaginyl-tRNA synthetase	CDS/syn
3.45	5	3954845	+	nars	Cell cycle	asparaginyl-tRNA synthetase	CDS/syn
3.46	5	3956515	+	nars	Cell cycle	asparaginyl-tRNA synthetase	3'UTR
2.72	5	4499566	+	LOC110523126	Cell cycle	cyclin-I	5'UTR
2.49	5	4515345	-	LOC110523127	Cell cycle	cyclin-G2	3'UTR
2.48	5	4517902	-	LOC110523127	Cell cycle	cyclin-G2	CDS/syn
2.31	5	4523733	+	LOC110523126	Cell cycle	cyclin-I	mRNA
2.30	5	4529514	+	LOC110523126	Cell cycle	cyclin-I	CDS/nonsyn
2.30	5	4533058	+	LOC110523126	Cell cycle	cyclin-I	CDS/nonsyn
2.30	5	4539962	+	LOC110523126	Cell cycle	cyclin-I	CDS/nonsyn
2.31	5	4545000	+	LOC110523126	Cell cycle	cyclin-I	CDS/nonsyn
2.09	5	4547885	+	LOC110523126	Cell cycle	cyclin-I	3'UTR
2.08	14	74268218	+	LOC110489167	Cell cycle	cyclin-G1	3'UTR
2.08	14	74743050	-	LOC110489177	Cytoskeleton	serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform	3'UTR
2.00	14	75046230	-	LOC110489191	Cytoskeleton	annexin A6	CDS/syn
2.00	14	75047544	-	LOC110489191	Cytoskeleton	annexin A6	CDS/syn
2.05	25	23765983	-	LOC110504922	Cytoskeleton	tubulin beta-4B chain	CDS/syn
2.04	25	23766034	-	LOC110504922	Cytoskeleton	tubulin beta-4B chain	CDS/syn
2.04	25	23766334	-	LOC110504922	Cytoskeleton	tubulin beta-4B chain	CDS/syn
2.05	25	23766912	-	LOC110504922	Cytoskeleton	tubulin beta-4B chain	CDS/syn
2.09	25	23967070	+	m1ip1	Cytoskeleton	Mid1-interacting protein 1	5'UTR
2.09	25	23968673	+	m1ip1	Cytoskeleton	Mid1-interacting protein 1	3'UTR
2.11	25	23969016	+	m1ip1	Cytoskeleton	Mid1-interacting protein 1	3'UTR
2.05	25	23969082	+	m1ip1	Cytoskeleton	Mid1-interacting protein 1	3'UTR
2.05	25	23969158	+	m1ip1	Cytoskeleton	Mid1-interacting protein 1	3'UTR
2.02	29	12409282	+	LOC110509670	Cytoskeleton	tubulin-specific chaperone A	CDS/syn
2.02	29	12410990	+	LOC110509670	Cytoskeleton	tubulin-specific chaperone A	3'UTR
2.02	29	12653356	-	LOC110509674	Cytoskeleton	proteinase-activated receptor 2	3'UTR
2.02	29	12654062	-	LOC110509674	Cytoskeleton	proteinase-activated receptor 2	CDS/syn
2.07	29	12654462	-	LOC110509674	Cytoskeleton	proteinase-activated receptor 2	CDS/nonsyn
2.10	29	12665454	-	LOC110509677	Cytoskeleton	proteinase-activated receptor 1	3'UTR

359

360 A strong positive correlation between moisture and protein content has been established
361 in different species. A simultaneous decline in protein and moisture content was previously
362 reported in mammals [24]. Moisture content in rainbow trout exhibited a bidirectional
363 relationship with protein content depending on physiological/metabolic status. For
364 example, a negative correlation between moisture and protein content were previously

365 reported under muscle catabolic conditions associated with full sexual maturation (R^2 =
366 0.994, $p < 0.01$) [25]; whereas, a positive correlation was reported in female trout, on a
367 high plane of nutrition, that were approaching spawning [26]. This was explained by
368 selective mobilization of either protein during spawning or fat before spawning; in either
369 case, the depleted macromolecule was replaced by water. It is noteworthy that protein
370 content variation of the current study was not statistically significant between the 4 high-
371 ranked families versus 4 low-ranked families (data not shown). The current WssGBLUP
372 analysis indicated that thirteen SNPs in genes involved in protein degradation were
373 involved in the additive genetic variance of moisture content (Table 4). Briefly, E3
374 ubiquitin-protein ligase RNF170 is an E3 ubiquitin-protein ligase that plays an essential
375 role in the ubiquitination and degradation of inositol 1,4,5-trisphosphate receptor type 1
376 (ITPR1) [59, 86]. The latter controls the calcium release from the endoplasmic reticulum
377 [87], which affects the muscle protein content in rainbow trout [39] and has a profound
378 effect on the regulation of cytoskeleton [88]. Cystatin-1, which possesses a peptidase
379 inhibitor activity, had a single 5'UTR SNP. Thioredoxin-like 1 (TXNL1) had two
380 synonymous SNPs. The knockdown of TXNL1 moderately stabilizes the ubiquitin-protein
381 conjugates suggesting a connection between protein reduction and proteolysis [89]. Pre-
382 mRNA-processing factor 19 (PRPF19) and ubiquitin-conjugating enzyme E2 D2
383 (UBE2D2) had four SNPs. These ligases catalyze polyubiquitin chain assembly and play
384 a role in proteasomal protein degradation [90, 91]. Nuclear factor NF-kappa-B p105
385 subunit (NFKB1) had two 3'UTR SNPs. NFKB1 is involved in the negative regulation of
386 cellular protein metabolic process [92] and apoptotic process [93].

387 Table 4. SNP markers in genomic sliding windows explaining at least 2% of the genetic
388 variance for moisture content and involved in proteolytic activities. A color gradient on
389 the left indicates differences in additive genetic variance explained by windows containing
390 the representative SNP marker (green is the highest and red is the lowest). SNPs are sorted
391 according to their chromosome positions.

Var%	CHR	Position	Strand	Gene ID	Function	Gene annotation	Region/effect
3.42	5	1402874	-	LOC110523084	Ubiquitin-protein ligase	E3 ubiquitin-protein ligase RNF170	3'UTR
3.43	5	2249297	+	LOC110523107	peptidase inhibitor	cystatin-1	5'UTR
3.37	5	3931855	-	txnl1	Redox homeostasis	thioredoxin like 1	CDS/syn
3.34	5	3941358	-	txnl1	Redox homeostasis	thioredoxin like 1	CDS/syn
2.05	25	23666377	-	LOC110504917	Ubiquitin-protein ligase	pre-mRNA-processing factor 19	CDS/syn
2.05	25	23690277	+	LOC110504919	Ubiquitin-protein ligase	ubiquitin-conjugating enzyme E2 D2	5'UTR
2.05	25	23698043	+	LOC110504919	Ubiquitin-protein ligase	ubiquitin-conjugating enzyme E2 D2	3'UTR
2.06	25	23698573	+	LOC110504919	Ubiquitin-protein ligase	ubiquitin-conjugating enzyme E2 D2	3'UTR
2.06	25	23700241	-	LOC110504918	Transcription factor	nuclear factor NF-kappa-B p105 subunit	3'UTR
2.06	25	23700272	-	LOC110504918	Transcription factor	nuclear factor NF-kappa-B p105 subunit	3'UTR
2.12	29	11141837	+	LOC110509643	Phagosome	ras-related protein rab7	3'UTR
2.03	29	11416104	+	LOC110509654	Phagosome	V-type proton ATPase subunit B, brain isoform	3'UTR
2.03	29	12313543	+	LOC110509669	Lysosome	AP-3 complex subunit beta-1	CDS/syn

392 In addition to the ubiquitin-protein ligases, SNPs in three genes involved in
393 lysosomal/phagosomal pathways were identified. Ras-related protein rab7 (RAB7A)
394 harbored a 3'UTR SNP. RAB7A is a major regulator of endo-lysosomal maturation/
395 trafficking and protein targeting to lysosome inducing autophagosome formation [94].
396 Thus, RAB7A positively regulates the protein catabolic process [95]. V-type proton
397 ATPase subunit B (ATP6V1B2) had a 3'UTR SNP. V-ATPase is responsible for acidifying
398 the intracellular compartments, including lysosomes [96]. The gene encoding the β chain
399 of the adaptor protein-3 (AP-3) complex had a single synonymous SNP. Deletion in
400 AP3B1 perturbs assembly of AP-3 complex and, in turn, trafficking of transmembrane
401 lysosomal proteins [97].

402 In this study, most of the common genomic loci affecting the highest proportion of the
403 additive variance were involved in lipid metabolism, suggesting a common mechanism
404 underlying intramuscular fat and moisture content and, partially, explaining the strong
405 negative correlation between the fat and moisture content in this selectively bred rainbow
406 trout population. Unique loci affecting moisture content were primarily involved in
407 cytoskeleton regulations and protein turnover. Inhibition of protease activity, such as
408 calpains, reduced degradation of proteins responsible for cell membrane-cytoskeleton
409 attachments and postmortem drip channel formation in muscle [98]. The presence of
410 calcium enhances proteolysis, by μ-Calpain, of myofibrillar and other cytoskeletal proteins
411 during postmortem storage [45, 99]. Further investigation is warranted to determine QTL
412 that could be prioritized in breeding programs to achieve optimal moisture content with
413 low enzymatic activity and drip loss, and optimal fat content that can meet consumer
414 preferences.

415 Overall, the WssGBLUP analysis has enriched the current understanding of the genetic
416 architecture of the fat and moisture content in rainbow trout. Common SNP windows
417 explained a high proportion of the additive genetic variance associated with both fat and
418 moisture content, suggesting common regulatory mechanisms. Knowledge of the
419 heritability of fat and moisture content and their correlations with other traits is needed for
420 establishment of sustained multi-trait selection programs. The aquaculture industry is
421 interested in implementing genomic selection in the breeding programs; however, applying
422 high-density SNP chips is cost-prohibitive for small-sized hatcheries and companies. SNPs
423 with a high-effect on genetic variances of fat and moisture content, identified in this study,
424 could be prioritized to reduce SNP panel density needed to evaluate the predictive abilities
425 for both traits. In another study, we found that prioritizing SNPs based on the proportion
426 of variance explained for muscle yield and firmness allowed to reduce the SNP panel
427 density down to ~800 SNPs. Reduced SNP panels outperformed the traditional PBLUP
428 model in predicting the future fish performance, and maintained predictive abilities
429 comparable to the 50K SNP panel (data will be published elsewhere).

430

431 **Single marker GWA analyses**

432 To identify single SNP markers associated with variation in fat and moisture content,
433 we analyzed SNPs that passed QC filtration (n = 29,451) using a generalized score test;
434 this test incorporates multiple covariates in the analysis and accounts for family structure
435 using a kinship matrix [100]. In this study, 8 and 24 significant SNPs, surpassing the
436 genome-wide significance level, had a potential impact on the fat and moisture content
437 (Bonferroni-corrected $p < 1.69E-06$; Figures 4 & 5 and Tables S5 & S6), respectively.
438 Whereas, 29 and 46 SNPs surpassing the suggestive significance level (Bonferroni-
439 corrected $p < 1E-05$; Figures 4 & 5 and Tables S5 & S6) were detected in association with
440 fat and moisture content, respectively. Suggestive significant SNPs were not considered
441 for the downstream analysis.

442 SNPs associated with the fat content were mainly located on chromosome 5 (n = 7),
443 and SNP-harboring genes have roles in lipid metabolism (Table 5). The list includes 78
444 kDa glucose-regulated protein (GRP78), spindle and kinetochore associated complex
445 subunit 1 (SKA1), apelin receptor B (APLNR-B), desmoplakin, podocan, and calcium-
446 binding mitochondrial carrier protein SCaMC-1 (SLC25A24). Briefly, two missense
447 mutations were identified in genes coding for GRP78 and SKA1. GRP78 is essential for
448 adipocyte differentiation and a balanced secretion of adipokines. Deletion of GRP78
449 causes lipoatrophy in mice observed as a dramatic reduction in gonadal and subcutaneous
450 adipose tissue [101]. SKA1 was downregulated in adipose tissues between samples from
451 obese and healthy control children and has been suggested as a candidate biomarker for
452 childhood obesity [102]. Three synonymous mutations were identified in genes encoding
453 APLNR, desmoplakin, and podocan. APLNR knockout mice demonstrated excess fatty
454 acid accumulation in skeletal muscle [103]. Abnormalities in desmoplakin have been
455 associated with changes in lipid metabolism [104]. Podocan belongs to the small leucine-
456 rich proteoglycans (SLRPs) that bind to low-density lipoprotein receptor-related protein
457 (LRP-1) [105]. A 3'UTR SNP was identified in a gene coding for SLC25A24. Mice fed a
458 high-fat diet exhibited increased expression level of SLC25A24; whereas, adipocyte
459 differentiation was suppressed in Slc25a24- knockout [106].

460 Puromycin-sensitive aminopeptidase (NPEPPS), on chromosome 17, had a
461 nonsynonymous SNP explaining the highest variability in fat content ($R^2 = 3.2\%$) (Table
462 5). NPEPPS impacts different physiological processes, including protein turnover and cell
463 cycle regulation. NPEPPS was upregulated in mitten crabs fed with a linseed oil rich in
464 linoleic acid [107]. However, the effect NPEPPS on lipid metabolism in fish needs further
465 investigations.

466

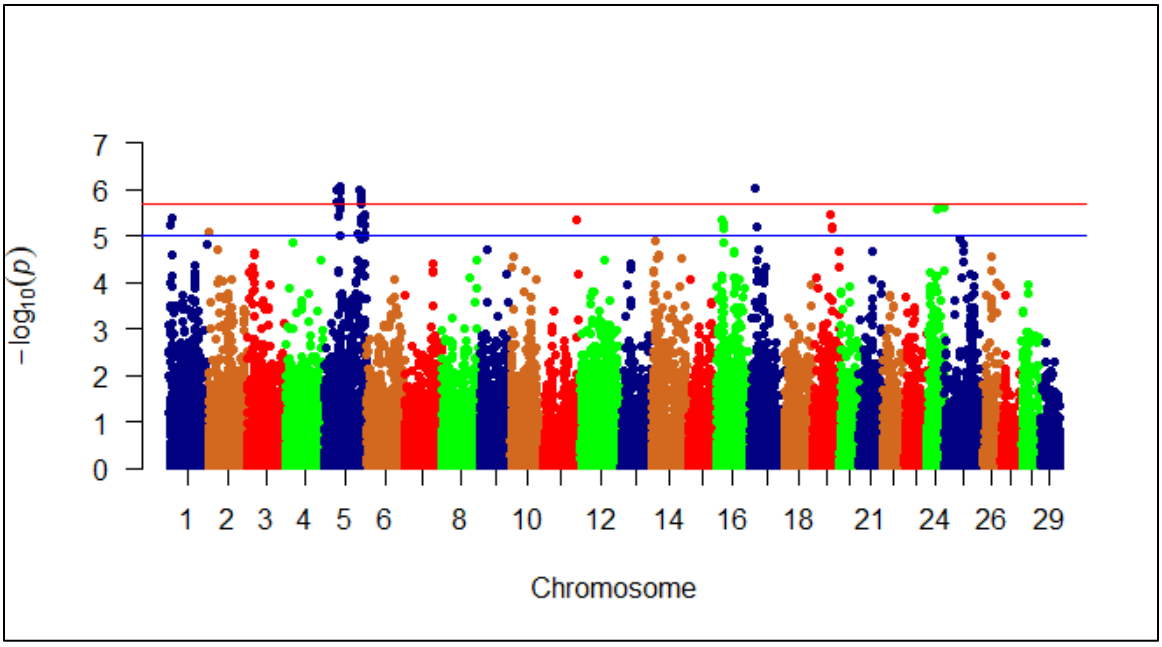

Figure 4. Manhattan plot showing single SNP markers significantly associated with variations in muscle fat content using family-based association analysis. Many of the significant SNPs are located on chromosome 5. Blue and red horizontal lines represent suggestive (1e-05) and significant (1.69e-06) threshold p-values, respectively.

SNPs associated with moisture content (n = 24) were associated with protein turnover, calcium metabolism, and cytoskeleton integrity (Table S6). Most of these SNPs (n = 11; ~46%) were located on chromosome 17. An SNP in a gene coding for acylphosphatase 2 ranked at the top of the list (R^2 = 7.4%) (Table 5); however, its physiological role is not clear. Eight SNPs associated with moisture content were identified in five genes engaged in protein metabolism (Table 5). These genes are NPEPPS, eukaryotic initiation factor 4A-III (EIF4A3), eukaryotic translation initiation factor 4B (EIF4B), ribosome binding protein 1 (RRPB1), and F-box only protein 46 (FBXO46). Briefly, the aminopeptidase, NPEPPS, was associated with variation in fat and moisture content, suggesting a correlation between moisture and fat content. Five SNPs were identified in two genes encoding EIF4A3 and EIF4B, suggesting a role for the protein translation machinery in determining variation in moisture content. RRPB1 is an ER integral membrane protein implicated in polysome assembly and, therefore, protein synthesis [108]. RRPB1 has been suggested as essential in regulation of UPR signaling molecules and autophagy [109]. Finally, the F-box family SCF-E3 ubiquitin ligase, FBXO46, had a single 3'UTR SNP.

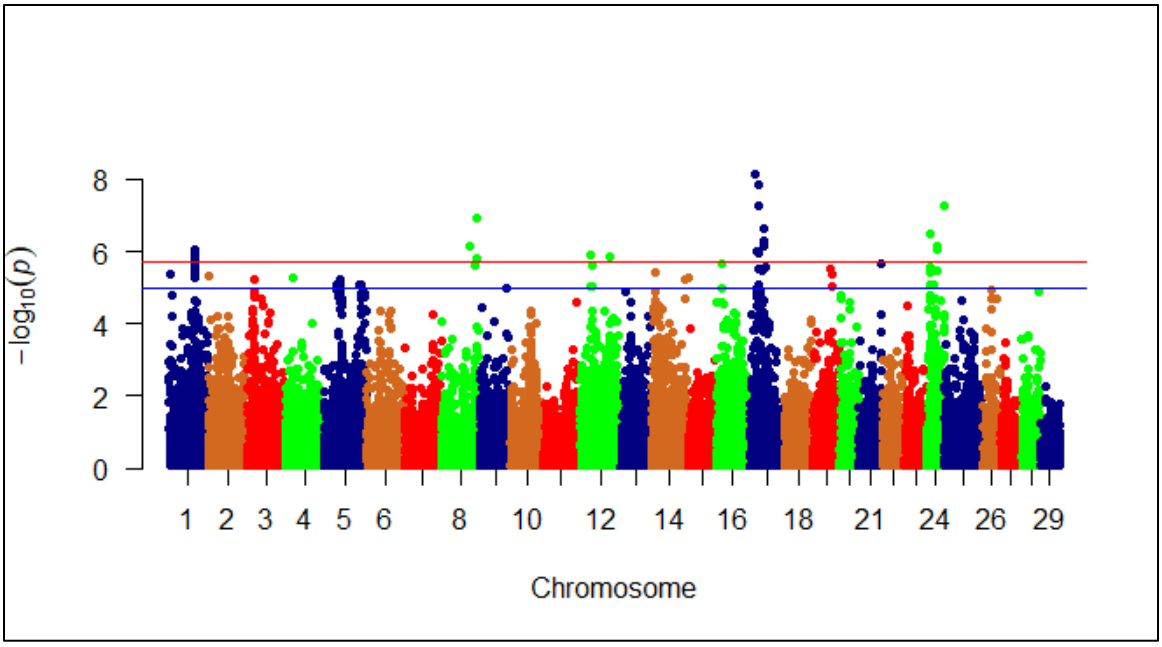

Figure 5. Manhattan plot showing single SNP markers associated with variations in moisture content using family-based association analysis. Most of the significant SNPs are located on chromosome 17. Blue and red horizontal lines represent suggestive (1e-05) and significance (1.69e-06) threshold p-values, respectively.

A previous report showed that expression of genes involved in the actin cytoskeleton and cytoskeletal organization is positively correlated with drip loss in pig [74]. In this study, a total of 10 SNPs were associated with moisture content were identified in seven genes engaged in cytoskeleton regulation (Table 5). These genes are encoding serum response factor (SRF), kinesin-1 heavy chain (KIF5B), inositol-trisphosphate 3-kinase C (IP3KC), supervillain (SVIL), calcineurin subunit B type 1 (PPP3R1), eIF4A and EIF4B. Briefly, SRF is a master regulator of the actin cytoskeleton [110]. Mutations in KIF5A caused cytoskeletal defects in humans [111]. Two, 3'UTR SNPs were identified in IP3KC and PPP3R1. IP3K and calcium/calcineurin signaling play critical roles in maintaining Ca^{2+} homeostasis that has a profound effect on the cytoskeleton [88, 112, 113]. Supervillin (SVIL) is one of the first components of the costameric membrane skeleton to assemble during muscle formation. It establishes a high-affinity connection between the membranes and actin cytoskeleton [114]. Translation initiation factors, including eIF4A and EIF4B, associate with the actin cytoskeleton, which affects protein synthesis [115].

506 Table 5. SNP markers significantly associated with variability in fat and moisture content
507 using family-based association analysis. A color gradient on the left shows the phenotypic
508 variation explained by each SNP marker (green is the highest and red is the lowest). SNPs
509 associated with the phenotypes are sorted according to their chromosome positions.

510

R^2	CHR	Position	P_RAO	Region|effect	Strand	Gene ID	Gene annotation	Trait
0.016	1	54958459	1.26E-06	CDS|syn	-	LOC110525667	serum response factor-like	Moisture content
0.019	5	27016969	9.94E-07	CDS|nonsyn	+	LOC110523600	78 kDa glucose-regulated protein-like	Fat content
0.019	5	31174527	9.94E-07	3'UTR	-	LOC110523653	uncharacterized LOC110523653	Fat content
0.021	5	32672947	8.47E-07	CDS|nonsyn	-	ska1	spindle and kinetochore associated complex subunit 1	Fat content
0.021	5	33025927	1.11E-06	CDS|syn	-	LOC110523701	apelin receptor B-like	Fat content
0.019	5	77138939	9.94E-07	CDS|syn	+	LOC110524668	desmoplakin-like	Fat content
0.019	5	78880913	1.08E-06	CDS|syn	-	podn	podocan	Fat content
0.019	5	79116860	1.37E-06	3'UTR	-	LOC110524700	calcium-binding mitochondrial carrier protein SCaMC-1	Fat content
0.029	8	75925893	1.15E-07	5'UTR	+	LOC110530752	kinesin-1 heavy chain-like	Moisture content
0.025	12	24047528	1.31E-06	3'UTR	+	LOC110537317	inositol-trisphosphate 3-kinase C-like	Moisture content
0.020	12	65629971	1.45E-06	5'UTR	-	LOC110538950	supervillin-like	Moisture content
0.032	17	13538660	9.70E-07	CDS|nonsyn	+	LOC110493605	puromycin-sensitive aminopeptidase-like	Fat content
0.042	17	13538660	6.92E-09	CDS|nonsyn	+	LOC110493605	puromycin-sensitive aminopeptidase-like	Moisture content
0.030	17	17622851	9.55E-07	3'UTR	-	LOC110493736	calcineurin subunit B type 1	Moisture content
0.055	17	20335252	1.05E-06	3'UTR	-	LOC110493798	eukaryotic initiation factor 4A-III	Moisture content
0.057	17	20678734	1.11E-06	CDS|nonsyn	+	rrbp1	ribosome binding protein 1	Moisture content
0.074	17	21029747	1.37E-08	3'UTR	-	acyp2	acylphosphatase 2	Moisture content
0.034	17	30097354	7.50E-07	3'UTR	-	LOC110493954	eukaryotic translation initiation factor 4B-like	Moisture content
0.055	17	30097441	7.32E-07	3'UTR	-	LOC110493954	eukaryotic translation initiation factor 4B-like	Moisture content
0.055	17	30098943	6.96E-07	CDS|nonsyn	-	LOC110493954	eukaryotic translation initiation factor 4B-like	Moisture content
0.056	17	30108957	5.40E-07	CDS|syn	-	LOC110493954	eukaryotic translation initiation factor 4B-like	Moisture content
0.019	24	9580897	3.27E-07	3'UTR	+	LOC110503690	F-box only protein 46-like	Moisture content

511 In our previous work, we profiled transcriptome expression of fish families (YC 2010)
512 showing contrasting phenotypes in fat content, which revealed only 17 differentially
513 expressed transcripts associated with fat content [31]. About 90% of the genetic variation
514 among individuals comes from SNPs [116], and therefore, identifying SNP markers
515 associated with complex traits is most suitable for genetic evaluation in selection programs.
516 Few previous GWA studies identified a small number of SNPs responsible for the additive
517 variance for fat content in Atlantic salmon and Common Carp [11, 34, 38]. The current
518 GWA analysis identified a total of 137 SNPs in windows explaining at least 2% of the
519 additive genetic variance for fat content, suggesting a better characterization of the genetic
520 basis underlying variation in fat content. The discrepancies among the different GWA
521 studies might be due to; 1) usage of different algorithms in the GWA studies, 2) variation
522 in population size, 3) substantial difference in the capacity of the SNP arrays, 4) polygenic
523 nature of intramuscular fat content, 5) different thresholds in each study including sliding
524 window size [35].

525 Compared to our WssGBLUP analysis, the single marker GWA analysis revealed a
526 smaller number of SNP markers associated with variation in intramuscular fat and moisture
527 content. Besides, these two GWA approaches revealed different significant peaks
528 associated with traits of interest. Aguilar et al. [117] showed that the highest peak based
529 on the p-value was not the same based on the proportion of variance explained, and this is
530 because the latter depends on allele frequency, i.e., high effect but low frequency decreases
531 the variance explained. This result is consistent with other studied traits, such as fillet
532 firmness, protein content [39], and bodyweight gain [40] in rainbow trout. The potential
533 factors associated with observed heterogeneity between the two approaches are different
534 algorithms, thresholds, and windows size used in each approach. For instance,
535 WssGBLUP uses a flexible default HWE threshold as it assumes selection may have
536 caused a departure from equilibrium, and therefore only extreme outliers would be
537 excluded in order to keep the information content of haplotypes. The WssGBLUB was
538 more effective than the single marker GWA in examining the genetic architecture of
539 studied traits and identifying common QTL between traits. This method has proven to be
540 optimal for breeding populations given the data structure: phenotyped individuals may not
541 have genotypes, and there is a long history of pedigree recording [117]. Common QTL
542 identified in this study may explain the high negative correlation between fat and moisture
543 content. The recombinational progression of QTL and nearby markers determines the
544 information content of haplotypes [118]. However, SNP-harboring genes identified by the
545 two approaches had similar biological functions and were involved in lipid metabolism,
546 protein turnover, and cytoskeletal remodeling. Routine use of single-SNP and multi-
547 makers for GWA analysis was previously recommended to take advantage of the complete
548 information content of the genotypes [118].

549 Taken together, controlling muscular fat content can help the aquaculture industry to
550 produce a final product of expected quality, including moisture content, drip loss, firmness,
551 and shelf-life. Dietary lipids are used to increase fillet fat to improve fillet sensory
552 characteristics [119]. However, this can also elevate the feeding costs, increase visceral
553 fat, and accelerate lipid oxidation which increases fillet degradation. Alternatively,
554 genetic/genomic selection can be used to control fillet fat content. The findings of the
555 current study can help breeders where GEBV for muscular fat and moisture content can be
556 added to multi-trait selection indices that reflect the various needs of producers and
557 consumers [120].

558

559 **CONCLUSIONS**

560 The current GWA analyses identified novel genomic regions associated with additive
561 genetic variance for fat and moisture content in rainbow trout. These genomic loci code
562 for proteins involved in lipid metabolism, actin cytoskeleton remodeling, and protein

563 synthesis/degradation. This work reveals significant QTL associated with fat content,
564 which appears to be a polygenic trait. The top common windows affecting additive genetic
565 variance for fat and moisture content are mainly on chromosome 19. These findings
566 provide a genetic basis for description of the molecular mechanisms underlying fat and
567 moisture content in teleost fish. Variation in moisture content is likely to be driven by
568 changes in fat content in an antagonistic fashion. This work provides putative markers that
569 could be prioritized when estimating genomic breeding values for fat and moisture content.
570 GEBV for muscular fat and moisture content can be included in multi-trait selection indices
571 that meet the various producers' and consumers' demands [135].

572 **METHODS**

573 **Fish population, tissue sampling, and phenotypic traits**

574 The fish population used in the current GWA analyses was previously described in
575 detail [121]. Briefly, a selective breeding program was established at NCCCWA in 2002
576 by intercrossing seven domesticated strains of rainbow trout; this fish population has gone
577 through five generations of selection for improved growth performance [30]. Full-sib
578 families collected from third- and fourth-generation fish (Year-class; YC 2010 and 2012)
579 were used for the current GWA analyses. Phenotypic data for muscle fat and moisture
580 content were obtained from 789 fish representing 197 families produced from two
581 generations (YC 2010 and 2012). Single-sire×single-dam matings occurred over 6 weeks
582 to produce full-sib families. Individuals from each family were reared together in a 200-L
583 tank in order to keep the pedigree information. Tagging fish, at ~5-months post-hatch with
584 a passive integrated transponder (Avid Identification Systems Inc., Norco, CA) allowed
585 husbandman to rear different fish families together in 800-L communal tanks (~13°C) until
586 ~13 months post-hatch. A commercial fishmeal-based diet was provided, and the feeding
587 schedule was previously described [122]. Fish were purged for 5 days before harvest to
588 facilitate viscera removal.

589 Whole-body weight (WBW) was measured, and fish were sorted within family
590 accordingly. For sampling, the 2nd or 3rd fish from each sorted family was selected in order
591 to keep the distribution of WBW around the median of each family. For each YC, fish
592 were randomly assigned to one of five harvest groups allowing a single fish per family per
593 harvest group (~100 fish each). The harvest groups were sampled over five weeks (one
594 group/week). Fish groups from the two-year classes were harvested at different ages. Fish
595 produced from YC 2010 were harvested between 410- and 437-days post-hatch (mean
596 body weight = 985 g; SD = 239 g), whereas, fish produced from YC 2012 were harvested
597 between 446- and 481-days after hatching (mean body weight = 1,803 g; SD = 305 g). Fish
598 were euthanized with tricaine methanesulfonate (Tricaine-S, Western Chemical, Ferndale,
599 WA), harvested, and eviscerated. Head-on gutted carcasses were shipped on ice to the

West Virginia University Muscle Foods Processing Laboratory (Morgantown, WV) where carcasses were manually processed into skinless fillets.

Proximate analyses, including crude lipid and moisture content (water content), were previously described [123]. In brief, crude lipid and moisture analyses were performed using AOAC-approved methods (AOAC 2000). Crude lipid content was determined using Soxhlet extraction with petroleum ether. Moisture content was assessed by weighing the sample before and after drying at 110°C for 18 hrs. When muscle fat and moisture content were regressed on body weight, coefficient of determination (R^2) values of 0.23 and 0.38 were observed, respectively. Heritability was estimated for fat and moisture content using genomic relationship matrix (GRM) calculated from the variant data [100].

SNP genotyping and quality control

A 50K, transcribed gene SNP-chip was recently developed and used in identifying genomic loci responsible for additive genetic variance in fillet yield [36]. SNPs used to build the SNP chip were identified and reported in our previous study [121]. Briefly, the chip included ~21K SNPs showing potential allelic imbalances with growth, muscle quality, and susceptibility/resistance to Bacterial Cold Water Disease (BCWD) [36, 121]. About 5K nonsynonymous SNPs were included in the chip. Additional SNPs were added to the chip to have a minimum of 2 SNPs per each SNP-harboring gene. The array contains 50,006 SNPs.

In total, 1,728 rainbow trout fish were used for genotyping and quality assessment of the SNP chip. Genotyped fish were collected from the USDA/NCCCWA growth- and BCWD-selection lines [36]. Samples and genotypes had undergone quality control checks. The Affymetrix SNPolisher software was used for QC assessment and filtration of samples/genotypes at the default settings [36]. Genotyped samples were filtered using a call rate of 0.97 and Dish QC (DQC) threshold of 0.82. In the current study, 789 genotyped fish, with available phenotypic data for intramuscular fat and moisture content, were used for GWA analyses.

Fifty-SNP window GWA analysis

Estimates of SNP effects from weighted single-step GBLUP (WssGBLUP) were used to conduct the current GWA analysis, as we previously described [36]. The WssGBLUP combines phenotypes, genotypes, and pedigree information into a single evaluation. Genomic relationship matrix was created based on VanRaden equation [124].

$$G = \frac{MDM'}{2\sum Pj(1-p_j)'}$$

Where D represents the diagonal matrix of SNPs weights; M is a matrix of genotype centered by allele frequency (p) and j is the locus.

SNPs used to construct the genomic relationship matrix are weighted according to the proportion of additive variance they explain. The following single-trait model was used:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_1\mathbf{a} + \mathbf{Z}_2\mathbf{w} + \mathbf{e}$$

where **y** is the vector of phenotypes, **b** is the vector of fixed effects (harvest group and hatch-year), **a** is the vector of random animal effect, **w** is the vector of random family effect, and **e** is the vector of residual effects. Random and fixed effects were determined according to [35, 125]. $\mathbf{X}$, $\mathbf{Z}_1$, and $\mathbf{Z}_2$ are incidence matrices for the effects contained in **b**, **a**, and **w**, respectively. Whereas the family and residual random effects were considered uncorrelated, the animal effect was correlated. The covariance structure for the animal effect was given by $\mathbf{H}\sigma_a^2$, where **H** is a matrix that combines pedigree- and genomic-based relationships [126] and σ_a^2 is the additive variance.

$$\mathbf{H}^{-1}=\mathbf{A}^{-1}+\begin{bmatrix}0 & 0\\ 0 & \mathbf{G}^{-1}-\mathbf{A}_{22}^{-1}\end{bmatrix}$$

where $\mathbf{H}^{-1}$ is the inverse of the realized relationship matrix (**H**), $\mathbf{A}^{-1}$ is the inverse of the relationship matrix based on pedigree information, $\mathbf{A}_{22}^{-1}$ is the inverse of the pedigree relationship matrix for genotyped animals only, and $\mathbf{G}^{-1}$ is the inverse of the genomic relationship matrix. The covariance structure is given by $\mathbf{I}\sigma_w^2$, where **I** is an identity matrix and σ_w^2 is the family variance.

The variance components were estimated using AIREMLF90 [127]. The inbreeding coefficient was calculated by INBUPGF90 [128] using pedigree data representing 63,808 fish produced from five consecutive generations by the NCCCWA breeding program. Genomic data were edited using PREGSF90 [127], and samples or SNP were kept according to the following parameters: minor allele frequency (MAF) > 0.05, call rate > 0.90, and a default value of Hardy-Weinberg equilibrium (HWE) < 0.15. Out of 50,006 SNPs, 35,322 SNPs (70.6%) passed QC and were used for the WssGBLUP analysis.

Two iterations were used in the current WssGBLUP analysis. All SNPs passing QC were assigned, by default, the same weight in the first iteration (i.e., weight = 1.0). SNP effects ($\hat{u}$) were determined using POSTGSf90 (part of GBLUPf90 software family) according to

$$\hat{u} = qDZ'(ZDZ'q)^{-1}\,\hat{a}$$

Where q is a weight factor based on SNPs frequency, D is a weight matrix of SNPs, Z is a matrix of gene content adjusted for allele frequencies, and â is Genomic Breading Values of genotyped animals [129].

In the second iteration, SNP weights were determined according to SNP effects ($\hat{u}$) calculated in the first iteration as $\hat{u}^2 2p(1-p)$, where p is the allele frequency in the current genotyped population. In brief, three steps were conducted for each iteration: 1) weights were assigned to the SNPs; 2) genomic estimated breeding values (GEBV) were calculated, based on $\mathbf{H}^{-1}$ [128]; and 3) SNP effects and updated weights were computed by POSTGSF90 [128] using genomic sliding windows of 50 adjacent SNPs. This window size (n = 50 SNPs) was adopted instead of physical size (e.g., specific number of nucleotides) because SNPs were not evenly distributed over the whole genome. Percentage of genetic variance explained by i-th region was computed using the following equation [129]:

$$\frac{var(a_i)}{\sigma_a^2} * 100\% = \frac{var\left(\sum_{j-1}^{50} z_j\, \hat{u}_j\right)}{\sigma_a^2} * 100\%$$

Where a_i is the genetic value of the i-th region that consists of contiguous 50 SNPs, σ^2_a is the total genetic variance, Z_j is the vector of the gene content of the j-th SNP for all individuals, and $\hat{u}_j$ is marker effect of the j-th SNP within the i-th region.

The qqman package [130] in R was used to generate Manhattan plots showing the proportion of additive variance explained by the 50 SNP windows.

Single marker GWA analysis

PLINK [131] was used to filter the genomic data before performing a single marker association analysis. The filtering criteria included MAF > 0.05 and a default value of HWE < 0.001. The command, --assoc, was used to retrieve R-squared values (R^2) of association between the quantitative traits and genotypes. R^2 (coefficient of determination) is the proportion of variance in the phenotype that is explained by the genetic factors in a linear regression model and it could be calculated as follows: $R^2 = 1 - (SS_{regression} / SS_{total})$; where $SS_{regression}$ is the sum of squares due to regression, and SS_{total} is the total sum of squares [132]. A generalized test score in ONETOOL [100] was used to perform family-based association analysis. This test allows for multiple covariates and accounts for family structure using a kinship matrix. The ONETOOL uses kinship2 package [133] to calculate the kinship traditional matrix. For computation, the kinship function uses a recursive algorithm that assumes not inbred founders. Covariates were incorporated in the linear model to account for fixed effects (harvest group and hatch-year) and population structure. The first two principal components (PCs) were included in the model to account for

population structure. P-values with Bonferroni correction were used to account for multiple testing. Bonferroni corrected p-values were calculated as (α/total number of variants), where α = 0.05 was used for a genome-wide significance level, and α = 0.3 was used for the suggestive significance level. Manhattan plots showing single SNP markers associated with variations in intramuscular fat and moisture content were generated using qqman package [130].

Gene annotation

To annotate the SNP-harboring genes, a bed file containing SNPs associated with the quality trait of interest was intersected with the rainbow trout genome gff/gtf file using Bedtools [134]. SNPs were classified as genic or intergenic according to their physical position relative to the body of the gene. Genic SNPs exist in coding DNA sequence (CDS), introns, or untranslated regions (5'UTR and 3'UTR). Intergenic SNPs are defined as SNPs located in the region between genes.

Abbreviations

BCWD: Bacterial Cold Water Disease

GO: Gene ontology

GWA: Genome-wide association

HWE: Hardy–Weinberg equilibrium

MAF: Minor allele frequency

NCCCWA: USDA National Center of Cool and Cold Water Aquaculture

QC: Quality control

QTL: Quantitative trait loci

SNP: Single nucleotide polymorphism

UTR: Untranslated region

WssGBLUP: Weighted single-step GBLUP

YC: Year class

DECLARATIONS

Ethics approval and consent to participate

Institutional Animal Care and Use Committee of the United States Department of Agriculture, National Center for Cool and Cold Water Aquaculture (Leetown, WV) specifically reviewed and approved all husbandry practices used in this study (IACUC protocol #056).

Consent for publication

Not applicable.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors' Contributions

MS, TL, and BK conceived and designed the experiments. RA-T, MS, TL, and BK performed the experiments. RA-T, AA, DL, BK, and MS analyzed the data. AA and MS wrote the manuscript.

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Availability of data and materials

All datasets generated for this study are included in the manuscript and/or the Additional Files. The genotypes (ped and .map files) and phenotypes are available in our previous publication [39].

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Supplementary information

Additional file 1: Table S1-Genetic variance for fat content explained by all SNP markers in genomic sliding windows. Table S2-SNP markers in genomic sliding windows explaining at least 2% of the genetic variance for fat content. Table S3-Genetic variance for moisture content explained by all SNP markers in genomic sliding windows. Table S4-SNP markers in genomic sliding windows explaining at least 2% of the genetic variance for moisture content. Table S5-SNP markers significantly associated with variability in fat content (highlighted in yellow) using family-based association analysis. Table S6-SNP markers significantly associated with variability in moisture content (highlighted in yellow) using family-based association analysis.

Figures

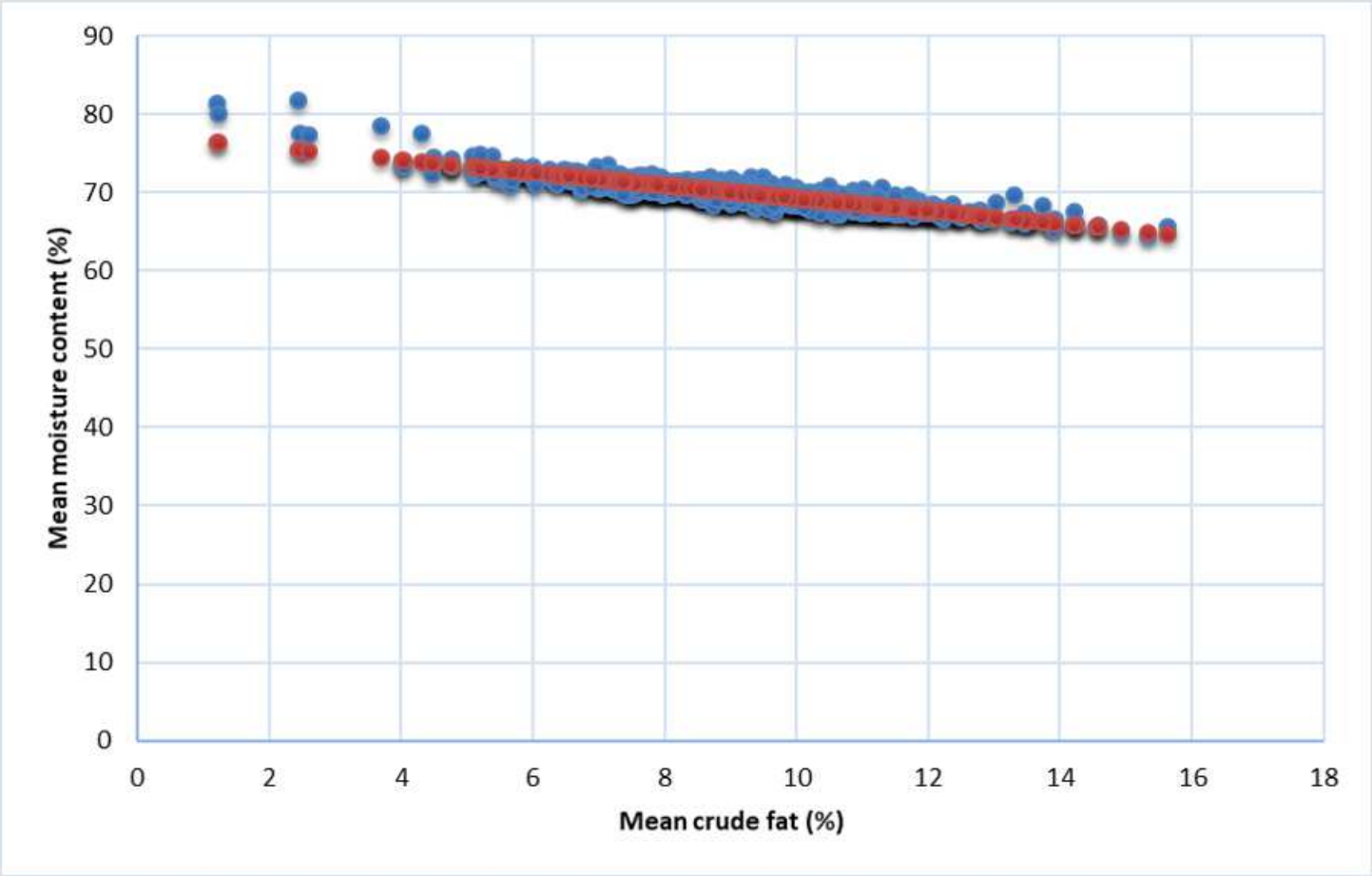

Figure 1

Reverse relationship between intramuscular fat and moisture content in fish used for GWA analyses.

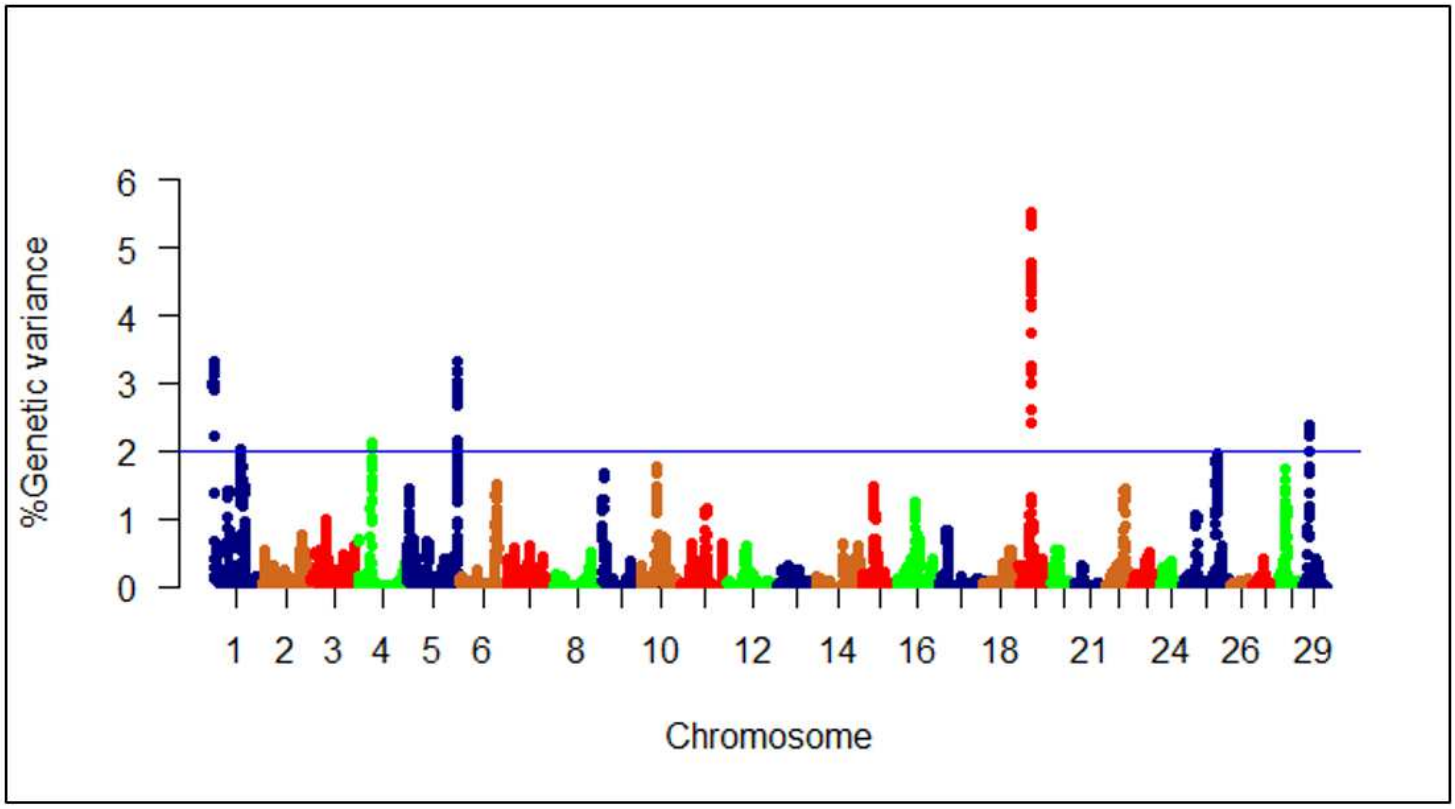

Figure 2

Manhattan plot showing association between 50 SNP-genomic sliding windows and muscle fat content. Chromosome 19 showed the highest peaks with genomic loci explaining up to 5.51% of the additive genetic variance. The basal blue line represents 2% of the genetic variance explained by the sliding windows.

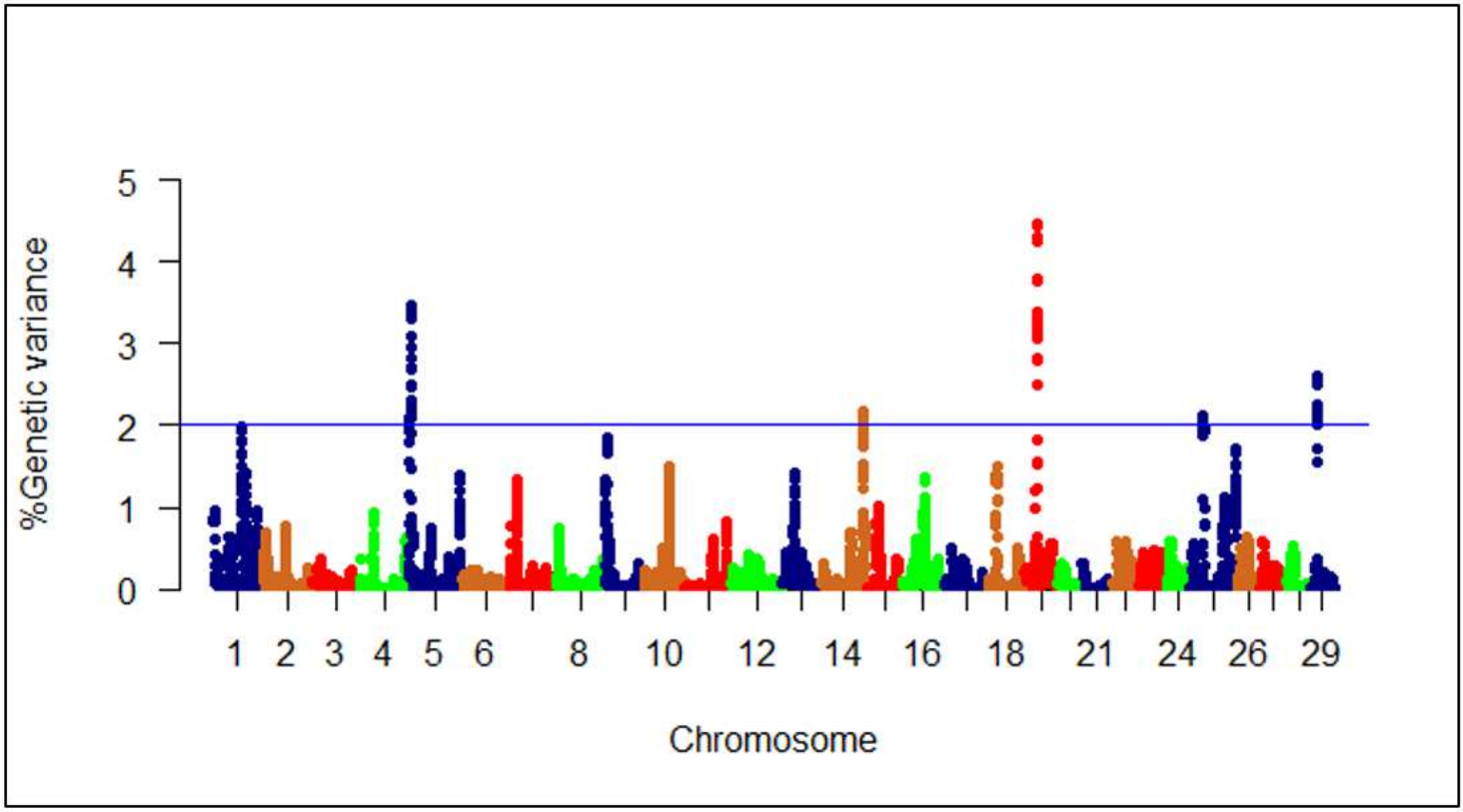

Figure 3

Manhattan plot showing association between 50 SNP-genomic sliding windows and muscle moisture content. Chromosome 19 showed the highest peaks with genomic loci explaining up to 4.46% of the additive genetic variance. The basal blue line represents 2% of the genetic variance explained by the sliding windows.

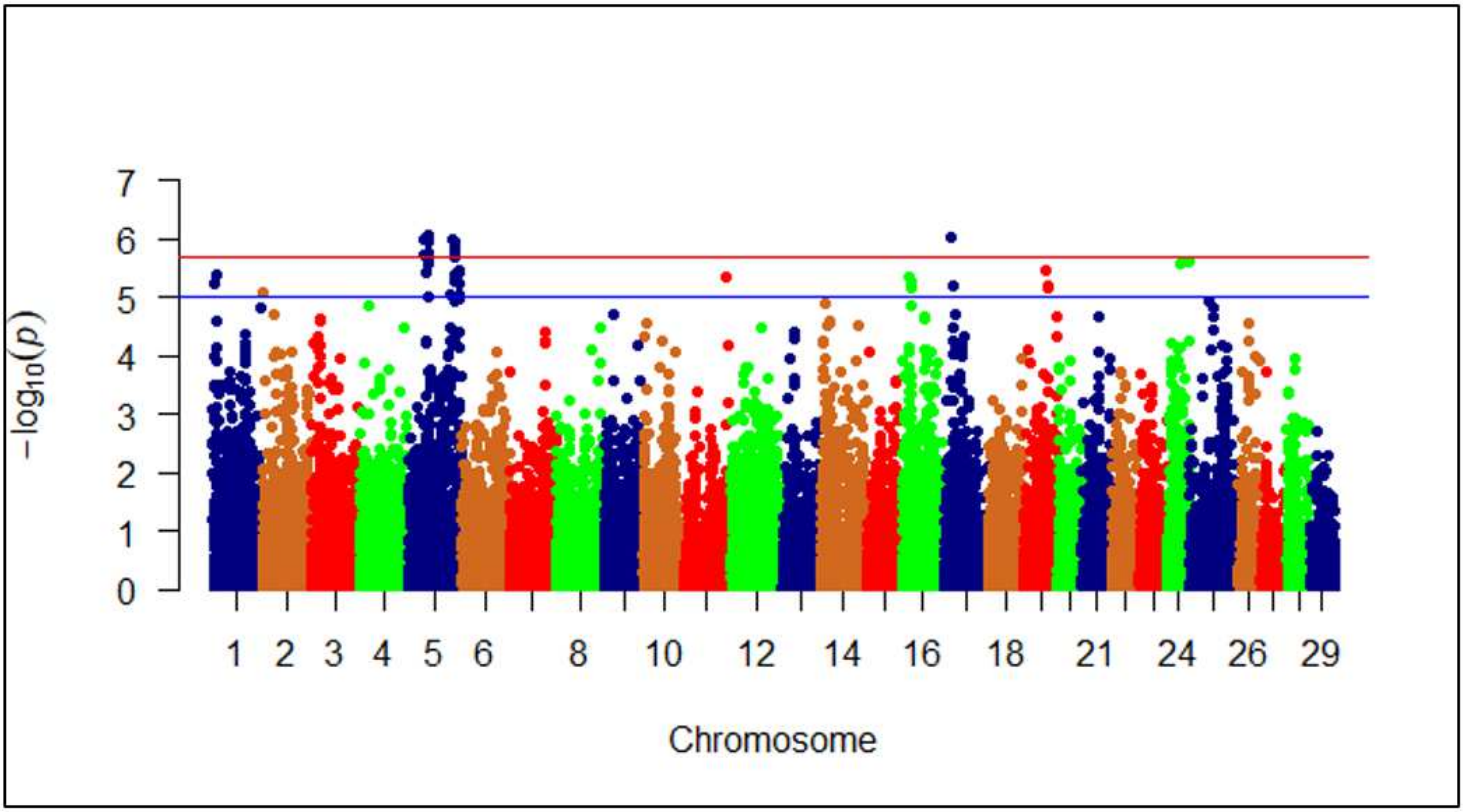

Figure 4

Manhattan plot showing single SNP markers significantly associated with variations in muscle fat content using family-based association analysis. Many of the significant SNPs are located on chromosome 5. Blue and red horizontal lines represent suggestive (1e-05) and significant (1.69e-06) threshold p-values, respectively.

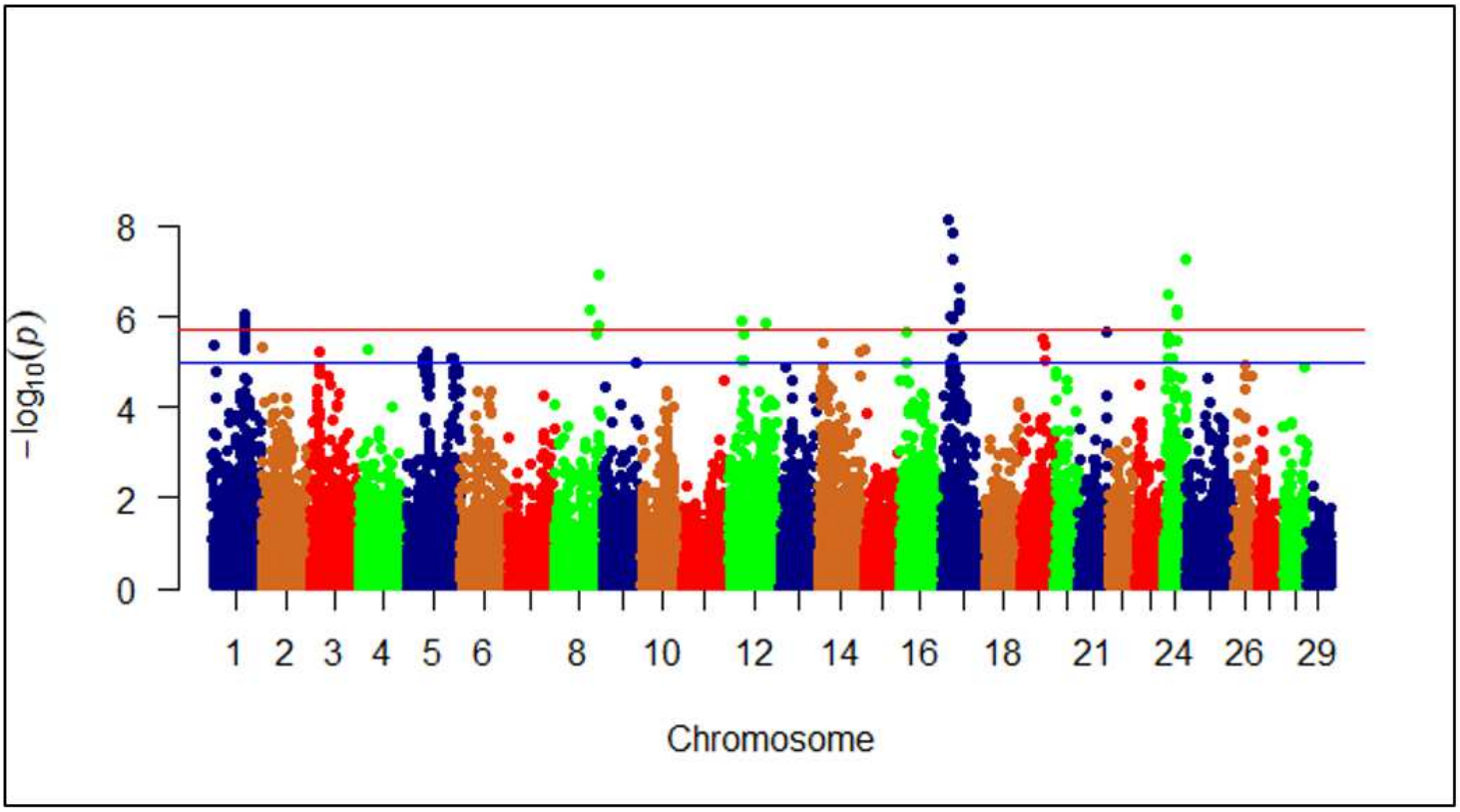

Figure 5

Manhattan plot showing single SNP markers associated with variations in moisture content using family-based association analysis. Most of the significant SNPs are located on chromosome 17. Blue and red horizontal lines represent suggestive (1e-05) and significance (1.69e-06) threshold p-values, respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- table2.png
- table1.png
- table4.png
- table3.png
- table5.png
- Supplementaryfatmoisture.xlsx