Research Note: The effect of selection for 16-week body weight on turkey serum metabolome

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ABSTRACT The phenotype of modern commercial turkeys is substantially different than that of unselected, heritage turkey lines. These phenotypic changes have arisen from alterations in the genome/transcriptome, as well as the influence of many external factors on growth performance including nutrition, environment, and management. To investigate the phenotypic changes resulting from genetic selection for increased body weight, The Ohio State University maintains 2 unique genetic turkey lines: the randombred control (RBC2) line, which is comprised of genetics from 1960 era commercial turkeys and has been maintained without conscious selection for any trait; and the F line, which was originally selected from the RBC2 line and has been selected for increased 16 wk body weight for over 50 generations. This study used broadspectrum mass-spectrometry profiling techniques to identify and quantify differences in the metabolome of the serum of F and RBC2 turkey lines. Serum samples from both F and RBC2 turkeys were subject to quantitative time of flight liquid chromatography tandem mass spectrometry analyses. Principle component analyses showed distinct populations of metabolites in the F vs. RBC2 serum, suggesting that increased body weight is associated with the accumulation of several metabolites. Comparing the spectral features to online databases resulted in the selection of 104 features with potentially identifiable chemical structures. Of these 104 features, 25 were found at higher levels in the serum of the RBC2 line turkeys, while 79 were found at a greater abundance in the F line turkeys. A more detailed analysis of these 104 features allowed for the putative identification of 49 compounds, which were clustered into 6 functional groups: 1) energy metabolism; 2) vitamins; 3) hormones and signaling molecules; 4) lipid derivatives, fatty acid metabolites, and membrane components; 5) amino acid/protein metabolism; and 6) microbial metabolites. Further validation and experimentation is needed to confirm the identity of these metabolites and understand their biological relevance and association with selection for increased body weight.

Key words: Growth selection, metabolome, serum, turkey

INTRODUCTION

Due to an increase in consumer demand and pressure to reduce input costs, the commercial turkey industry has continually selected for higher yielding, more energy-efficient turkeys. As a result, the phenotype of modern turkeys has changed substantially when compared to heritage lines of similar ancestry. Modern commercial turkeys reach a market weight approximately 3 times heavier, and in a shorter period of time compared to 50 yr prior (Clark et al., 2019). The economic impact and importance of increasing turkey body weights while decreasing production time has resulted in significant emphasis being placed on understanding how these genetic selection strategies have impacted muscle growth. To date, most of these efforts have focused on investigating the positive and negative effects of selection on muscle mass accretion, muscle tissue development, and the breast muscle characteristics of heavy, high-yielding commercial turkeys. However, it is likely that the phenotypic and cellular changes occurring as a result of genetic selection are not solely associated with the breast muscle, but also impact, either directly or indirectly, nearly every organ system in the body.

The Ohio Agricultural Research and Development Center (OARDC) within The Ohio State University (**OSU**) maintains 2 unique turkey lines which are excellent models to study the impact of growth selection. The randombred control line (RBC2) is a composite turkey line comprised of genetics from 4 1950s

strains of Large White commercial turkeys (Nestor et al., 1967). The F line was originally selected for increased 16 wk body weight from the RBC2 line in 1966, and it has been continually selected for increased 16 wk body weight for over 50 generations. In contrast, the RBC2 control line has been maintained without conscious selection for any trait during that same period of time. Since the F and RBC2 lines are derived from an identical genetic background, any phenotypic, cellular, or genetic differences can be directly attributed to selection for increased body weight.

The F and RBC2 turkey lines have been used for many years to define not only the changes related to muscle mass accretion of the breast muscle (Velleman et al., 2000, 2003; Velleman and Nestor, 2004; Clark et al., 2019), but also changes that may be associated with embryogenesis (Christensen et al., 1993; Lilburn and Antonelli, 2012), nutrient absorption (Fan et al., 1997), skeleton (Zhong et al., 2012), reproduction (Nestor et al., 1982; Nestor and Bacon, 1986), genetics (Sporer et al., 2011), and immune status (Nestor et al., 1999; Li et al., 2000; Huff et al., 2005). These studies were driven primarily by the gross phenotypic differences readily observable when comparing the lines or in some cases, by prior results detailing changes to the immune system function or nutrient absorption associated with growth selection. However, selection for increased body weight has likely impacted many other organ systems in ways that are, to date, only poorly understood, and which have gone undetected in previous studies. These differences may have subtle effects on the improvements in breast muscle growth of faster-growing commercial turkeys. Furthermore, these alterations to turkey physiology and development may also contribute to the negative side effects associated with growth selection, including increased incidence of myopathies (Barbut, 1996; McCurdy et al., 1996), insufficient skeletal support (Sullivan, 1994; Crespo et al., 1999), and cardiopulmonary defects (Shivaprasad et al., 2004), all of which occur with increased frequency in modern commercial turkey flocks.

Recently, new advances in analytical chemistry techniques have allowed scientists to simultaneously identify and quantify numerous metabolites within a single cell, tissue, or biofluid. Since these metabolites are the end products or intermediaries of complex biochemical reactions occurring in the cell, a comprehensive understanding of these metabolites can uncover cellular mechanisms and pathways related to differences in the genome which previously may have gone undetected. Therefore, the objective of this research was to characterize and describe the differences in the abundance of serum metabolites between the F and RBC2 turkey lines. The blood is not only a primary carrier of small molecules throughout the body, but it also serves a critical role as a transporter for dissolved gases, nutrients, metabolites (both essential cellular components and waste products), and signaling compounds/hormones. In addition, the blood also regulates pH, carries immune system components involved in the

defense against toxins and biotic infection, contributes to the control of body temperature and, perhaps most importantly, serves as a communication medium between all organ systems within the body (Psychogios et al., 2011). Identifying and understanding changes in the turkey serum metabolome will expose many undiscovered mechanisms which have been altered as a result of selection for increased body weight.

MATERIALS AND METHODS

Turkeys

All bird activities were approved by the Institutional Animal Care and Use Committee of OSU, and levels of care met or exceeded the guidelines set forth by the Guide for the Care and Use of Agriculture Animals in Research and Teaching. Turkey eggs from both the F and RBC2 lines were collected, incubated, and hatched in accordance to standard operating procedures common to the turkey industry. Details about the selection history and reproduction of the lines are described by Nestor et al. (Nestor, 1977, 1984; Nestor et al., 2000, 2008). After hatch, the poults were wing banded and vent sexed. Both F and RBC2 males were then group housed together and given ad libitum access to standard commercial turkey diets which met or exceeded NRC recommendations (National Research Council, 1994). At 7 wk of age, 10 F and 10 RBC2 turkeys were moved to individual pens and allowed to familiarize themselves to the new environment. Seven weeks of age was chosen as the turkey's rate of gain and musculature deposition is near maximum at this point in development. After 36 h in the new pen, access to feed was removed overnight (15 h). The following morning, blood was collected from the brachial wing vein. The blood was then allowed time to clot at room temperature and spun at 1,900 x g for 10 min to separate serum. Aliquots of serum were then stored at −80°C until thawed for metabolomics analyses. At the time of sample collection, the F line birds had an average body weight of 3.5 kg (SD = 0.64) whereas the RBC2 had an average body weight of 1.6 kg (SD = 0.15).

Broad-Spectrum Metabolomics

Organic, non-protein components of serum samples were extracted in methanol. Briefly, serum samples were thawed on ice and maintained at 4°C throughout the extraction process. Methanol was added at 3.5 volumes to each serum sample (250 μL), along with 700 ng of internal standard (3.5 μL deuterated tryptophan at a concentration of 200 ng/μL; OlChemim, s.r.o, Czech Republic), for a final volume of 1128.5 μL. To precipitate proteins, samples were incubated for 12 h at −20°C, then spun at 14,000 × g for 15 min at 4°C. Following centrifugation, the supernatants, containing organic metabolites, were harvested and filtered through a 0.2 μm, 5 mm PTFE disc syringe filters directly into target vials. Samples were then sealed and subjected to
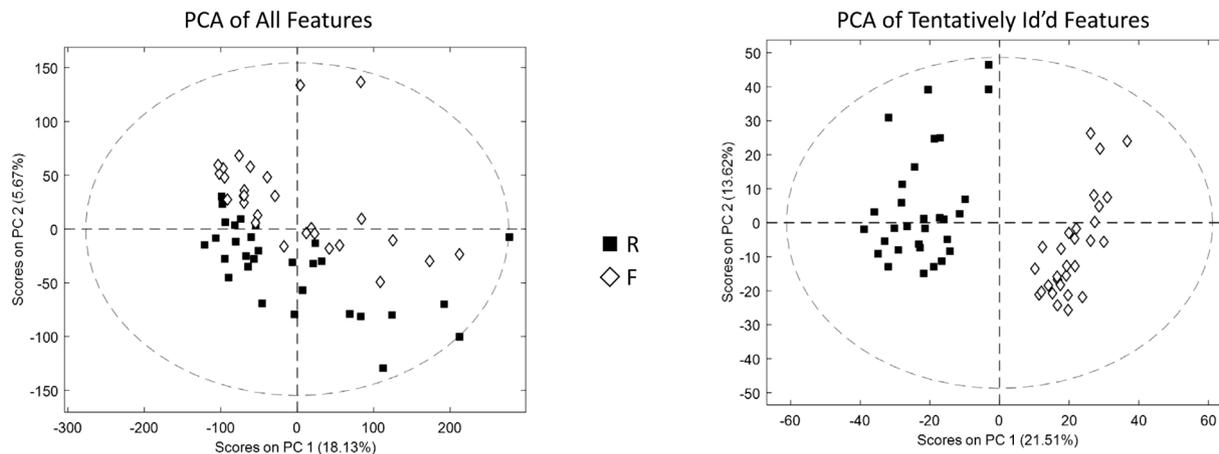
Figure 1. Principle component analyses (PCA) of features found in the serum of randombred control line 2 (RBC2) (control; squares) and F (selected for increased muscle mass; diamonds) turkey lines. (A) PCA plot of the approximately 2,700 features identified with a cut-off value of $P \leq 0.5$. Analyses show distinct populations of metabolites in RBC2 vs. F lines. (B) PCA plot of putatively identified metabolites (104 features) in RBC2 (control; squares) and F (increased muscle mass; diamonds) lines. The putatively identified metabolites show clear separation (differential accumulation) between the 2 lines.

quantitative time of flight liquid chromatography tandem mass spectrometry (**Q-TOF LC-MS/MS**) analyses. LC-MS/MS analyses were performed on an Agilent 6545 Q-TOF (Santa Clara, CA) in positive ion mode with a dual AJS ESI system and an Agilent 1290 Infinity LC system. Briefly, 5 μL of each sample was separated on an Agilent C-18 Poroshell column using the following program: 0 min 2% B, 15 min 90% B, hold for 1 min, 17 min, 2% B, 32 min 2% B (buffer A: H_2O 0.1% formic acid; buffer B: MeOH 0.1% formic acid). The mass spectrometer was run in MS^2 scan mode, with a range of 50 to 1,700 amu. Ion source parameters were as follows: gas temp. 250°C, source gas flow 8 L/min., nebulizer 25 psi, sheath gas temp. 350°C, sheath gas flow 10 L/min, capillary voltage 3,500 V (positive ion mode), nozzle voltage 500 V (positive ion mode). Features were selected using data-dependent acquisition, in which the top 5 ions at a given time point were retained, used to generate a mass spectral profile, then excluded for a 30-s window.

Data Analysis

Mass spectral data were analyzed using Agilent MassHunter Software. Peaks were normalized to the area of the internal standard (deuterated tryptophan). Features were identified using the Progenesis software package (Durham, NC). Features with a P-value of less than 0.05 were retained for downstream analyses. Features were identified using Progenesis software and searching against the Kyoto Encyclopedia of Genes and Genomes (**KEGG**), Nature Chemistry, and Human Metabolite Database (**HMDB**) metabolite libraries.

RESULTS AND DISCUSSION

A total of 2,700 features with a P-value of less than 0.05 and a fold-change cut-off of approximately 1.4

were detected and tentatively matched in our broad-spectrum metabolomics analyses. Principle component analyses showed a clear separation of features between the F and RBC2 turkey lines (Figure 1), indicating the presence of a population of distinct metabolites accumulating in each of the 2 lines. Comparison to the KEGG, Nature Chemistry, and HMDB databases allowed us to select 104 features with potential chemical structures. Of these 104 features, 25 were found at higher levels in the serum of the RBC2 line turkeys, while 79 instances were found at a greater abundance in the F line turkeys. More detailed analyses of the spectral profiles and chemical structures of these features allowed us to putatively identify 49 of these 104 compounds. Pathway-based analyses revealed that the identified metabolites clustered into 6 functional groups: 1) energy metabolism; 2) vitamins; 3) hormones and signaling molecules; 4) lipid derivatives, fatty acid metabolites, and membrane components; 5) amino acid/protein metabolism; and 6) microbial metabolites.

Energy Metabolism

Unsurprisingly, metabolites involved in energy metabolism exhibited distinct differences in the serum of F line turkeys with increased body weight and muscle mass (Table 1). For example, levels of diadenosine diphosphate, a derivative of ATP, were increased 1.8-fold in F line serum compared to RBC2 serum. Although the precise function of diadenosine diphosphate in poultry is not well researched, human biomedical studies suggest that diadenosine polyphosphates act as vasodilators, neurotransmitters, signaling molecules, and may have a role in mediating metabolic, heat, and oxidative stress (Lee et al., 1983; Varshavsky, 1983; Bochner et al., 1984; Hoyle, 1990; Baxi and

Table 1. Putative serum metabolites associated with energy metabolism, vitamins, and hormones or signaling molecules in a turkey line selected for 16 wk body weight (F) and a randombred control line 2 (RBC2).

Metabolite	HMBD ID number	Mean fold change[1]	Retention time (min)
Energy metabolism			
Oxalosuccinic acid	HMDB03974	−2.07	1.08
QH(2)	HMDB59661	−2.01	3.90
Diadenosine diphosphate	HMDB01312	1.85	9.61
6-Hydroxy flavin adenine dinucleotide	HMDB11612	2.30	8.72
Vitamins			
Cob(I)yrinate a,c diamide[2]	HMDB06904	1.55	9.54
Hormones and signaling molecules			
Estradiol[3]	HMDB00151	−2.16	1.92
Neuromedin C	HMDB13020	−1.82	17.04
18-Oxocortisol[4]	HMDB00332	1.64	9.10

[1]Negative values indicate higher expression in the RBC2 line compared to the F line, whereas positive values indicate greater expression in the F line.

[2]Indistinguishable from Oxytocin 1–8, HMDB13033.

[3]Likely a terpine molecule; however, there is a possibility that this feature may correspond to a carnitine derivative.

[4]Likely oxocortisol; however, this compound may be another polyterpenoid.

Vishwanatha, 1995; Ralevic et al., 1995; Flores et al., 1999).

F line turkeys also had a 2.3-fold increase in the levels of 6-hydroxy flavin adenine dinucleotide (6-hydroxy FAD) in serum compared to the RBC2 turkeys. 6-Hydroxy FAD is derived from the reduction of flavin adenine dinucleotide (**FAD**) by NADPH in the presence of oxygen (Marshall et al., 2005). FAD is a co-coenzyme critical for the function of multiple cellular enzymes and is essential for the redox reactions of base carbon metabolism (Marshall et al., 2005). Specific reactions involving the reduction of FAD include the conversion of succinate to fumarate in the TCA cycle, and the conversion of malate to oxaloglutarate in pyruvic acid metabolism. The role of 6-hydroxy FAD is less understood, although in humans the AMID (AIF-homologous mitochondrion-associated inducer of death) flavoprotein has been shown to induce cellular apoptosis and to form a stoichiometric cofactor protein complex with 6-hydroxy FAD (Marshall et al., 2005). Given the role of 6-hydroxy FAD in apoptosis in humans, it is interesting that the presence of this compound may indicate increased cellular turnover or death in F lines. However, further research is needed to determine whether or not 6-hydroxy FAD plays a significant role in apoptosis of poultry cells.

Oxalosuccinic acid and ubiquinol (QH$_2$) were increased 2.1- and 2.0-fold, respectively, in the serum of RBC2 turkeys compared to F line serum. While both of these metabolites have a significant role in energy metabolism (oxalosuccinate is an intermediate in the conversion of isocitrate to alpha-keto-glutarate), their role in serum remains unclear. Ubiquinol is a fully reduced form of coenzyme Q10 (COQ10) and has also been implicated in redox reactions in which COQ10, in its fully oxidized ubiquinone or partially oxidized semiquinone form, accepts electrons and is reduced to ubiquinol. In this role, ubiquinol is the end product of antioxidant reactions and protects against lipid perox-

idation and the formation of reactive oxygen species independently of vitamin E (Ernster and Forsmark-Andrée, 1993). Lower levels of ubiquinol in the serum of F line turkeys may indicate either decreased oxidative stress or decreased antioxidant protection in the serum of these turkeys. However, as CO10 levels in serum are associated primarily with diet, and corn oil contains relatively high levels of COQ10, increased levels of ubiquinol in RBC2 turkeys may also reflect differences in dietary intake, digestion, or processing between the 2 lines.

Vitamins

Cob(I)yrinate a, c diamide is an intermediary of vitamin B12 biosynthesis and was likely increased in the serum of F line turkeys compared to RBC2 turkeys (Table 1). The increased levels may be a result of the increased feed consumption associated with F line turkeys, which consume significantly more feed than RBC2 turkeys (Clark et al., 2019) as the mineral vitamin premix contained vitamin B12 supplement (Provimi, Brookville, OH). It is important to note, however, that there is also a small chance that the spectra associated with this peak may instead correspond to oxytocin 1–8, a fragment of the neurotransmitter oxytocin. While this possibility is less likely, further investigation is needed to confirm the identity of this peak.

Hormones and Signaling Molecules

Estradiol The serum of the RBC2 line showed a 2.1-fold increase in levels of a triterpenoid-derived molecule which was most likely estradiol, a steroid hormone (Table 1). However, given the structural similarity and potential overlap in the spectra of longer-chain hydrocarbons, there is a small possibility that this molecule may also be a carnitine-fatty acid derivative. It would be of no surprise that estradiol would be altered by

the selection of 16 wk body weight as the reproduction traits of the F and RBC2 turkeys are distinctly different (Strong and Nestor, 1980; Nestor et al., 1982). Interestingly, although the turkeys used in this trial were immature (7 wk of age) males, it is possible that increased body weight is associated with increases in the synthesis of testosterone, which can be converted into estradiol. To investigate this question further, it would be useful to use a targeted metabolomic approach to quantify levels of steroid hormones in F and RBC2 lines during growth and development.

Neuromedin C Neuromedin C is a bombesin like decapeptide closely associated with gastrin-releasing peptide (Gajjar and Patel, 2017), and is synthesized from the same precursor as the appetite suppressing polypeptide, neurotensin (Richards, 2003). Similar to neurotensin, neuromedin C is also an anorexigenic compound (Tachibana et al., 2010) and was increased 1.8-fold in the serum of RBC2 turkeys compared to the F line. The lower levels of neuromedin C in the serum of F line turkeys may contribute to greater feed intake in these fast-growing tom turkeys, as the F line turkeys consume approximately 2.6 times more feed than the RBC2 line (Clark et al., 2019). In mammals, neuromedin C also has additional physiological functions, including the stimulation of gastrin release and regulation of blood pressure, and it increases the release of somatostatin (Gajjar and Patel, 2017). Given that somatostatin is a growth hormone inhibitor (Scanes et al., 1984), this physiological role of neuromedin C may have significant implications for body weight gain in poultry.

18-Oxocortisol Serum levels of 18-oxocortisol were increased by 1.6-fold in the serum of F line turkeys compared to the RBC2 line. 18-Oxocortisol is an analog of aldosterone and can be synthesized through the metabolism of cortisol. Most of the scientific literature concerning 18-oxocortisol relates to primary aldosteronism (Gomez-Sanchez et al., 1985; Lenders et al., 2018), which causes high blood pressure as a result of excess aldosterone production. Additionally, patients with primary aldosteronism produce excess 18-cortisol (Gomez-Sanchez et al., 1985; Lenders et al., 2018), which like aldosterone can act as both a mineralocorticoid and glucocorticoid.

Lipid Derivatives, Fatty Acid Metabolites, and Membrane Components

A number of lipids, fatty acids, and membrane components were found to accumulate differentially between the F and RBC2 turkey lines (Table 2). Several classes of acyl glycerides, primary components of both serum and body fats, were different between RBC2 and F lines. For example, a 14:0 monoacylglyceride derivative was 1.5-fold greater in RBC2 turkey serum compared to the F line. Conversely, a number of different triglycerides were found to be elevated in the serum of F line turkeys compared to the RBC2 line. It is of no surprise that triglycerides are found at higher levels in the faster-growing F line, as fast-growing poultry often have increased fat stores, increased low-density lipoprotein cholesterol, and greater serum triglyceride levels (Leclercq, 1984; March, 1984; Musa et al., 2008; Baéza and Bihan-Duval, 2013). It should be noted, however, that the sample extraction and LC-MS/MS methods used in this study were not optimized for the isolation, separation, and quantification of lipids and fatty acids. While the data presented here are interesting, further research using a chloroform: methanol extraction system and an ionization source more suited to ionizing non-polar lipids (atmospheric pressure chemical ionization, for example) would be necessary to better characterize and quantify the specific fatty acid species found in the acyl glyceride molecules discovered in our study.

Similar to mono-, di-, and triacylglycerides, several membrane phospholipids were also found at different levels within the serum of F and RBC2 turkeys. Glycerophospholipids of unknown head group composition containing fatty acid chains with lengths and saturations most likely of 18:3 and 22:5 were increased 1.8-fold in the RBC2 serum compared to the serum of F line turkeys, while the serum of F line turkeys contained greater amounts of glycerophospholipids of unknown head group composition exhibiting putative 18:1 and 18:0 fatty acid chain lengths, compared to the RBC2 turkeys. However, it should be noted that glycerophospholipids with 18:1/18:0 fatty acid species share several similar spectral features with diglycerides, particularly under the separation and ionization conditions used in this study. These data therefore need to be interpreted very cautiously, as there is a chance that the compounds which we have tentatively identified as 18:0/18:1 glycerophospholipids may actually be diglycerides with esterified unsaturated fatty acids. Two specific glycerophospholipid species which showed altered levels in F vs. RBC2 turkeys were phosphatidylethanolamine (**PE**) and phosphatidylcholine (**PC**). One specific PE species, PE(DiMe (13,5)/DiMe (13,5)), was increased in the serum of RBC2 turkeys compared to F line turkeys; but PE species containing fatty acid chains with carbon lengths of 20 and 18 showed the opposite, and were increased in the serum of F line turkeys compared to RBC2 turkeys. Several PC compounds, which are also glycerophospholipids, were also enriched in the serum of F line turkeys. As noted above, further investigation using methods optimized for phospholipid quantification are needed to better understand the changes and potential impacts of phospholipids in F vs. RBC2 turkey serum.

Other lipid and membrane components exhibiting altered levels in F vs. RBC2 serum were cardiolipins and sphingolipids. Cardiolipins consist of 2 phosphatidic acid moieties, each linked to a glycerol backbone. As a result, each cardiolipin molecule contains 2 phosphodiester linkages and 4 fatty acid chains (tails).

Table 2. Putative serum metabolites associated with lipid derivatives and fatty acid metabolism in a turkey line selected for 16 wk body weight (F) and a randombred control line 2 (RBC2).

Metabolite	HMBD ID number	Mean fold change[1]	Retention time (min)
Lipid derivatives and fatty acid metabolism			
Decenoyl-CoA, derivative	HMDB01291	−1.87	9.62
Glycerophospholipid (18:2/22:6), derivative[2]	HMDB10659	−1.85	3.23
2,4-Decadienoyl-CoA	HMDB01168	−1.83	9.44
Phosphatidylethanolamine (DiMe(13,5)/DiMe(13,5))	HMDB61491	−1.83	9.43
Triacylglyceride(20:5/18:2/20:5), derivative[3,4]	HMDB10536	−1.81	9.65
Monoacylglyceride (14:0), derivative[3,5]	HMDB11530	−1.52	8.34
Cardiolipin (18:1/18:0/18:1/18:2), derivative[6]	HMDB57894	1.34	17.15
Triacylglyceride (15:0/18:3/18:4), derivative[3,7]	HMDB43447	1.48	9.96
Cardiolipin (i-12:0/i-22:0/i-24:0/i-24:0), derivative[6]	HMDB89194	1.52	9.48
Triacylglyceride (15:0/18:0/o-18:0), derivative[3]	HMDB43075	1.52	9.91
Lignocericyl coenzyme A[8]	HMDB60239	1.63	11.20
1,2-Di-(octadecatrienoyl)-3-(galactosyl-alpha-1-6-galactosyl-beta-1)-glycerol	HMDB11127	1.64	9.54
Ganglioside GT2 (d18:0/12:0)[9]	HMDB12022	1.65	10.32
Ganglioside GQ1c (d18:0/18:0)	HMDB11935	1.70	5.77
Ganglioside GD1a (d18:1/12:0)[10]	HMDB11792	1.74	9.61
Triacylglyceride (18:4/18:4/18:4)	HMDB55501	1.79	6.79
Phosphatidylcholine (22:4/20:2), derivative[11]	HMDB08637	1.81	8.72
Phosphatidylcholine (22:0/24:0), derivative[12]	HMDB08289	1.85	8.63
Triacylglyceride (20:5/18:2/20:5), derivative[3,4]	HMDB10536	1.86	9.61
Phosphatidylcholine (o-14:0/16:0)	HMDB13341	1.87	5.87
Phosphatidylethanolamine (20:2/P-18:1),derivative[13]	HMDB09314	1.88	9.58
Ganglioside GD2 (d18:0/14:0)	HMDB11838	1.95	4.21
Acetoacetyl-CoA	HMDB01484	1.95	6.11
Cardiolipin (i-13:0/i-24:0/i-24:0/i-24:0), derivative[14]	HMDB74900	1.97	6.23
Shingolipid, ceramide (d18:1/23:0)	HMDB00950	2.05	6.44
Glycerophospholipid (18:1/18:0), derivative[15]	HMDB10617	2.06	5.61
Cholesterol ester (15:0)	HMDB60057	2.11	5.87

[1]Negative values indicate higher expression in the RBC2 line compared to the F line, whereas positive values indicate greater expression in the F line.

[2]May correspond to a glycerophospholipid (18:3/22:5) derivative.

[3]The fatty acid chains may be esterified at any of the 3 positions on the glycerol molecule.

[4]May correspond to a number of triacylglycerides or a combination of triacylglycerides which other esterified fatty acids.

[5]May correspond to other fatty acid metabolites (HMDB00701, HMDB00747, or HMDB02087).

[6]May correspond to a number of other cardiolipins with a variety of other esterified fatty acids.

[7]May correspond to a triacylglyceride (15:0/22:6/14:1) derivative (HMDB43778).

[8]May correspond to tetracosanoyl-CoA (HMDB06526).

[9]This compound also corresponds to 2 other spectral features with a fold change of 1.56 and 1.65.

[10]May correspond to ganglioside GD1b (d18:1/12:0), HMDB11822.

[11]May correspond to other phosphatidylcholine derivatives with 2 fatty acids of carbon lengths 20 and 22 with a total of 6 double bonds.

[12]May correspond to a variety of triglycerides esterified to one 15:0 fatty acid and at least 1 polyunsaturated fatty acid.

[13]May correspond to phosphatidylethanolamine (20:3/P-18:0) derivative.

[14]May correspond to a cardiolipin (i13-0/i-22:0/a-25:0/a-25:0) derivative.

[15]May correspond to a diacylglyceride (22:4/22:5) derivative.

In other words, cardiolipins have 4 fatty acid tails connected to a central glycerol moiety and a 1,3-diacylglycerol chain (Wishart et al., 2018). Several of these lipid molecules, which are most commonly found in the inner mitochondrial membranes, exhibited increased accumulation in the serum of F line turkeys compared to the RBC2 turkeys. This is interesting, as more recent research has linked cardiolipin release to increased apoptosis and cell death (Maguire et al., 2017).

Additionally, several sphingolipid gangliosides exhibited increased levels in F vs. RBC2 serum. Gangliosides contain a ceramide and oligosaccharide, collectively termed a glycosphingolipid, along with one or more sialic acid residues (Wishart et al., 2018). Gangliosides GT2, GQ1c, GD1a (or GD1b), and GD2 were all increased in the F line turkeys compared to the RBC2 turkeys, each with a fold-change of 1.6 or greater. The purpose or rationalization for the increased levels of these gangliosides within the serum of growth-selected poultry is not understood, but could be related to ganglioside interactions with growth factors (Bremer et al., 1984, 1986) or their functional role in insulin sensitivity (Nojiri et al., 1991; Yamashita et al., 2003).

Finally, we noted differences in the levels of metabolites associated with lipid degradation, specifically beta-oxidation products, between the control and growth-selected turkey lines. Specifically, RBC2 turkeys had greater amounts of decenoyl-CoA and decadienoyl-CoA in the serum compared to F turkeys; on the other hand, the serum from F line turkeys had greater amounts of acetoacetyl-CoA and lignocericyl CoA (or tetracosanoyl CoA) than did that of RBC2 birds. These unique fatty

Table 3. Putative serum metabolites associated with protein metabolism and microbial fermentation in a turkey line selected for 16 wk body weight (F) and a randombred control line 2 (RBC2).

Metabolite	HMBD ID number	Mean fold change[1]	Retention time (min)
Protien metabolism			
Hydroxyprolyl-tyrosine[2]	HMDB28875	−4.17	8.33
Tryptophyl-glutamine[3]	HMDB29081	−2.22	17.13
Gamma-glutamyl glutamine	HMDB11738	−1.66	6.10
L-alpha-glutamyl-L-hydroxyproline	HMDB11161	1.48	1.34
Microbial fermentation			
2,3-Butanediol	HMDB03156	1.47	1.40
O-Desmethylangolensin	HMDB04629	1.63	2.83

[1]Negative values indicate higher expression in the RBC2 line compared to the F line, whereas positive values indicate greater expression in the F line.

[2]Indistinguishable from the dipeptide glutamylphenylalanine (HMDB00594).

[3]Indistinguishable from the dipeptides tryptophyl-gamma-glutamate (HMDB29097) and tryptophyl-serine (HMDB29092).

acid intermediaries in the F and RBC2 turkeys, which have been fed the same diet, suggest that long-term selection for increased body weight has impacted fatty acid metabolism. Growth selection-induced changes in fatty acid metabolism are also supported by the work of Boschetti et al. (2016), who demonstrated that desaturating enzyme activity was also different in birds with different growth rates.

Protein Metabolism

Our metabolomic screening found that there were a number of dipeptides, likely a result of incomplete protein breakdown, found at higher levels in the RBC2 serum compared to the F line serum (Table 3). Dipeptides putatively comprised of hydroxyproline and tyrosine, gamma-glutamate and phenylalanine, tryptophan and glutamine, tryptophan and gamma-glutamate, tryptophan and serine, and glutamic acid and glutamine were all increased in the serum of RBC2 turkeys compared to the F line turkeys. Further confirmation of the composition of these dipeptides will require the application of more targeted QTOF LC-MS/MS methods, and potentially the use of nuclear magnetic resonance to confirm bond structures and linkages. Despite this limitation, however, it is clear that a number of dipeptide compounds exhibited increased in the serum of RBC2 turkeys, with some compounds showing levels of accumulation up to 4.2-fold higher than those observed in the F line. There was only one dipeptide increased in the serum of F line turkeys compared to the RBC2 turkeys, which we putatively identified as L-alpha-glutamyl-L-hydroxyproline, a dipeptide comprised of glutamate and hydroxyproline which may arise as a result of collagen break down (Jandke and Spiteller, 1986). While the functional relevance of these dipeptides in the serum is not known, the increased levels of dipeptides observed in F line turkey serum may be associated with growth selection effects on the rate of protein catabolism, protein metabolism, or dipeptide absorptive capacity.

Microbial Fermentation

Interestingly, 2 metabolites with differential expression patterns in F vs. RBC2 serum were putatively identified as compounds associated with bacterial fermentation (Table 3). Serum from the RBC2 turkeys had greater levels of a compound tentatively identified as 2,3-butanediol, whereas serum from the F line had greater levels of a compound putatively identified as O-desmethylangolensin. While the biological functions of these bacterial fermentation products are not understood, these findings may suggest that the intestinal microbiome is impacted by the genetic changes associated with selection for increased body weight. Further research is needed to not only determine how genetic changes that arise as a result of growth selection can alter the intestinal microbiome when housed in identical environments, but also how these microbial shifts may regulate body weight gain.

CONCLUSION

Comparison of the F and RBC2 serum metabolome suggests that selection for increased 16 wk body weight has significantly impacted many metabolic processes of the turkey. Our data do not allow us to conclude which metabolic changes lead to increased body weight, and which are potentially indirect effects resulting from increased body weight (i.e., downstream of the phenotype selected for during breeding efforts) or other, non-body weight associated changes in the genome. Additionally, while our analyses have putatively identified several metabolites, further research is needed to validate the metabolites which have been identified in this study, as well as to identify any additional metabolites involved in body weight regulation. For example, extraction procedures designed to optimize the extraction of non-polar lipids (i.e., the use of chloroform: methanol extraction, rather than methanol precipitation of sera) would allow a more complete characterization of fatty acid, sterol, and phospholipid species, while the use of normal phase qTOF LC-MS/MS or nuclear magnetic

resonance would provide increased resolution of dipeptide and other proteinaceous features of the serum. Our data do, however, highlight several compounds and metabolic pathways associated with increased body weight. These metabolites can inform future studies to further dissect their impact on growth, muscle mass accretion, and overall changes in the phenotype of modern commercial turkeys.

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