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Muscle growth affects the metabolome of the *pectoralis major* muscle in red-winged tinamou (*Rhynchotus rufescens*)

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ABSTRACT The aim of the present study was to identify and quantify the metabolites (metabolome analysis) of the *pectoralis major* muscle in male red-winged tinamou (*Rhynchotus rufescens*) selected for growth traits. A selection index was developed for females [body weight (**BW**), chest circumference (**CC**), and thigh circumference (**TC**)] and males [BW, CC, TC, semen volume, and sperm concentration] in order to divide the animals into 2 experimental groups: selection group with a higher index (TinamouS) and commercial group with a lower index (TinamouC). Twenty male offspring of the 2 groups (TinamouS, $n = 10$; TinamouC, $n = 10$) were confined for 350 d. The birds were slaughtered and *pectoralis major* muscle samples were collected, subjected to polar and apolar metabolites extractions and analyzed by proton nuclear magnetic resonance (**^{1}H NMR**) spectroscopy. Analysis of the polar metabolomic profile identified 65 metabolites; 29 of them were differentially expressed between the experimental groups ($P < 0.05$). The TinamouS groups exhibited significantly higher concentrations ($P < 0.05$) of 25 metabolites, including anserine, aspartate, betaine, carnosine, creatine, glutamate, threonine, 3-methylhistidine, NAD+, pyruvate, and taurine. Significantly higher concentrations of cysteine, beta-alanine, lactose, and choline were observed in the TinamouC group ($P < 0.05$). The metabolites identified in the muscle provided information about the main metabolic pathways (higher impact value and $P < 0.05$), for example, phenylalanine, tyrosine and tryptophan biosynthesis; alanine, aspartate and glutamate metabolism; D-glutamine and D-glutamate metabolism; β-alanine metabolism; glycine, serine and threonine metabolism; taurine and hypotaurine metabolism; histidine metabolism; phenylalanine metabolism. The NMR spectra of apolar fraction showed 8 classes of chemical compounds. The metabolome analysis shows that the selection index resulted in the upregulation of polyunsaturated fatty acids, unsaturated fatty acids, phosphocholines, phosphoethanolamines, triacylglycerols, and glycerophospholipids. The present study suggests that, despite few generations, the selection based on muscle growth traits promoted changes in metabolite concentrations in red-winged tinamou.

Key words: muscle growth, selection index, metabolomics, NMR, Tinamidae

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INTRODUCTION

Brazil has one of the richest avifaunas in South America. However, the illegal trade and hunting of wild birds represents the national reality and is directly associated with culture, economy, and human nutrition. The wild bird products market serves niche consumption, such as the production of meat, eggs, feathers/plumes, leisure, and ornaments, arousing the interest of small producers (Piacentini et al., 2015; Pacheco et al., 2021). Thus, the commercial production of wild birds must consider conservationist principles and the demands and needs of consumers.

The red-winged tinamou (*Rhynchotus rufescens*) is a terrestrial bird and its free-living population can be

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found in semiopen areas throughout Brazil, with predominance in the Cerrado region (Sick, 1997). Although only few studies have investigated the meat of red-winged tinamou, this bird provides good carcass and breast yields when bred in captivity (Moro et al., 2006; Tholon, 2008; Correia et al., 2018; Hata et al., 2018).

Investments in animal improvement are one of the most important factors for increasing meat production (Mpenda et al., 2019). In the case of the red-winged tinamou, the implementation of genetic breeding programs is still necessary to improve meat production. In this scenario, a selection index should be used, which permits to improve the aggregate breeding value of a given population by thoughtfully combining multiple traits of economic interest (Hazel and Lush, 1942; Cunningham and Tauebert, 2009). A previous study from our group using the same red-winged tinamou population found that the use of a selection index promotes greater body growth while preserving meat quality of the birds (Martins et al., 2023).

Although animal selection promotes significant improvements in growth rates and meat production, a detailed understanding of muscle development is necessary in order to prevent physiological disorders, changes in composition of the muscle fiber type, and metabolic alterations that could influence meat quality (Deeb and Lamont, 2002; Ruusunen and Puolanne, 2004; Aliabad et al., 2011; Tang et al., 2021). Within this context, metabolomics has been used to elucidate the biological mechanisms underlying muscle development related to the production of different bird species (Wang et al., 2017; Ceribeli et al., 2018; Cônsolo et al., 2020; Ma et al., 2020; Basile et al., 2021), since metabolites are the intermediate products or end-products of complex cellular reactions and are sensitive markers of physiological activity (Fontanesi, 2016; Zhang et al., 2022). The present study reports for the first time the metabolomic profile of the *pectoralis major* muscle of 2 groups of male red-winged tinamou (*Rhynchotus rufescens*) bred in captivity and selected for growth using proton nuclear magnetic resonance (^{1}**H NMR**) spectroscopy.

MATERIALS AND METHODS

Animal Production and Sample Collection

The animals used in the experiment belong to the Commercial Wild Fauna Breeding Center (authorization number 0000035744) of the Wild Animal Sector, School of Veterinary Medicine and Animal Science (**FMVZ**), Unesp, Botucatu, Brazil. All procedures were conducted in accordance with guidelines for animal welfare and humane slaughter and were approved by the Ethics Committee on Animal Experimentation of FMVZ (Protocol 0083/2020).

Red-winged tinamous were classified using a phenotypic selection index established in 2017 (Martins et al., 2023). Briefly, the selection index was composed of growth traits such as live weight and chest and thigh circumference at 180 d of life and male reproductive traits such as semen volume and sperm concentration. Birds with the highest indices were selected and formed the selection group (TinamouS), with a selected fraction of 14.3% (selection intensity (i) = 1.5), while animals that did not undergo selection formed the commercial group (TinamouC).

Ten couples from the TinamouS group were housed in 2-m^2 pens, 1 couple per pen, designed in such a way as to avoid inbreeding. Animals from the TinamouC group were housed in 6-m^2 pens at different male/female ratios. During the breeding season, eggs were collected, disinfected, identified, and artificially incubated at 36°C and 60% humidity (Premium Ecológica IP 70). On d 18, the eggs were transferred to individual net bags and sent to the hatchery where they were incubated at 37.5°C and 85% humidity for a period of 21.3 ± 0.948 d.

After hatching, as previously reported by Martins et al. (2023), the chick weight of the second generation of the 2 experimental groups was measured with a digital scale (TinamouS = 38.9 ± 3.6 g; TinamouC = 39.7 ± 3.3 g; $P = 0.64$). The birds were identified with a ring on the right wing, submitted to a sanitary protocol, and allocated to same pen- with coast cross hay bedding, which was changed every 30 d. The animals were provided with a prestarter phase commercial feed containing 230 g/kg of crude protein. Feed and water were offered ad libitum in specific bird feeders and drinkers, which were cleaned and supplied daily. After 30 d of life, 20 male animals (n = 10 per group) were randomly selected and allocate into 2 pens, with 5 TinamousS and 5 TinamouC animals/each. A commercial broiler chicken diet was offered, which contained 150 g/kg crude protein and 2.65 kcal/kg metabolizable energy.

Twenty male animals (n = 10 per group) were weighed at 180 d (TinamouS = 579.67 ± 63.48 g; TinamouC = 494.05 ± 63.81 g; $P = 0.02$) and at 350 d of life (TinamouS = 697.39 ± 47.22 g; TinamouC = 612.10 ± 45.76 g; $P = 0.004$) (Martins et al., 2023). The animals were sent to the slaughterhouse where they were stunned, bled, scalded at 57°C for up to 2 min, eviscerated, and identified. The hot carcass weight, weights of skinless breast, back, wing, thigh, drumstick, heart, liver and intestine, carcass yield, and breast yield data had been collected and published in a previous study from our research group (Martins et al., 2023). During slaughter, samples of the *pectoralis major* muscle were collected from each bird, immediately immersed in liquid nitrogen, and stored at −80°C for the metabolomic assays.

Nontargeted Metabolomics

Preparation of Metabolite Samples Metabolites were extracted using approximately 200 mg of muscle sample of each animal. The samples were homogenized in methanol:chloroform:water (2:1:1, v/v/v) in a homogenizer tube containing ceramic beads for 1 min at 5 ms^{-1} in a cell disruptor (Fast Prep, MP Biomedicals, Solon, OH) at 4°C according to Zawadzki et al. (2017), with modifications. The homogenate was centrifuged at 13,000 × g

for 10 min at 4°C and the upper (hydroalcoholic phase; polar metabolites) and lower phases (chloroform phase; apolar metabolites) were transferred to new microtubes. Next, 300 μL chloroform was added to the upper phase to extract possible lipid residues. The tubes were homogenized in a vortex, centrifuged at 13,000 × *g* for 10 min at 4°C, and the phases were separated. The samples were kept on ice throughout the procedure. The microtubes were dried in a Speed-Vac concentrator (ThermoSavant, Holbrook, NY) for 12 h.

After drying, 1 mL deuterium oxide phosphate buffer (0.10 M, pD 7.4) containing 0.05% 3-trimethylsilyl-2,2,3,3-d4-propionate sodium salt as internal chemical shift standard (**TMSP-d4**, Sigma-Aldrich St. Louis, MO, USA) was added to the polar metabolite extract. The mixture was homogenized in a vortex, and 600 μL was transferred to a 5 mm NMR tube. For the apolar metabolite extract, 1 mL chloroform was added, the mixture was homogenized in a vortex, and 600 μL was transferred to a 5 mm NMR tube.

^{1}H NMR Spectrum Acquisition *Polar Metabolites* The ^{1}H NMR spectra were acquired at 298 K with a 14 T Bruker Avance III NMR spectrometer (Bruker BioSpin, Rheinstetten, Germany) equipped with a 5-mm PABBO probe head. First, a protocol was developed for the quality control (**QC**) samples, which consisted of a pool of aliquots of the metabolite extracts from the *pectoralis major* muscle (calibrated 90° pulse and irradiation at water frequency). The protocol was performed in fully automated mode using the Bruker routine (load, autotuning, lock phase, shimming, acquisition, and process) via the ICON-NMR interface (Bruker Biospin). Proton NMR spectra were acquired using a 90° pulse (zg sequence) and 64 K data points, with a spectral width of 20 ppm, acquisition time of 2.726 s, recycle delay of 4 s, and 16 scans were used.

Water suppression was obtained by the 1D NOESY pulse sequence (Bruker 1D noesygppr1d) using the same data, spectral with and acquisition time as in the experiments with irradiation at water frequency (O1 at 2,821.41 Hz depending on the QC) and mixing time of 0.005 s. The FIDs were multiplied by an exponential multiplication function of 0.3 Hz before Fourier transform. Only 0-order phase correction was allowed and the TMSP-d4 signal was calibrated at $\delta = 0.00$ ppm using the Topspin 3.6 software for NMR analysis (Bruker Inc., Karlsruhe, Germany).

The 2D NMR experiments with J-resolved (**JRES**), ^{1}H−^{13}C HSQC and ^{1}H−^{1}H COSY experiments were performed on 2 samples of each group for the validation of the assignments of metabolites signals.

Apolar Metabolites The ^{1}H NMR spectra were also obtained with a Bruker Avance III NMR 600 MHz NMR spectrometer (Bruker BioSpin, Germany). The spectra were recorded at 25°C, with an acquisition time of 2.66 s, spectral width of 26 ppm, and recycle decay of 2 s and 128 scans.

Identification and Quantification of Metabolites *Polar Metabolites* The ^{1}H NMR spectra were processed and analyzed using the Chenomx NMR Suite 8.2 (Chenomx, Edmonton, Canada). The identified and quantified metabolites were also defined based on the Human Metabolome Database (**HMDB**, http://www.hmdb.ca) and the Biological Magnetic Resonance Data Bank (**BMRB**, http://bmrb.wisc.edu).

Apolar Metabolites The compounds were identified based on chemical shift, peak multiplicity and coupling constants according to the NMR lipid library (HMDB, http://www.hmdb.ca). Individual metabolite peaks were integrated and the ^{1}H NMR data were binned to 0.04 ppm and were transformed into a data matrix using the MNova software.

Statistical Analysis

The metabolites quantification were analyzed using the MIXED procedure of the SAS statistical program (2011, SAS Institute, Cary, NC) to obtain the *P* value. In addition, the metabolite data were imported into MetaboAnalyst 5.0 (http://www.metaboanalyst.ca) for correlation analysis between metabolites, principal component analysis (**PCA**), partial least squares discriminant analysis (**PLS-DA**), and calculation of variable importance in projection (**VIP**) scores in order to identify differentially expressed metabolites ($P < 0.05$), according to the method proposed by (Xia and Wishart, 2011). The metabolic pathways for polar compounds were analyzed based on the Kyoto Encyclopedia of Genes and Genomes (**KEGG**, http://www.kegg.jp) using the *Gallus gallus* pathway library. The network diagrams between the metabolites found and the impact values obtained by topology-based pathway analysis (Xia et al., 2009), as well as the integration of the metabolic pathways, are displayed graphically.

RESULTS

The metabolomics profiles of the *pectoralis major* muscle of red-winged tinamou were analyzed by ^{1}H NMR. Sixty-five polar metabolites were identified and quantified. These metabolites were classified as amino acids, peptides, and analogs (43.1%), purine ribonucleotides/purines and purine derivatives/nucleotides (18.5%), carboxylic acids and derivatives (9.2%), carbohydrates (6.2%), alcohols (3.1%), fatty acyls (3.1%), hydroxy acids and derivatives (3.1%), imidazoles and imidazolines (3.1%), quaternary ammonium salts (3.1%), alpha-keto acids (1.6%), amines (1.6%), organic nitroso compounds (1.6%), organosulfonic acids (1.6%), and pyridinecarboxylic acids (1.6%) (Table 1).

The concentration of 29 polar metabolites showed a difference ($P < 0.05$) between the experimental groups. Compared to the TinamouC group, the TinamouS group exhibited higher concentrations ($P < 0.05$) of amino acids, peptides, and analogs (anserine, arginine, aspartate, betaine, carnosine, creatine, creatine phosphate, creatinine, glutamate, leucine, proline, threonine,

Table 1. Metabolites identified in the proton nuclear magnetic resonance (^{1}H NMR) spectra of the *pectoralis major* muscle of captivity-bred red-winged tinamou (*Rhynchotus rufescens*) of the selection (TinamouS) and commercial (TinamouC) groups.

Polar metabolites	ID[1]	Formula	TinamouS (mg/dL)[2]	TinamouC (mg/dL)[2]	*P* value
Amino acids, peptides, and analogs					
Alanine	HMDB00161	$C_3H_7NO_2$	4.18 ± 1.22	3.96 ± 1.54	0.7258
Anserine	HMDB00194	$C_{10}H_{16}N_4O_3$	4.44 ± 0.83	0.99 ± 0.70	<0.0001
Arginine	HMDB00517	$C_6H_{14}N_4O_2$	3.30 ± 0.62	1.61 ± 0.40	<0.0001
Aspartate	HMDB00191	$C_4H_7NO_4$	3.66 ± 0.49	1.49 ± 0.39	<0.0001
Betaine	HMDB00043	$C_5H_{11}NO_2$	3.79 ± 0.62	2.02 ± 0.51	0.0005
Carnosine	HMDB00033	$C_9H_{14}N_4O_3$	7.41 ± 0.96	4.12 ± 0.68	<0.0001
Creatine	HMDB00064	$C_4H_9N_3O_2$	227.17 ± 21.52	133.12 ± 33.56	<0.0001
Creatine phosphate	HMDB01511	$C_4H_{10}N_3O_5P$	128.92 ± 42.65	44.31 ± 21.24	0.0264
Creatinine	HMDB00562	$C_4H_7N_3O$	4.53 ± 0.89	2.42 ± 0.62	0.0036
Cysteine	HMDB00574	$C_3H_7NO_2S$	2.06 ± 0.70	3.67 ± 1.08	0.0009
Glutamate	HMDB00148	$C_5H_9NO_4$	4.73 ± 0.68	2.46 ± 0.65	0.0004
Glutamine	HMDB00641	$C_5H_{10}N_2O_3$	4.37 ± 1.20	3.81 ± 1.54	0.5150
Glutathione	HMDB00125	$C_{10}H_{17}N_3O_6S$	2.20 ± 0.66	2.36 ± 0.91	0.6593
Glycine	HMDB00123	$C_2H_5NO_2$	1.23 ± 0.55	1.08 ± 0.59	0.6946
Guanidinoacetate	HMDB00128	$C_3H_7N_3O_2$	549.12 ± 142.83	528.20 ± 108.90	0.8066
Histidine	HMDB00177	$C_6H_9N_3O_2$	0.53 ± 0.68	0.39 ± 0.11	0.5385
Isoleucine	HMDB00172	$C_6H_{13}NO_2$	1.61 ± 0.30	1.49 ± 0.66	0.6096
Leucine	HMDB00687	$C_6H_{13}NO_2$	3.28 ± 0.67	1.89 ± 0.55	<0.0001
Lysine	HMDB00182	$C_6H_{14}N_2O_2$	1.14 ± 0.56	0.73 ± 0.50	0.2825
Methionine	HMDB00696	$C_5H_{11}NO_2S$	1.24 ± 0.39	1.12 ± 0.43	0.5527
Phenylalanine	HMDB00159	$C_9H_{11}NO_2$	1.88 ± 0.62	1.65 ± 0.53	0.3742
Proline	HMDB00162	$C_5H_9NO_2$	1.59 ± 0.34	0.94 ± 0.25	0.0001
Sarcosine	HMDB00271	$C_3H_7NO_2$	0.98 ± 0.39	0.83 ± 0.33	0.6411
Threonine	HMDB00167	$C_4H_9NO_3$	6.20 ± 1.47	2.78 ± 1.22	0.0046
Tyrosine	HMDB00158	$C_9H_{11}NO_3$	2.24 ± 0.40	2.01 ± 0.68	0.3665
Valine	HMDB00883	$C_5H_{11}NO_2$	2.42 ± 0.43	2.18 ± 0.89	0.4498
Beta-alanine	HMDB00056	$C_3H_7NO_2$	0.44 ± 0.13	0.75 ± 0.22	0.0013
3-Methylhistidine	HMDB00479	$C_7H_{11}N_3O_2$	4.53 ± 0.76	2.37 ± 0.33	0.0062
Purine ribonucleotides/purines and purine derivatives/nucleotides					
ADP	HMDB01341	$C_{10}H_{15}N_5O_{10}P_2$	4.43 ± 0.82	1.81 ± 0.70	0.0005
AMP	HMDB00045	$C_{10}H_{14}N_5O_7P$	44.58 ± 13.36	5.70 ± 3.57	<0.0001
ATP	HMDB00538	$C_{10}H_{16}N_5O_{13}P_3$	9.94 ± 1.96	5.45 ± 1.34	<0.0001
Adenosine	HMDB00050	$C_{10}H_{13}N_5O_4$	0.30 ± 0.01	0.16 ± 0.04	0.0037
GTP	HMDB01273	$C_{10}H_{16}N_5O_{14}P_3$	2.07 ± 0.88	1.98 ± 0.80	0.8454
IMP	HMDB00175	$C_{10}H_{13}N_4O_8P$	87.95 ± 12.68	32.69 ± 10.13	0.0120
Inosine	HMDB00195	$C_{10}H_{12}N_4O_5$	14.91 ± 2.50	8.93 ± 1.70	0.0001
NADH	HMDB01487	$C_{21}H_{29}N_7O_{14}P_2$	0.94 ± 0.60	0.72 ± 0.16	0.3177
NADPH	HMDB00221	$C_{21}H_{30}N_7O_{17}P_3$	1.31 ± 0.76	0.96 ± 0.27	0.2135
NAD+	HMDB00902	$C_{21}H_{28}N_7O_{14}P_2$	15.12 ± 2.53	6.50 ± 2.68	0.0011
Oxypurinol	HMDB00786	$C_5H_4N_4O_2$	2546.68 ± 399.92	3151.27 ± 370.12	0.3849
Uridine	HMDB00296	$C_9H_{12}N_2O_6$	0.26 ± 0.08	0.29 ± 0.07	0.3688
Carboxylic acids and derivatives					
Acetate	HMDB00042	$C_2H_4O_2$	1.20 ± 0.20	0.67 ± 0.16	0.0052
Citrate	HMDB00094	$C_6H_8O_7$	0.53 ± 0.24	0.51 ± 0.17	0.8234
Fumarate	HMDB00134	$C_4H_4O_4$	0.29 ± 0.11	0.25 ± 0.10	0.3756
Malonate	HMDB00691	$C_3H_4O_4$	71.43 ± 12.48	61.29 ± 12.03	0.1939
Methylmalonate	HMDB00202	$C_4H_6O_4$	3.92 ± 1.06	3.19 ± 0.67	0.2992
Succinate	HMDB00254	$C_4H_6O_4$	0.20 ± 0.08	0.15 ± 0.05	0.0945
Carbohydrates					
1,3-Dihydroxyacetone	HMDB01882	$C_3H_6O_3$	5.41 ± 1.02	2.86 ± 0.86	<0.0001
Glucose	HMDB00122	$C_6H_{12}O_6$	17.20 ± 2.50	4.70 ± 1.09	0.0009
Glucose-6-phosphate	HMDB01401	$C_6H_{13}O_9P$	19.61 ± 8.29	19.01 ± 7.35	0.9238
Lactose	HMDB00186	$C_{12}H_{22}O_{11}$	6.46 ± 2.55	17.52 ± 2.21	0.0001
Alcohols					
Ethanol	HMDB00108	C_2H_6O	6.44 ± 1.21	4.12 ± 1.03	0.4065
Methanol	HMDB01875	CH_4O	74.29 ± 13.67	40.24 ± 19.33	0.2402
Fatty acyls					
Citraconate	HMDB00634	$C_5H_6O_4$	0.13 ± 0.06	0.09 ± 0.04	0.0818
4-Hydroxybutyrate	HMDB00710	$C_4H_8O_3$	0.29 ± 0.13	0.30 ± 0.17	0.8566
Hydroxy acids and derivatives					
Lactate	HMDB00190	$C_3H_6O_3$	431.87 ± 103.90	397.26 ± 95.12	0.5136
Malate	HMDB00156	$C_4H_6O_5$	1.57 ± 0.53	1.05 ± 0.40	0.0896
Imidazoles and imidazolines					
Imidazole	HMDB01525	$C_3H_4N_2$	1.44 ± 0.56	1.17 ± 0.25	0.1809
N-Methylhydantoin	HMDB03646	$C_4H_6N_2O_2$	1.29 ± 0.31	1.06 ± 0.26	0.0806
Quaternary ammonium salts					
Carnitine	HMDB00062	$C_7H_{15}NO_3$	1.86 ± 0.59	1.55 ± 0.60	0.2638
Choline	HMDB00097	$C_5H_{14}NO$	0.66 ± 0.29	1.37 ± 0.45	0.0005
Alpha-keto acids					
Pyruvate	HMDB00243	$C_3H_4O_3$	0.29 ± 0.06	0.20 ± 0.04	0.0008

(*continued*)

Table 1 (*Continued*)

Polar metabolites	ID[1]	Formula	TinamouS (mg/dL)[2]	TinamouC (mg/dL)[2]	*P* value
Amines					
Dimethylamine	HMDB00087	C_2H_7N	8.49 ± 1.64	7.38 ± 1.59	0.1408
Organic nitroso compounds					
N-Nitrosodimethylamine	HMDB31419	$C_2H_6N_2O$	2.40 ± 1.79	3.60 ± 1.87	0.1582
Organosulfonic acids					
Taurine	HMDB00251	$C_2H_7NO_3S$	14.26 ± 2.42	6.30 ± 2.74	0.0001
Pyridinecarboxylic acids					
Niacinamide	HMDB01406	$C_6H_6N_2O$	3.06 ± 0.96	2.77 ± 0.67	0.4475

[1]ID: Human Metabolome Database (HMDB, http://www.hmdb.ca).
[2]Mean ± standard deviation.

and 3-methylhistidine), purine ribonucleotides/purines and purine derivatives/nucleotides (ADP, AMP, ATP, adenosine, IMP, inosine, and NAD+), carboxylic acids and derivatives (acetate), carbohydrates (1,3-dihydroxyacetone and glucose), alpha-keto acids (pyruvate), and organosulfonic acids (taurine). However, the concentrations of lactose, cysteine, beta-alanine, and choline were lower ($P < 0.05$) in *pectoralis major* muscle of the TinamouS group.

The polar metabolites were submitted to PCA and PLS-DA. The former demonstrated divergent metabolite concentrations in the TinamouS and TinamouC groups, with a total variance of 55.9% [PC1 (33.2%) vs. PC2 (22.7%)] (Figure 1A). The total variance was 44.7% in PLS-DA [component 1 (23%) vs. component 2 (21.7%)] (Figure 1B). In addition, the cross-validation parameters (accuracy = 0.95, $Q^2 = 0.69$ and $R^2 = 0.83$ for component 1 and accuracy = 1.0, $Q^2 = 0.80$ and $R^2 = 0.91$ for component 2) suggest differences between the experimental groups, which can be observed by the evident separation of the TinamouS and TinamouC groups.

A VIP score >1.0 indicates the top 15 most influential metabolites in the PLS-DA model (Figure 1C). Among these metabolites, higher concentrations of AMP, anserine, creatine phosphate, glucose, aspartate, threonine, glutamate, and IMP were observed in the TinamouS group. On the other hand, the TinamouC group exhibited higher concentrations of oxypurinol, lactose, choline, N-nitrosodimethylamine, cysteine, beta-alanine, and guanidinoacetate.

The present study also explored the correlations between polar metabolites, with 197 significant positive (≥ 0.5; $P < 0.05$) and 79 significant negative correlations (≥−0.5; $P < 0.05$) (Figure 1D, Supplementary Tables 1 and 2). We highlight the positive correlations of carnosine dipeptide with glutamate (0.71) and aspartate (0.66). Glutamate also showed positive correlations with pyruvate (0.57) and ATP (0.57) and a negative correlation with choline (−0.55). Aspartate was correlated with anserine (0.77), pyruvate (0.57), ATP (0.58), choline (−0.57), beta-alanine (−0.49), and cysteine (−0.61). Anserine showed a negative correlation with cysteine (−0.59) and beta-alanine (−0.56). Beta-alanine was also correlated with cysteine (0.77), threonine (0.59), and choline (0.62). Choline showed positive correlations with threonine (0.71) and cysteine (0.61). Finally, betaine was correlated with NAD+ (0.57).

To better understand the polar metabolomic profile, the metabolic pathways were analyzed using the *Gallus gallus* database (Figure 2). In general, 20 significant pathways were detected ($P < 0.05$), including 8 main pathways with an impact value >0.35: phenylalanine, tyrosine and tryptophan biosynthesis (gga00400); alanine, aspartate and glutamate metabolism (gga00250); D-glutamine and D-glutamate metabolism (gga8964539); β-alanine metabolism (gga00410); glycine, serine and threonine metabolism (gga00260); taurine and hypotaurine metabolism (gga00430); histidine metabolism (gga00340); phenylalanine metabolism (gga00360) (Table 2). Integration of these main pathways in a single map revealed a high level of connectivity and interconnection between pathways (Figure 3).

We also obtained the ^{1}H NMR spectra of the lipid fraction of red-winged tinamou *pectoralis major* muscle. Identification of apolar metabolites revealed peaks of cholesterol (**CH**), free fatty acids (**FFA**), polyunsaturated fatty acids (**PUFA**), unsaturated fatty acids (**UFA**), phosphocholines (**PCh**), phosphoethanolamines (**PE**), triacylglycerols (**TAG**), and glycerophospholipids (**PL**). PCA and PLS-DA were also used to visualize differences in apolar metabolites (Figure 4A and B). In general, PCA [PC1 (71.4%) vs. PC2 (16.2%)] and PLS-DA [component 1 (38.6%) vs. component 2 (48.9%)] revealed a clear separation between the TinamouS and TinamouC groups. The goodness-of-fit measures were accuracy = 0.9, $R^2 = 0.65$, and $Q^2 = 0.45$ for the first component of PLS-DA and accuracy = 1.0, $R^2 = 0.86$, and $Q^2 = 0.83$ for the second component.

The differences between TinamouS and TinamouC can also be clearly seen in the clusters of the heatmap obtained by hierarchical cluster analysis (Figure 4C). Lipids of the TinamouS group were richer in PUFA, UFA, PCh, PE, TAG, and PL. Different patterns were found for lipids of the TinamouC group, with high levels of free fatty acids and cholesterol. The VIP score was calculated and a score ≥1.0 defined the most influential apolar compounds in the PLS-DA model (Figure 4D). This procedure permitted to identify the 6 key compounds that separated the red-winged tinamou groups (PUFA, PL, TAG, PCh, UFA, and cholesterol).

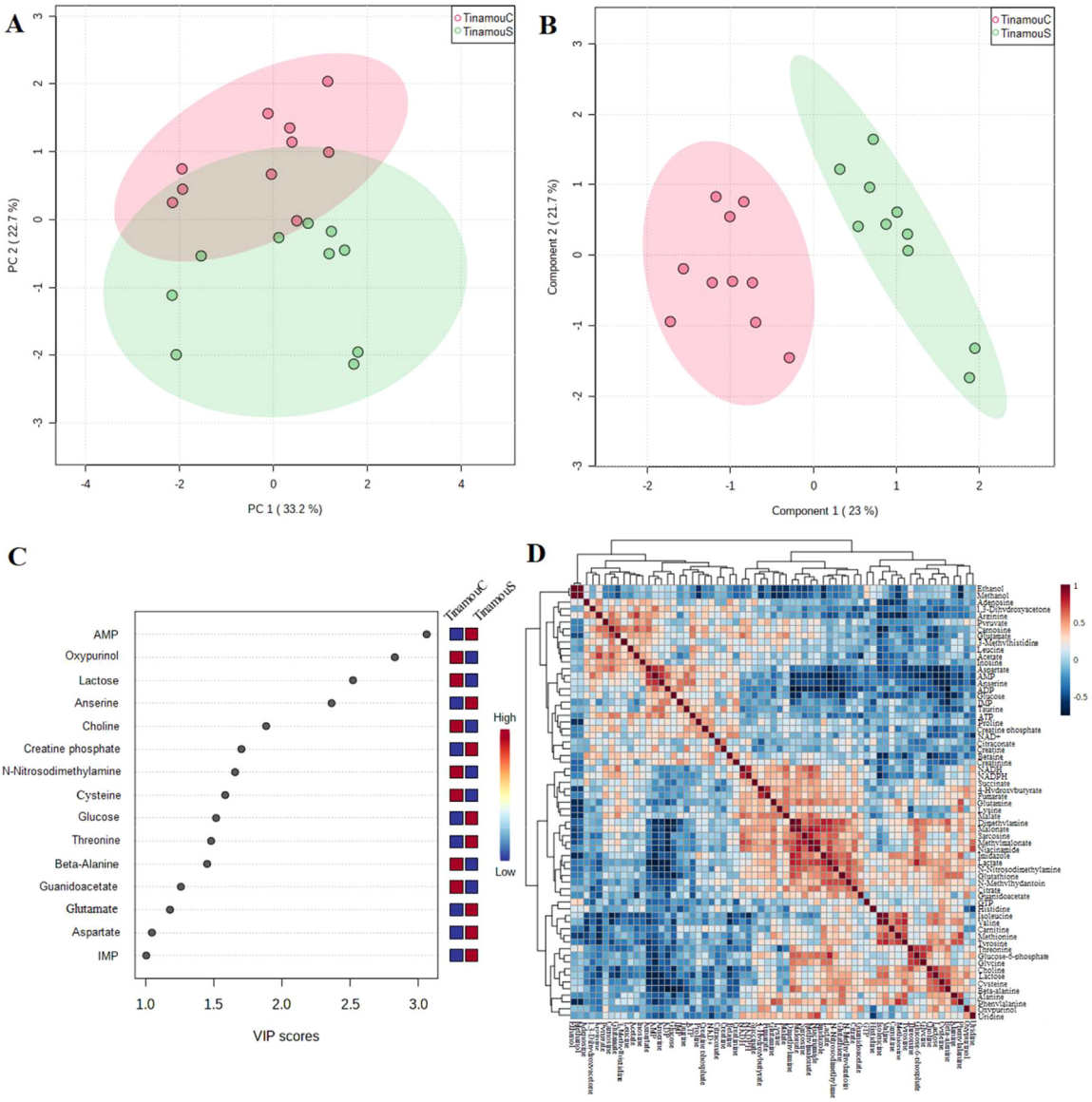

Figure 1. Profile of polar metabolites in the *pectoralis major* muscle of red-winged tinamou. (A) Principal component (PC) analysis of polar metabolites identified by ^{1}H NMR (1 data point represents 1 animal). (B) Partial least squares discriminant analysis of polar metabolites in the ^{1}H NMR spectra (1 data point represents 1 animal). (C) Top 15 metabolites (chemical spectrum shift—ppm) selected by a variable importance in the projection (VIP) score ≥1.0. (D) Correlation heatmap of all polar metabolites. Blue and red colors correspond to positive and negative correlations, respectively. The intensity of the correlation and *P* value are given in Supplementary Tables 1 and 2.

DISCUSSION

Using the same population of red-winged tinamou (*Rhynchotus rufescens*) adopted here, Martins et al. (2023) previously selected 20 birds for muscle growth based on a selection index (TinamouS = 10 animals with a higher index vs. TinamouC = 10 animals with a lower index). We used the same experimental groups in the present study and investigated the metabolome of the *pectoralis major* muscle by ^{1}H NMR spectroscopy. The muscle metabolite profile of red-winged tinamou has not yet been described in the literature.

Polar Metabolites

Amino Acids, Peptides, and Analogs In the present study, metabolomic analysis was able to identify differences in the concentrations of metabolites involved in multiple biochemical processes between the TinamouS and TinamouC groups. Our results indicated higher concentrations of some amino acids in the TinamouS group, providing evidence of an association of these compounds with greater muscle performance. Amino acids are of great importance for the synthesis of proteins of high nutritional value (production of meat, milk, and eggs) and are responsible for muscle growth, with insufficient amounts compromising protein synthesis and, consequently, animal performance (Church et al., 2020).

Arginine is an essential amino acid in birds that stimulates the release of hormones such as insulin and growth hormone (Murakami et al., 2012). Furthermore, this compound is used for the synthesis of different metabolites such as ornithine, polyamines (spermidine,

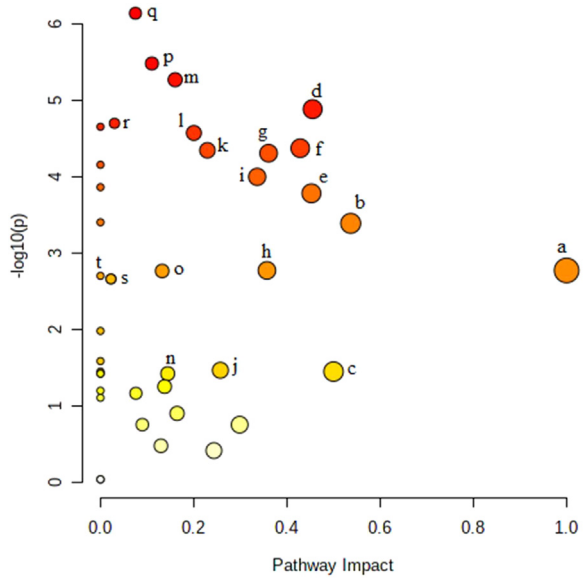

Figure 2. Analysis of metabolic pathways in the *pectoralis major* muscle of captivity-bred red-winged tinamou. Pathways are plotted according to significance (*y*-axis) and pathway impact value (*x*-axis). Larger circles represent greater pathway enrichment and darker colors represent greater significance. The letters indicate the pathways (see Table 2).

spermine, and putrescine), proline, creatine, nitric oxide, citrulline, glutamate, and agmatine, which are involved in skeletal muscle vasodilation, proteins synthesis, and immune response stimulation (Fernandes et al., 2009). In the study by Castro et al. (2019), broiler chickens supplemented with arginine exhibited greater body growth, lean mass deposition, and bone mineral density. Thus, the difference in arginine concentration between the experimental groups observed in the present study suggests that this amino acid contributes to the muscle performance and growth of red-winged tinamou subjected to selection (TinamouS).

Although a nonessential amino acid, aspartate is important for energy production, which, contributes to muscle growth (Kaneko et al., 2008). Aspartate aminotransferase is responsible for the synthesis of this metabolite. The enzyme is found at high concentrations in various organs and tissues, particularly in the heart, liver, skeletal muscle, kidney, and brain (Kaneko et al., 2008; Rezende et al., 2019). According to MacRae et al. (2006), serum activity of this enzyme serves as an enzymatic marker of muscle changes, including both muscle damage and increases in muscle mass. A study on broiler chickens demonstrated an increase in the serum levels of this enzyme during the final growth phase (Rezende et al., 2019).

In the present study, aspartate possibly contributed to the muscle development of TinamouS animals since this amino acid is a precursor for the biological synthesis of purines, pyrimidines, nucleic acids, and arginine. Furthermore, aspartate participates in processes such as the transport of cations (Mg^{+}, K^{+}, Zn^{2+}, and Ca^{2+}), osmosis, and the production of energy for muscle work as a substrate (Krebs cycle) or as a stimulant (purine nucleotide cycle) (Kaneko et al., 2008).

The amino acid betaine is derived from glycine or is synthesized from choline (Paniz et al., 2005; Ratriyanto et al., 2009; Ribeiro et al., 2015). This compound acts through 2 main metabolic mechanisms: exclusive action as a methyl group donor for different transmethylation reactions, with benefits for production at a lower cost, and increasing the osmotic resistance of cells, thereby reducing energy demand and preventing cellular dehydration and ionic balance (Liu et al., 2013; Park and

Table 2. Metabolic pathways in the *pectoralis major* muscle of captivity-bred red-winged tinamou.

Letter[1]	Pathway name	Total cmpd[2]	Hits[3]	*P* value[4]	−Log (*p*)[5]	Impact[6]
a	Phenylalanine, tyrosine and tryptophan biosynthesis	4	2	0.00167000	2.7768	1.00
b	Alanine, aspartate and glutamate metabolism	28	8	0.00040500	3.39	0.54
c	D-Glutamine and D-glutamate metabolism	6	2	0.03520700	1.45	0.50
d	*β*-Alanine metabolism	21	5	0.00001300	4.89	0.45
e	Glycine, serine and threonine metabolism	34	9	0.00016400	3.79	0.45
f	Taurine and hypotaurine metabolism	8	2	0.00004210	4.38	0.43
g	Histidine metabolism	16	6	0.00004890	4.31	0.36
h	Phenylalanine metabolism	8	2	0.00167200	2.78	0.36
i	Nicotinate and nicotinamide metabolism	15	3	0.00009960	4.00	0.35
j	Arginine and proline metabolism	38	7	0.03386000	1.47	0.26
k	Purine metabolism	62	8	0.00004480	4.35	0.23
l	Cysteine and methionine metabolism	33	3	0.00002650	4.58	0.20
m	Arginine biosynthesis	13	5	0.00000534	5.27	0.16
n	Starch and sucrose metabolism	16	1	0.03745700	1.43	0.14
o	Glutathione metabolism	28	4	0.00169900	2.77	0.13
p	Galactose metabolism	27	1	0.00000327	5.48	0.11
q	Pantothenate and CoA biosynthesis	19	4	0.00000072	6.14	0.07
r	Glycerophospholipid metabolism	35	1	0.00001990	4.70	0.03
s	Valine, leucine and isoleucine degradation	40	4	0.00216100	2.66	0.02
t	Pyrimidine metabolism	40	3	0.00216300	2.66	0.02

[1]Letters correspond to the information shown in Figure 2.
[2]Total cmpd corresponds to the total number of compounds in the pathway.[3]Hits correspond to the actually matched number from the user uploaded data.
[4]*P* value corresponds to the original *P* value calculated from the enrichment analysis.
[5]−Log(*p*) corresponds to the *P* value logarithm.
[6]Impact corresponds to the pathway impact value calculated by topology-based pathway analysis.

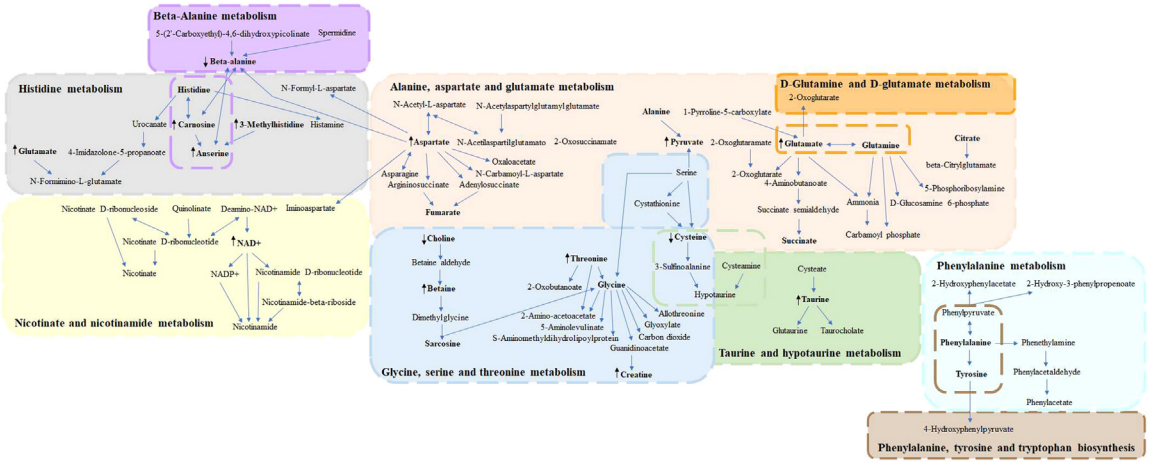

Figure 3. Integration of the 8 main metabolic pathways in the *pectoralis major* muscle of red-winged tinamou according to the *Gallus gallus* database obtained with MetaboAnalyst 5.0. Bold metabolites were identified in the ^{1}H NMR spectra. The black arrows indicate a significant increase or decrease ($P < 0.05$) in metabolites in the TinamouS group compared to TinamouC.

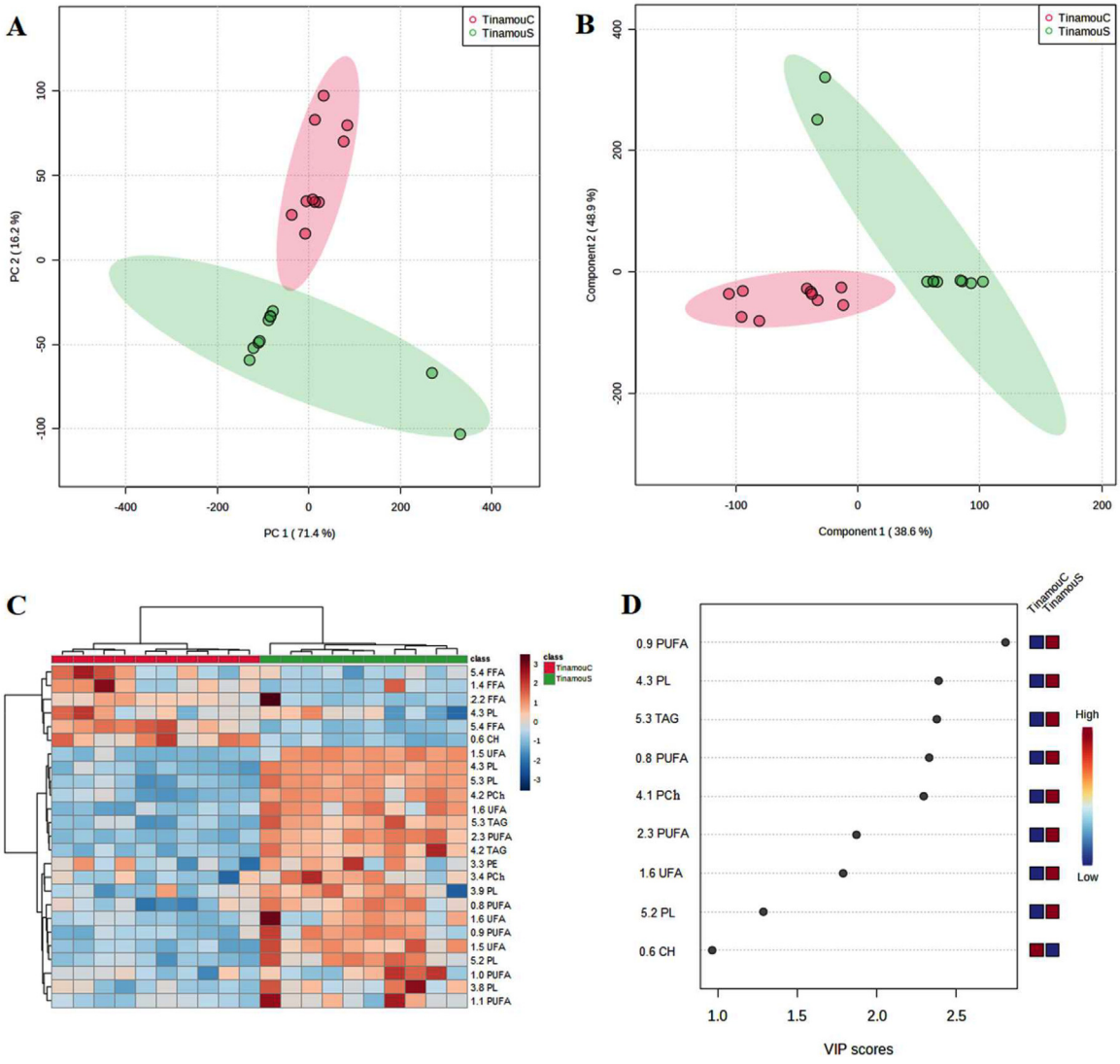

Figure 4. Apolar metabolite profile of the *pectoralis major* muscle of red-winged tinamou (*Rhynchotus rufescens*). (A) PCA score plot of the apolar metabolite profile between TinamouS vs. TinamouC (1 data point represents 1 animal). (B) PLS-DA score plot of the apolar metabolite profile between TinamouS vs. TinamouC (1 data point represents 1 animal). (C) Hierarchical cluster analysis (heatmap) of metabolomic differences between TinamouS vs. TinamouC by *t* test/ANOVA (chemical spectrum shift—ppm). (D) Top metabolites (chemical spectrum shift—ppm) selected by a VIP score ≥ 1.0.

Cholesterol (CH), free fatty acids (FFA), polyunsaturated fatty acids (PUFA), unsaturated fatty acids (UFA), phosphocholines (PCh), phosphoethanolamines (PE), triacylglycerols (TAG), and glycerophospholipids (PL).

Park, 2017; Chen et al., 2020; Wang et al., 2020a). In broiler production, betaine supplementation has been used to improve muscle growth and to delay the effects of lipid metabolism, decreasing the amount of abdominal fat and promoting body fat distribution. Additionally, betaine can elevate muscle creatine content, improving resistance and energy performance (Wang et al., 2004; Hoffman et al., 2009; Lever and Slow, 2010; Chen et al., 2020). Liu et al. (2013) found that the administration of betaine affected meat traits and the growth rate of ducks. In geese, betaine supplementation increased the feed conversion ratio (Yang et al., 2022). In addition to feed conversion, this compound increased breast muscle yield in broiler chickens (Nutautaitė et al., 2020). Within this context, our study also indicated an important role of betaine in the muscle growth of red-winged tinamou.

Creatine is a key compound that plays important roles in energy metabolism and muscle performance, as well as in reducing lactate accumulation in muscle (Zhang et al., 2014). It is therefore widely used as a supplement in humans and birds (Wyss and Kaddurah-Daouk, 2000; Beauclercq et al., 2016; Kreider et al., 2017). After its absorption by muscle tissue, creatine is immediately phosphorylated and transformed into creatine phosphate, which is a rapidly mobilizable reserve of phosphate for ATP production. In addition, this metabolite is essential when there is a need for short bursts of energy, providing maximum muscle power and preserving muscle glycogen (Roschel et al., 2010; Mousavi et al., 2013; Terenzi, 2013).

Creatinine is derived from the degradation of muscle creatine and creatine phosphate and is considered a biomarker of muscle mass in a steady state (Virgili et al., 1994). Rosa et al. (2018) found that the inclusion of creatinine in the broiler chicken diet increased feed conversion and breast weight. These findings confirm that the high concentrations of creatine, creatine phosphate, and creatinine in the TinamouS group are closely associated with muscle growth of the birds.

The present study also showed higher concentrations of glutamate and proline in the TinamouS group. These compounds are derived from the metabolism of arginine (Fernandes and Murakami, 2010; Fouad et al., 2012). Glutamate is an anabolic precursor for muscle growth, participates in protein synthesis, is responsible for regulating the acid-base balance in the kidney, and ensures interorgan nitrogen transport (Newsholme et al., 2003). In addition to these functions, this amino acid is involved in the neural control of feed intake and body weight in birds by influencing the expression of orexigenic (related to appetite) and anorexigenic (related to loss of appetite) neuropeptides in the central nervous system (Paul et al., 2012). Thus, glutamate supplementation has also been used in birds to achieve maximum growth, production, and feed efficiency (He et al., 2021a).

Proline plays an important role in the production of collagen (which accounts for 30% of all proteins deposited in the body), regulation of gene expression, cell differentiation, wound healing, antioxidant reactions, immune response, and the synthesis of polyamines and glutamate, in addition to contributing to the growth of birds (Wu et al., 2011). In addition, proline is an essential amino acid since arginine catabolism supplies less than 7% of the proline needed to meet the requirements of birds, particularly during the initial production phase (Pistollato et al., 2010; Wu et al., 2011). Thus, the increase in the concentrations of this amino acid observed in the TinamouS group indicates higher protein production and consequently greater animal growth.

Threonine is an essential and limiting amino acid. Since birds cannot synthesized this amino acid, it must be supplemented in poultry feed (Atencio et al., 2004; Mandal et al., 2006; de Araújo Campos et al., 2012). This metabolite plays a key role in protein synthesis. In the present study, the high concentration of threonine in the TinamouS group indicates greater muscle development in view of the requirement of this amino acid for protein synthesis and maintenance of body protein turnover. In a study of meat-type quails, Silva et al. (2018a, b) reported that the animals required 1.37% of threonine in the diet from birth to 21 d of age, while the requirement for better animal development was 1.32% during the growth phase of quails. Other authors included different levels of threonine in the diet of broiler chickens and reported that increasing the level of this amino acid improved the feed conversion ratio and body weight gain (Taghinejad-Roudbaneh et al., 2013). Higher dietary levels of threonine have also been linked to improvement in the gastrointestinal function of birds, increasing the absorption of nutrients (Chen et al., 2017).

3-Methylhistidine is synthesized only in muscle by histidine residue methylation and is considered a marker of the degradation of myofibrillar protein not reincorporated and necessarily excreted in urine (Virgili et al., 1994). According to Baldi et al. (2021), histidine is a good indicator of energy supply in chicken muscle. Furthermore, Kochlik et al. (2018) indicates the use of plasma 3-methylhistidine as a biomarker of muscle protein turnover in humans who do not consume meat for 24 h. Based on these findings, we suggest that the higher concentration of this possible biomarker in *pectoralis major* muscle of animals of the TinamouS group is probably the result of protein turnover.

The essential amino acid leucine serves as a substrate for protein synthesis and acts as a nutrient signal that regulates protein synthesis and the inhibition of protein degradation in skeletal muscle, as well as the activity of proteins involved in mRNA translation (Escobar et al., 2005; Wu et al., 2010; Wilkinson et al., 2013). Thus, the higher concentration of leucine in the *pectoralis major* muscle of red-winged tinamou may be an indicator of protein synthesis.

The peptides carnosine and anserine found at higher concentrations in the TinamouS group are endogenous bioactive compounds. These metabolites exert strong buffering and antioxidant activities (Peiretti et al., 2011, 2012; Sundekilde et al., 2017). In addition to its

function as a proton buffer, other roles have recently been attributed to carnosine, such as protection against oxidative damage, calcium binding, and regulation of calcium sensitivity (Trexler et al., 2015).

Anserine, which is found in muscle of broilers, turkeys, and ducks, has an important impact on the nutritional value and antioxidant status of meat due to its specific properties that prevent cell oxidation (Peiretti et al., 2011; Charoensin et al., 2021). Charoensin et al. (2021) observed that Thai native chicken meat contains more anserine than commercial broiler chicken meat. According to these authors, the differences in anserine and carnosine between chicken breeds can be attributed to different muscle fiber types. Recently, Baldi et al. (2021) demonstrated that the glycolytic metabolites glucose and lactate are positively correlated with anserine in chicken muscle. However, in the present study, anserine was negatively correlated with lactate (-0.63, $P < 0.05$) and showed no correlation with glucose.

The positive correlation between carnosine and glutamate observed in the present study may have been due to the synthesis of these compounds within the histidine metabolism pathway (Brosnan and Brosnan, 2020). In this pathway, histidine methylation is catalyzed by carnosine N-methyltransferase to form anserine. Furthermore, Drozak et al. (2013) described carnosine methyltransferase in chickens as a histamine N-methyltransferase-like enzyme. We also found a significant positive correlation between carnosine and aspartate in the muscle of red-winged tinamou. The alanine, aspartate, and glutamate metabolism, beta-alanine metabolism, nicotinate and nicotinamide metabolism, and histidine metabolism pathways are interconnected through aspartate. This metabolite is directly related to beta-alanine, which is synthesized into carnosine.

Purine Ribonucleotides/Purines and Purine Derivatives/Nucleotides Another interesting observation of the present study is the high concentrations of ADP, AMP, ATP, adenosine, IMP, inosine, NAD+, and acetate in the TinamouS group. These metabolites are involved in cell growth and division, modulation of the immune system and the maintenance of intestinal health, participate directly in the metabolism of other compounds through biochemical processes, and are related to energy production, storage and expenditure (Chiofalo et al., 2011; Faveri et al., 2015; Wen et al., 2020). Some authors suggest that the lementation of broiler chicken diets with nucleotides can improve the physical and nutritional characteristics of breast meat and promote higher weight gain and feed conversion (Chiofalo et al., 2011; Jung and Batal, 2012; Faveri et al., 2015).

The coenzyme NAD+ is essential for all cells since it acts as an electron carrier in hundreds of reactions during the metabolism of carbohydrates, fatty acids, and amino acids (González and da Silva, 2019). Considering its participation in ATP production during mitochondrial respiration, it is also worth mentioning that NAD + is the coenzymatic form of niacin (vitamin B3), whose derivatives are important for cellular energy metabolism and DNA repair, and thus plays an indirect role in muscle growth (González and da Silva, 2019). In addition, the acetyl group is derived from acetate and, when bound to coenzyme A to form acetylCoA, NAD+ is essential for the metabolism of carbohydrates and fats. However, the accumulation of acetylCoA can inhibit the enzyme pyruvate dehydrogenase and thus reduce the availability of pyruvate as an oxidative substrate (Silveira and Curi, 2012). Wang et al. (2020b) reported that breed and age of the animals affected acetate concentration in duck breast. Therefore, in the present study, the increase in NAD+ and acetate concentrations may have contributed to muscle growth in red-winged tinamou subjected to selection.

Carbohydrates, Alpha-Keto Acids, and Organosulfonic Acids 1,3-Dihydroxyacetone is a simple carbohydrate whose phosphorylated form is called dihydroxyacetone phosphate. It is an important intermediate of glycolysis (Voet et al., 2016). The combination of 1,3-dihydroxyacetone with pyruvate results in a nutritional supplement that increases the biodegradation of fats and intensifies muscle mass gain (Ivy, 1998). Glucose is a fundamental carbohydrate used as an energy source and for the formation of metabolic intermediates. This compound is of great importance in broiler production because of the energy demand for muscle growth and development and is one of the factors that stimulate myofibrillar protein synthesis (Manda et al., 2010).

Pyruvate is the product of glucose degradation in the glycolytic pathway, where 1 glucose molecule is converted to 2 pyruvate molecules, with concomitant generation of 2 ATP molecules (Teslaa and Teitell, 2014). Pyruvate is an important metabolic intermediate with several potential destinations, including entry into the tricarboxylic acid cycle for production of NADH and $FADH_2$ and conversion to lactate, with concomitant regeneration of NAD+ and NADH (Teslaa and Teitell, 2014). Thus, the increased concentrations of 1,3-dihydroxyacetone, glucose and pyruvate observed in the *pectoralis major* muscle of red-winged tinamou are interrelated and may be associated with muscle growth.

In the present study, pyruvate was positively correlated with aspartate and glutamate. This result suggests a mutual relationship between these metabolites and alanine, aspartate and glutamate metabolism and D-glutamine and D-glutamate metabolism pathways. Glutamate is transaminated using pyruvate or oxaloacetate to produce alanine and aspartate, respectively (He et al., 2021b). Studies have shown the occurrence of the glutamine-glutamate cycle in skeletal muscle of birds, which regulates the synthesis and release of glutamine (Wu et al., 1991). In addition, glutamine is associated with the rapamycin signaling pathway, which is directly related to protein synthesis and muscle growth (He et al., 2021b).

Taurine exerts a wide variety of functions in biological systems such as antioxidant and anti-inflammatory activity, maintenance of mitochondrial integrity, energy metabolism, osmoregulation, thermoregulation,

detoxification, immunomodulation, and skeletal muscle homeostasis, as well as functions in the central nervous and cardiovascular systems (Walczewska et al., 2015; Grove and Karpowicz, 2017; Schaffer and Kim, 2018; Page et al., 2019; Qvartskhava et al., 2019; Seidel et al., 2019). Studies on chickens reported that taurine is related to muscle growth (Xiao et al., 2019a,b; Han et al., 2020). However, according to Surai et al. (2020), taurine may become semiessential for broilers under stress conditions. The concentration of taurine possibly affected the muscle performance of red-winged tinamou subjected to selection.

Cysteine, Beta-Alanine, Lactose, and Choline The concentrations of cysteine, beta-alanine, lactose, and choline were lower in the TinamouS group compared to TinamouC. Cysteine, a nonessential amino acid, is synthesized from methionine (Bunchasak, 2009). The latter has been reported to be a fundamental amino acid for protein deposition (Tesseraud et al., 2011; Wen et al., 2017). However, in the present study, there was no difference in methionine between the experimental groups. Beta-alanine is a nonessential amino acid that is found mostly in animal products. Its main function is the intramuscular synthesis of carnosine, which can contribute to muscle hypertrophy (Blancquaert et al., 2017; Kelly et al., 2017; Maté-Muñoz et al., 2018; Freitas et al., 2019; Roveratti et al., 2019; Cabral and Minakawa, 2020). In chickens, lactose is used as a bioactive compound (prebiotic, probiotic, or symbiotic) that stimulates more efficient production of the body, positively affecting animal health and reducing intestinal diseases (Dankowiakowska et al., 2019). Lactose has been added to chicken diets to reduce colonization with different intestinal bacteria, pathogenic or not, by increasing the amount of lactic acid, with a consequent reduction in pH (Fathima et al., 2022). Choline is essential in poultry nutrition and can be synthesized in the liver; however, it is commonly supplemented because of its high requirements (Combs Jr., 2008). This is due to the fact that choline contributes to various metabolic functions, including lipid transport, cell signaling, and biosynthesis of methylated compounds (Igwe et al., 2015; Gregg et al., 2022).

Metabolic Pathways Although pyruvate was not correlated with cysteine or threonine in red-winged tinamou muscle, these metabolites are intimately connected through 2 pathways: i) glycine, serine and threonine metabolism, and ii) taurine and hypotaurine metabolism. Sarcosine is rapidly metabolized to glycine by sarcosine dehydrogenase. Glycine can also be synthesized endogenously from threonine and serine via multiple pathways (Li and Wu, 2018). Serine is converted directly into glycine and vice versa (Sugahara and Kandatsu, 1976). Hence, we must consider the influence of the physiological pathways of all of these amino acids on muscle growth. Within this context, taurine is a product of cysteine catabolism but we found no significant correlation in the present study. However, our results showed positive correlations of choline with cysteine and threonine. Choline is an endogenous precursor of glycine, which, forms cysteine from methionine. Interactive effects between glycine and choline on broiler growth have been previously described (Siegert et al., 2015a). Additionally, studies have investigated supplementation of broiler chickens with glycine, serine, and threonine in order to better understand the role of these metabolites in muscle growth (Siegert et al., 2015b; Hilliar et al., 2019; Hofmann et al., 2020; Gregg et al., 2022). Taken together, the metabolome and the interactive effects between the metabolic pathways observed in *pectoralis major* muscle may contribute to the genetic selection of red-winged tinamou (*Rhynchotus rufescens*) bred in captivity.

Apolar Metabolites

Our results obtained for apolar metabolites indicated that the selection index resulted in the upregulation of PUFA, UFA, PCh, PE, TAG, and PL in the *pectoralis major* muscle of red-winged tinamou. PUFA are fatty acids with 2 or more double bonds that are classified based on the length of the carbon chain and the position of the first double bond relative to the methyl terminus (Saini and Keum, 2018). These lipids can be combined with triglycerides to produce phosphatidic acid and phospholipids in vivo (Zhang et al., 2022). Phospholipids are essential components of cellular and subcellular membranes (Gundermann et al., 2011). Within this context, the changes in the concentrations of these lipids observed in the present study may directly or indirectly affect physiological functions and alter muscle growth.

Lipids have also been the focus of research in birds because studies of these metabolites can help us to understand mechanisms of muscle growth. In the study by Cui et al. (2018), supplementation of broiler chickens with *Moringa oleifera* leaves decreased body weight and average daily gain and increased the feed conversion ratio. The authors also observed that supplementation increased the concentration of PUFA. In another study, Cui et al. (2020) found that supplementation of birds with these leaves reduced feed conversion and altered the composition of lipids such as PCh and PE. Mahiza et al. (2021) also reported higher concentrations of PUFA in breast and thigh muscles of different breeds of fast-growing chickens compared to slow-growing birds. Genetic selection for muscle mass production and threonine supplementation increased the PUFA content in duck breast muscle (Jiang et al., 2020). The concentration of PUFA can also vary according to genetics, as observed by Uhlířová et al. (2019) between the native breed Czech goose and commercial hybrid Novohradska goose.

In a recent study by Ma et al. (2021), broiler chickens fed 3 g/kg of mixed organic acids exhibited an increase in UFA concentrations. Liu and Kim (2018) reported that dietary supplementation with UFA-rich oils increases energy digestibility of the diet and positively affects muscle growth. Interestingly, the profile of polar metabolites observed here may contribute to and

simplify future studies on lipid metabolism. In summary, the characterization of the metabolomic profile of the *pectoralis major* muscle of male red-winged tinamou provides for the first time a comprehensive view on the biochemical mechanisms underlying muscle growth. Thus, strategies designed to obtain data on metabolites can help in the production and selection of these birds.

CONCLUSIONS

The results of the present study show that application of the selection index for muscle growth in the second generation led to differences in the concentrations of polar and apolar metabolites in red-winged tinamou. These differentiating metabolites may be used as potential biomarkers for the identification of birds with higher breast weight and yield. In addition, our findings suggest that the selection process was effective in improving the commercial production of red-winged tinamou for the meat market.

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DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2023.103104.

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