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CORRELATIONS BETWEEN FIRST ORDER ECHOTEXTURAL CHARACTERISTICS AND CHEMICAL COMPOSITION OF PECTORALIS MAJOR MUSCLES IN BROILER CHICKENS RECEIVING DIFFERENT DIETARY FAT SUPPLEMENTS*

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Abstract

This study examined the quantitative relationships among ultrasonographic image attributes and chemical composition of the pectoralis major muscles in broiler chickens that received four different dietary fat supplements (Group SO: soybean oil; Group FO: flaxseed oil; Group SO+FO: soybean oil+flaxseed oil; and Group BT: beef tallow; n=10 birds/group). Ultrasonograms of birds' pectoral muscles, in a transverse (T) and longitudinal (L) plane, were obtained just before slaughter at 6 weeks of age and were subjected to digital image analyses to determine mean pixel intensity (MPI) and pixel heterogeneity values (standard deviation of numerical pixel values; MPH; a.k.a first order echotextural characteristics). Thirty-eight chemical characteristics of the muscles were determined post-mortem (crude fat, protein, and dry matter as well as fatty acid profiles) and were analyzed for correlations with the echotextural variables. A total of 12 (L-MPI: 7; L-MPH: 4; and T-MPH: 1 correlation), 5 (L-MPI: 2; L-MPH: 2; and T-MPI: 1 correlation), 15 (L-MPI: 10; T-MPI: 4; and T-MPH: 1 correlation) and 8 (L-MPI: 2; L-MPH: 1; and T-MPH: 5 correlations) significant correlations were recorded in Groups SO, FO, SO+FO and BT, respectively. When the data were pooled for all 40 birds studied, significant correlations with echotextural attributes were recorded for eighteen different chemical constituents, with the strongest overall correlation found between crude fat content and T-MPI (r=0.52, P=0.0005). In conclusion, there exists a potential application for ultrasonographic imaging *in situ* combined with computerized image analysis to estimate certain chemical constituents of pectoralis major muscles in broiler chickens. However, the existence and strength of correlations among ultrasonographic image attributes and muscle composition are affected by the source of dietary fat and relative abundance ("threshold concentrations") of individual chemical components.

Key words: broiler chicken, pectoral muscle, ultrasonography, echotexture, chemical composition

Global meat production increased significantly over the past two decades and is still forecasted to grow (Delgado-Pando et al., 2019). In 2017, there were 22.85 billion chickens worldwide. According to the report prepared by Food and Agriculture Organization of the United Nations (FAO), the world's broiler meat production amounted to ~100.6 metric kilotons in 2020 and the projected global production will reach ~102.1 metric kilotons in 2021 (Shahbandeh, 2021). Nutritional value and chemical composition are the main factors determining meat quality (Bodwell and Anderson, 1986; Fletcher, 2002; Baracho et al., 2006). There is a continued public demand for high quality of fresh meat and processed meat products, especially since many nations are becom-

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ing aging societies, and senior citizens require healthy nutrition and a variety of functional foods (Cobos and Diaz, 2014).

Meat from domesticated animal species is generally composed of approximately 72–75% water, 21% nitrogenous compounds (19% proteins and 1.5% non-protein nitrogenous compounds such as nucleotides, peptides, creatine, and creatinine), 2.5–5% lipids, and 1% non-nitrogenous compounds (vitamins) and carbohydrates (mainly glycogen) (Cobos and Diaz, 2014). Meat contains unsaturated fatty acids, namely oleic (C18:1), linoleic (C18:2), linolenic (C18:3), and arachidonic (C20:4) acid, which have well-known health benefits (Azain, 2004; Lock and Bauman, 2004). Poultry meat, however, also contains multiple cholesterol-raising saturated fatty acids such as palmitic acid (C16:0) (Lock and Bauman, 2004). Therefore, accurate monitoring of meat composition at all stages of the production cycle remains one of the top priorities of modern food industry (Fletcher, 2002). Researchers investigating various aspects of meat quality and poultry producers require constant access to technologies that enable the most frequent and complete monitoring of the chemical composition of meat.

At present, the assessment of meat quality in poultry facilities is typically carried out post-mortem. This allows for high accuracy but restricts the ability of the producers to make "real-time decisions" and improve meat quality using nutritional and sanitary or technological adjustments (Pena-Gonzalez, 2017; Bogucka et al., 2018; Sugiharto et al., 2018; Marzec et al., 2020). Moreover, both the quality control in commercial farms as well as genetic selection of birds require constant monitoring of meat productivity traits; phenotypic data combined with whole genome data can provide "deeper insight into the control of growth and body/carcass composition of farm animals" and "add extra value for genomic selection" (Petracci and Baeza, 2010). There is currently no method of predicting the chemical composition of meat based on the non-invasive assessment of birds' muscles before slaughter.

Measurements of speed at which ultrasonic sound waves travel through the "chicken analogues" (prepared by homogenizing dried chicken powder, corn oil, and distilled water) provided first evidence that ultrasound technology could be used to characterize chicken meat composition (Chanamai and McClements, 1999). The content of crude fat, solids-nonfat and moisture in the chicken meat predicted from ultrasonic velocities was in a very close agreement with that determined with standard laboratory methods (Chanamai and McClements, 1999). Studies have also shown that both the fat content and the percentage of solids-nonfat could be determined using ultrasonic velocity and frequency measurements in fish fillets (Ghaedian et al., 1997, 1998), lambs (Miles et al., 1991) and beef (Park et al., 1994). Ultrasonographic imaging uses high-frequency (>20 kHz) sound waves to construct images of internal organs and tissues (Blitz, 1963). Ultrasonography is a fully non-invasive and risk-free modality of diagnostic imaging as images are a result of the reflectance or attenuation of non-ionizing and non-destructive sound beams by different tissue interfaces (Pillen, 2010). The transducer in an ultrasound scanner system functions both as a transmitter and a receiver of ultrasound waves (i.e., echoes) to generate images composed of multiple pixel intensity units (two-dimensional gray-scale matrix) (Walker et al., 2004). Ultrasound waves are transmitted through structures with a high-water content and reflected or scattered by more dense objects (Walker et al., 2004). A transmitted portion of a wave is displayed as dark, non-echogenic spots or regions, whereas reflected echoes cause the appearance of brighter, echogenic segments of the image. Ultrasound images are a composition of pixels with the numerical values ranging from 0 (absolute black) to 255 (absolute white), and different types of tissues exhibit distinctive echotextural properties.

Computerized analysis of ultrasound images is a promising area of biomedical research that has not been fully explored or utilized yet, but which shows the potential to predict the histochemical characteristics of internal organs and tissues (Liu et al., 2008; Giffin et al., 2009, 2014; Ahmadi et al., 2013). Skeletal muscles have heterogeneous echotexture as proteins generally "absorb" ultrasound waves and appear as dark areas, but the connective tissue structures that surround each muscle fiber deflect the ultrasound waves depending on the angle at which they are hit, thereby appearing as brighter spots with different shades of grey (Walker et al., 2004; Tiidus, 2008). The intramuscular lipid content contributes significantly to muscle echointensity as fat is highly echogenic (Walker et al., 2004; Tiidus, 2008). An application of software packages to analyze image echotexture enhances the precision and objectivity of image assessment. First order textural descriptors are the most frequently used quantitative echotextural variables as they are directly related to the greyscale content of an image (Materka and Strzelecki, 1998). Examples of the first order descriptors include the mean, standard deviation, variance, skewness, and kurtosis of numerical pixel values (Haralick, 1979; Molinari et al., 2015). It was shown that intramuscular fat content of the longissimus dorsi muscle in lambs and cattle (Slósarz et al., 2001; Newlacil et al., 2013) and certain physicochemical properties of pectoral muscles in broiler chickens (Schwarz et al., 2019) could be determined using ultrasonographic imaging combined with computer-assisted analyses of first order echotextural variables.

The angle at which the transducer is positioned affects the interaction of ultrasound beams with muscle fibers (Cady, 1983; Clague et al., 1995). Strong echoes are produced when the angle between the probe and muscular fasciculi is perpendicular (Cady, 1983). At 60 to 70 degrees, the echoes become irregular and not particularly strong, while the echoes are barely present at <40 degrees. Therefore, the angle or plane at which the probe is placed during ultrasound scanning of the muscle plays an impor-

tant part in the generation of the image because skeletal muscles have a "directional fiber structure" (Cady, 1983). There have been no earlier attempts to examine first order echotextural parameters of chicken skeletal muscles, obtained from different scanning planes, for correlations with their chemical composition.

Quality and consistency of image texture is one of the most important components used in identifying and characterizing objects or regions of interest within ultrasonograms. The commonly used texture analysis approaches are based on the probability distribution of gray-scale levels and texture pattern properties (Kim et al., 1998). However, the efficiency of texture analysis is strongly affected by image quality (Kociołek et al., 2020). Ultrasound image normalization is the main step to facilitate the comparison of information obtained from images captured using different instrumental settings, scanners, operators and/or any other technical factor (Pazinato et al., 2015). Indeed, many studies demonstrate the importance of normalization methods for ultrasound image analysis (Sabetai et al., 2000; Tegos et al., 2000).

There were three major objectives of the present study conducted in broiler chickens: i. to record and compare ultrasonographic characteristics of the pectoralis major muscles (*M. pectoralis superficialis*) scanned in the longitudinal and transverse plane; ii. to examine pectoral muscles *in situ* for correlations among their first order quantitative echotextural attributes (mean numerical pixel values and heterogeneity) and chemical composition determined post-mortem; and iii. to assess the influence of the dietary fat source (i.e., single or mixed oils of plant origin and fat of animal origin) on the relationships between echotexture and chemical composition of the muscles. We hypothesized that ultrasonographic image attributes of the pectoralis major muscle in broiler chickens (normalized first order echotextural variables) would be significant predictors of its chemical composition and that there would exist detectable echotextural differences between the two scanning planes due mainly to variations in the spatial orientation of muscle fibers.

Material and methods

Animals, facility, and experimental groups

The local Ethics Committee (First Local Ethics Committee for Animal Experimentation in Kraków) formally approved all experimental procedures performed on live birds in this study (based on the guidelines contained in the Regulation no. 22/2016 dated January 20, 2016). The present experiment utilized 40 fast-growing, male broiler chicks (Ross 308), hatched in a commercial poultry hatchery and transported to an experimental farm of the National Research Institute of Animal Production situated in Aleksandrowice near Kraków, Poland. The birds were allotted to four equinumerous groups and then kept on deep litter under automatically controlled environmental conditions. Light (intensity level 20 lux) was provided for 23 h per day (1–7 days), 20 h per day (8–39 days), and 23 h per day (40–42 days), and an initial temperature of 30°C within the non-windowed, ventilated building was gradually decreased to 20°C until the end of the study period. Birds were fed *ad libitum* with starter (1–21 days), grower (22–35 days) and finisher (36–42 days of age) dry mixes containing 22%, 20.5% and 20.5% crude protein and 2990, 3130 and 3130 kcal metabolizable energy per kg of feed mixes, respectively, and had unlimited access to drinking water. All the mixtures were prepared according to the dietary requirements of broiler chickens (Smulikowska and Rutkowski, 2005). The proportions of fat supplements added to the diet were 2.9%, 4.8% and 4.8% for the starter, grower, and finisher, respectively. Group SO received soybean oil containing a majority of PUFA n-6 fatty acids; Group FO received flaxseed oil, which is a source of PUFA n-3; Group SO+FO received a mixture of both oils of plant origin in a proportion of 44%:56%, which corresponds to an equal amount of both PUFAs in the diet; and Group BT's mixes were supplemented with beef tallow containing a majority of saturated fatty acids and minimal content of polyunsaturated fatty acids from the PUFA n-3 and PUFA n-6 families. At the end of the rearing period (mean±SEM body weight of 2.98±0.05 kg), all birds were sacrificed by decapitation after 10 h of fasting. The birds were suspended and allowed to bleed for a minimum of 90 seconds.

Ultrasonography and echotextural analyses

This study retrospectively analyzed the ultrasonographic records obtained by Schwarz et al. (2019). Briefly, ultrasound images of the birds' pectoralis major muscles were obtained just before slaughter, using a hand-held 7.5-MHz linear-array transducer connected to the Aloka PS2 ultrasound diagnostic system (Aloka Ltd., Tokyo, Japan) with constant settings for main gain, contrast, and focal points. Each pectoral muscle was scanned in a transverse and longitudinal plane (Figure 1), and still images containing the largest cross-sectional area of the pectoralis major muscle were saved as Digital Imaging and Communications in Medicine (DICOM) images with the resolution of 640 x 480 pixels. Echotextural analyses conducted at the University of Guelph, ON, Canada utilized commercially available ImageProPlus® 7.0 analytical software (Media Cybernetics Inc., Rockville, MD, USA). Python algorithms were used to convert original red, green and blue (RGB) images to grey-scale (8 bites), and also to crop the image to the selected window size whenever necessary due to the presence of excessive image artifacts, a scale bar and all characters in the ultrasound text area. Gray-level stretching (byte-scale transformation) was used for image normalization. Numerical pixel values were scaled to fill the entire range of display brightness; the byte-scale transformation was performed by scaling the input region of interest as follows: $G_i=T(f_i-f_{min})/(f_{max}-f_{min})$ where f_i was the original intensity in the range ($f_{min,}$ f_{max}) and G_i was the corresponding scaled intensity to lie in (0, T); in this study,

T value was 255 (Kim et al., 1998). A polygonal tool was used to compute mean pixel intensity (MPI) and mean pixel heterogeneity (or standard deviations of the mean pixel values – MPH) of each ultrasonogram of the left pectoralis major muscle that was subsequently dissected for chemical analyses. Despite image normalization, a special care was taken to exclude the reflection artifacts and large, apparent blood vessels or intramuscular blood discharges/bruises.

Chemical analyses

Dry matter was determined with the oven-drying technique (PN-ISO 1442 : 2000, 2013), crude protein was measured using the Kjeldahl method (nitrogen to protein conversion rate of 6.25; PN-75/A-04018/Az3, 2002), and crude fat content was estimated after the Soxhlet extraction (PN-ISO 1444: 2000, 2013). The fatty acid composition of intramuscular fat was determined using the method described in ISO 12966-2 (2017). The composition of fatty acid methyl esters was determined with a gas chromatograph (Varian 450-GC, Varian BV, Middelburg, The Netherlands) equipped with a fused silica capillary column (30 mm x 0.32 mm, film thickness – 0.25 μm). Oven temperature was set at 200°C, carrier gas (helium) velocity was 2.5 ml/min, temperature of the injection port was 250°C, and the detector was kept at 300°C. Fatty acid content in analyzed samples was calculated by comparison with fatty acid standards (Sigma-Aldrich, USA), using chromatogram peak areas, and were expressed as g/100 g of total fatty acids methyl esters (% of extractable lipids). The fatty acid profile consisted of the following 35 individual fatty acid components, fatty acid groups, and calculated proportions: caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0), palmitoleic acid (C16:1), oleic acid (C18:1), erucic acid (C22:1), linoleic acid (C18:2), γ-linolenic acid (C18:3γ), α-linolenic acid (C18:3α), arachidonic acid (C20:4), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), n-6 polyunsaturated fatty acids (PUFA 6), n-3 PUFA (PUFA 3), PUFA 6/3, total PUFA, conjugated linoleic acid (CLA) c9-t11, CLA t10-c12, CLA c9-c11, CLA t9-t11, total CLA, total saturated fatty acids (SFA), total unsaturated fatty acids (UFA), total monounsaturated fatty acids (MUFA), UFA/SFA, MUFA/SFA, PUFA/SFA, total hypocholesterolemic acids (DFA), total hypercholesterolemic acids (OFA), and DFA/OFA.

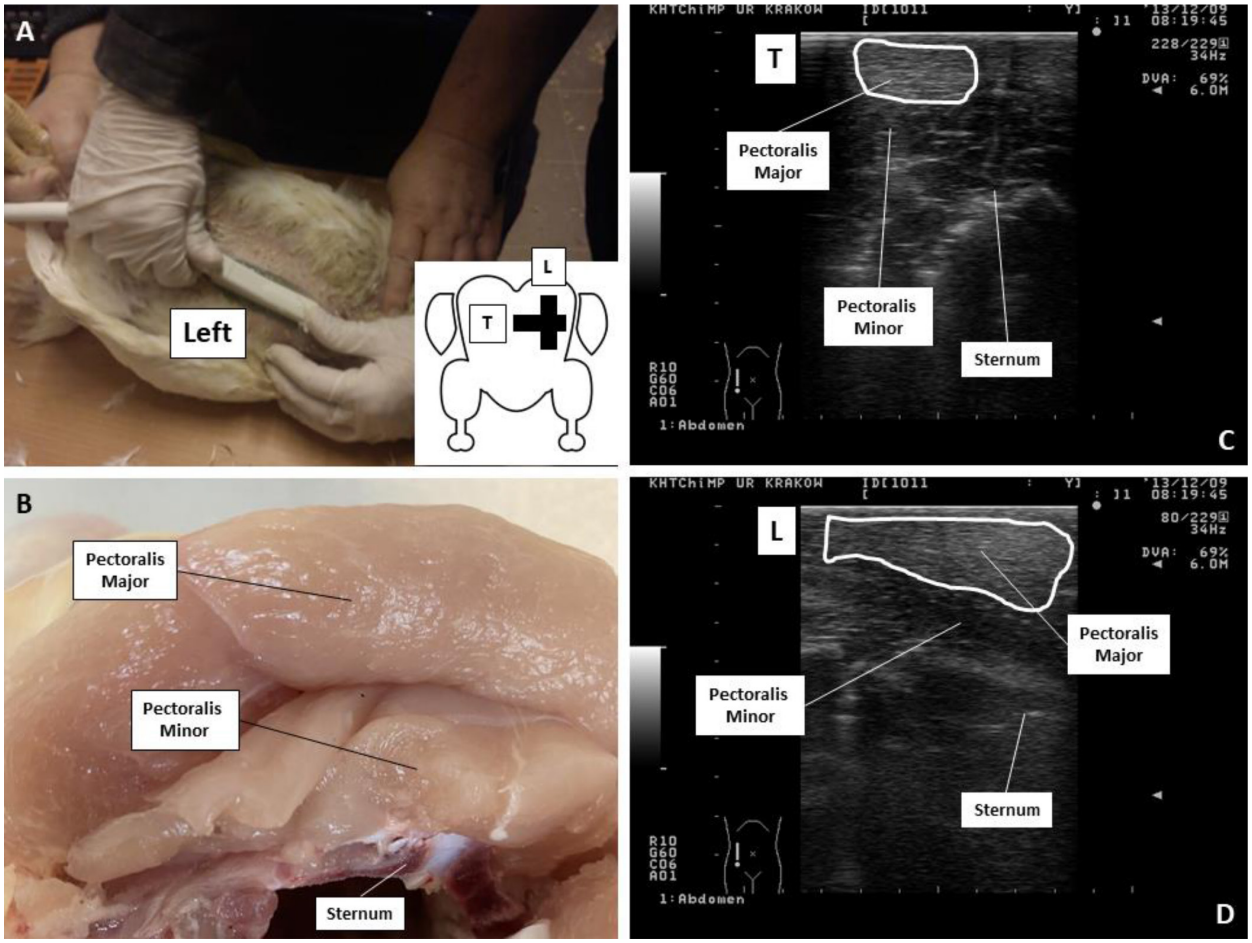


Figure 1. *In situ* ultrasonographic examinations (panel A) of the left pectoral muscle in broiler chickens (panel B; n=40) scanned in a transverse (T; panels C) and longitudinal (L; panels D) plane. White lines in panels C and D delineate potential placement of a polygonal area of interest used for echotextural analyses of muscle tissue

Statistical analyses

One-way analysis of variance (ANOVA) and Tukey test were used to compare chemical composition of pectoralis major muscles among the four groups of broiler chickens studied (SigmaPlot® 11.0; Systat Software Inc., San Jose, CA, USA). Echotextural differences between groups and scanning planes (transverse vs. longitudinal) were analyzed by two-way ANOVA (main effects of the group and scanning plane) followed by the least significant difference (LSD) test for comparisons of individual means. Proportions were compared by a χ^2 test (Brendt-Snedecor formula; Cochran and Cox, 1957). Pearson product moment analysis was used to examine correlations among first order echotextural attributes and chemical composition of pectoralis major muscles for the four fat-supplementation groups of birds. P value ≤0.05 was considered statistically significant and all results are expressed as mean±standard error of the mean (SEM).

Results

Muscle weight and chemical composition

Combined weight of pectoralis major muscles (674±13 g) did not vary (P>0.05) among the four group of broiler chickens studied. Total protein content of the pectoral muscles was greater (P<0.05) in Group BT compared with Group SO (Table 1). Groups SO and FO exceeded (P<0.05) Group BT in crude fat content of pectoralis major muscles. Relative content of dry matter, CLA *c*9-*c*11 and CLA *t*9-*t*11 did not vary (P>0.05) among the four groups of birds and the following fatty acids were non-detectable in three or all subsets of 42-day-old Ross 308 male broiler chickens studied (C8:0, C10:0, C20:0, C22:0, C22:1 and CLA *c*9-*t*11). Mean content of seven fatty acid constituents (C12:0, C14:0, C18:3γ, EPA, DHA, CLA *t*10-*c*12 and CLA) was ≤1% of total extractable fat in three or four groups of broiler chickens studied. Significant differences among the four fat-supplementation groups for the latter and all remaining fatty acids (C16:0, C20:0, C16:1, C18:1, C18:2, C18:3α, and C20:4), fatty acid groups (MUFA, EPA, DHA, PUFA 6, PUFA 3, PUFA, SFA, UFA, DFA, and OFA) and proportions (PUFA 6/3, UFA/SFA, MUFA/SFA, PUFA/SFA, and DFA/OFA) are summarized in Tables 1 and 2.

Differences in first order echotextural variables

There was a significant main effect of fat-supplementation group for MPI of pectoralis major muscles in the birds of the present study (Figure 2). Group FO birds exceeded (P<0.05) Group BT animals in mean MPI values of the left pectoralis major muscle in the transverse plane. The interaction of Group x Scanning plane was statistically significant for mean pixel heterogeneity (MPH) values of pectoralis major muscles. Mean pixel heterogeneity of the left pectoralis major muscle in the longitudinal plane was less (P<0.05) in Group SO compared with Groups FO, SO+FO and BT.

Table 1. Gross chemical composition and saturated fatty acid content of *pectoralis major* muscles in broiler chickens that received four different fat supplements (each group n=10). ND: non-detectable

Variable/Group	Group SO Soybean oil	Group FO Flaxseed oil	Group SO+FO Soybean+flaxseed oil	Group BT Beef tallow
Dry matter (%)	24.18±0.52	24.96±0.34	24.89±0.18	24.81±0.17
Total protein (%)	22.17±0.54 a	22.60±0.29 ab	23.02±0.16 ab	23.57±0.25 b
Crude fat (%)	2.13±0.10 b	2.28±0.20 b	1.85±0.11 ab	1.55±0.14 a
C8:0*	ND	ND	0.01±0.008	ND
C10:0	ND	ND	ND	ND
C12:0	0.05±0.008 ab	0.03±0.007 a	0.05±0.02 ab	0.09±0.02 b
C14:0	0.42±0.06 a	0.37±0.03 a	0.47±0.04 a	1.37±0.08 b
C16:0	18.35±0.41 b	16.45±0.48 a	18.97±0.37 b	22.90±0.17 c
C18:0	9.14±0.23 b	8.17±0.25 a	8.74±0.22 ab	10.51±0.15 c
C20:0	ND	ND	ND	ND
C22:0	ND	ND	ND	ND

a–c – within rows, mean values denoted by different letters are significantly different (P<0.05). *Fatty acid content expressed as % of extractable lipids.

Table 2. Mean (±SEM) mono- and polyunsaturated fatty acid content of pectoralis major muscles in broiler chickens that received four different fat supplements (each group n=10). ND: non-detectable

Variable/Group	Group SO Soybean oil	Group FO Flaxseed oil	Group SO+FO Soybean+flaxseed oil	Group BT Beef tallow
1	2	3	4	5
C16:1*	1.60±0.16 a	1.81±0.14 a	1.83±0.13 a	3.51±0.15 b
C18:1	26.9±40.71 a	28.7±60.51 a	27.26±0.35 a	37.07±0.86 b

Table 2 – contd.

1	2	3	4	5
C22:1	ND	ND	ND	ND
MUFA	28.5±0.9 a	30.6±0.6 a	29.1±0.4 a	40.6±1.0 b
C18:2	31.36±0.60 d	19.19±0.36 b	24.64±0.30 c	15.28±0.26 a
C18:3γ (γ-Linolenic acid)	0.20±0.009 c	0.06±0.01 a	0.11±0.006 b	0.11±0.01 b
C18:3α (α-Linolenic acid)	4.05±0.16 b	19.65±0.82 d	11.70±0.40 c	1.54±0.07 a
C20:4	7.2±0.6 b	3.5±0.3 a	4.6±0.2 a	6.6±0.7 b
EPA	0.2±0.02 a	1.4±0.1 c	1.0±0.07 b	0.4±0.04 a
DHA	0.3±0.02 a	0.5±0.06 ab	0.6±0.06 b	0.5±0.06 ab
PUFA 6	38.7±0.8 c	22.8±0.4 a	29.3±0.4 b	22.0±0.8 a
PUFA 3	4.6±0.1 b	21.6±0.8 d	13.3±0.4 c	2.4±0.1 a
PUFA 6/3	8.5±0.3 c	1.1±0.05 a	2.2±0.06 b	9.1±0.3 c
PUFA	43.5±0.9 b	44.4±0.8 b	42.7±0.4 b	24.5±0.9 a
CLA *c*9-*t*11	ND	ND	ND	0.03±0.01
CLA *t*10-*c*12	0.07±0.01 b	0.03±0.01 a	0.02±0.01 a	0.02±0.009 a
CLA *c*9-*c*11	0.05±0.01	0.02±0.01	0.02±0.009	0.05±0.02
CLA *t*9-*t*11	0.007±0.003	0.001±0.001	0.001±0.001	0.005±0.003
CLA	0.1±0.02 b	0.06±0.02 ab	0.04±0.02 a	0.1±0.03 ab
SFA	28.0±0.5 b	25.0±0.7 a	28.2±0.5 b	34.9±0.2 c
UFA	72.0±0.5 b	75.0±0.7 c	71.8±0.5 b	65.1±0.2 a
UFA/SFA	2.6±0.06 b	3.0±0.1 c	2.5±0.07 b	1.9±0.02 a
MUFA/SFA	1.02±0.04 a	1.2±0.04 b	1.03±0.03 a	1.2±0.03 b
PUFA/SFA	1.6±0.05 a	1.8±0.07 b	1.5±0.04 a	0.7±0.03 c
DFA	81.2±0.4 c	83.2±0.5 b	80.5±0.4 c	75.6±0.2 a
OFA	18.8±0.4 b	16.8±0.5 a	19.5±0.4 b	24.4±0.2 c
DFA/OFA	4.3±0.1 a	5.0±0.2 b	4.1±0.1 a	3.1±0.03 c

a–d – within rows, mean values denoted by different letters are significantly different (P<0.05).
*Fatty acid content expressed as % of extractable lipids.

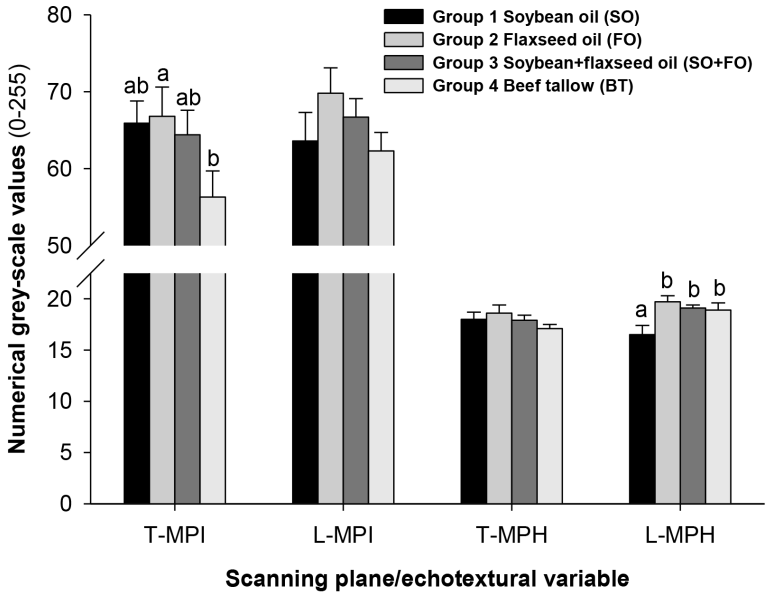


Figure 2. First order echotextural characteristics of the left pectoralis major muscle in four fat-supplementation groups of Ross 308 broiler chickens (each n=10). T: transverse plane; L: longitudinal plane; MPI: mean numerical pixel values (pixel intensity); and MPH: mean pixel heterogeneity (standard deviation of numerical pixel values). For each scanning plane/echotextural variable, the means denoted by different letters are significantly different (P<0.05)

Table 3. Summary of significant correlations between quantitative echotextural variables and chemical composition of pectoralis major muscles for each group of 10 broiler chickens that received four different types of dietary fat. R: coefficient of correlation; T: transverse plane; L: longitudinal plane; MPI: mean pixel intensity; and MPH-mean pixel heterogeneity (standard deviation of numerical pixel values)

Input variable (x)	Output variable (y)	R	P value	Regression equation
Group SO Soybean oil				
L-MPI	C16:1	–0.78	0.007	y=4.1–0.04x
L-MPI	C18:1	–0.65	0.04	y=36.1–0.14x
L-MPI	C18:2	0.87	0.001	y=21.2+0.15x
L-MPI	PUFA/SFA	0.68	0.03	y=0.9+0.009x
L-MPI	MUFA	–0.69	0.03	y=40.2–0.20x
L-MPI	PUFA	0.81	0.004	y=29.8+0.21x
L-MPI	PUFA 6	0.80	0.006	y=26.5+0.19x
L-MPH	C16:1	–0.69	0.03	y=3.8–0.13x
L-MPH	C18:2	0.84	0.002	y=21.6+0.58x
L-MPH	MUFA	–0.62	0.05	y=39.0–0.62x
L-MPH	PUFA 6	0.62	0.05	y=29.1+0.57x
T-MPH	Crude fat	–0.65	0. 04	y=3.4–0.068x
Group FO Flaxseed oil				
L-MPI	Crude fat	0.72	0.02	y=–0.7+0.04x
L-MPI	C18:0	–0.63	0.05	y=11.5–0.05x
L-MPH	C18:2	–0.66	0.04	y=27.0–0.40x
L-MPH	PUFA 6	–0.68	0.03	y=32.1–0.47x
T-MPI	Crude fat	0.64	0.05	y=0.07+0.03x
Group SO+FO Soybean+flaxseed oil				
L-MPI	PUFA 3	0.65	0.04	y=5.6+0.12x
L-MPI	DFA	0.66	0.04	y=72.3+0.13x
L-MPI	OFA	–0.66	0.04	y=27.6–0.13x
L-MPI	DFA/OFA	0.68	0.03	y=1.9+0.03x
L-MPI	PUFA/SFA	0.64	0.04	y=0.7+0.01x
L-MPI	CLA	0.78	0.008	y=-0.4+0.007x
L-MPI	PUFA	0.79	0.007	y=31.7+0.17x
L-MPI	C16:0	–0.69	0.03	y=26.8–0.12x
L-MPI	CLA *t*10-*c*12	0.70	0.03	y=–0.2+0.004x
L-MPI	CLA *c*9-*c*11	0.73	0.02	y=–0.2+0.003x
T-MPI	C16:1	–0.67	0.03	y=23.5–0.07x
T-MPI	PUFA	0.75	0.01	y=36.5+0.10x
T-MPI	DFA/OFA	0.63	0.05	y=2.9+0.02x
T-MPI	PUFA/SFA	0.65	0.04	y=0.7+0.01x
T-MPH	Crude fat	–0.65	0.04	y=0.7+0.06x
Group BT Beef tallow				
L-MPI	C18:0	–0.66	0.04	y=13.1–0.04x
L-MPI	C18:3γ (γ-Linolenic acid)	0.78	0.008	y=–0.2+0.004x
L-MPH	CLA *t*10-*c*12	0.66	0.04	y=–0.1+0.009x
T-MPH	Total protein	0.71	0.02	y=19.8+0.22x
T-MPH	DFA	0.75	0.01	y=70.1+0.32x
T-MPH	OFA	–0.75	0.01	y=29.9–0.32x
T-MPH	DFA/OFA	0.74	0.01	y=2.2+0.06x
T-MPH	PUFA/SFA	0.67	0.03	y=–0.006+0.04x

Table 4. Significant overall correlations between echotextural variables and chemical composition of pectoralis major muscles for 40 Ross 308 broiler chickens that received four different types of dietary fat. r: coefficient of correlation; T: transverse plane; L: longitudinal plane; MPI: mean pixel intensity; and MPH-mean pixel heterogeneity (standard deviation of numerical pixel values)

Input variable (x)	Output variable (y)	R	P value	Regression equation
L-MPI	Crude fat	0.46	0.003	y=1.8+0.001x
L-MPI	C12:0	–0.33	0.04	y=0.1–0.001x
L-MPI	C16:0	–0.39	0.01	y=26.6–0.11x
L-MPI	C18:3α (α-Linolenic acid)	0.33	0.04	y=0.09+0.0005x
L-MPI	SFA	–0.39	0.01	y=40.1–0.17x
L-MPI	OFA	–0.39	0.01	y=28.5–0.13x
L-MPI	UFA/SFA	0.41	0.009	y=1.1+0.02x
L-MPI	DFA/OFA	0.41	0.008	y=1.8+0.03x
L-MPI	PUFA/SFA	0.36	0.02	y=0.2+0.02x
L-MPH	C18:2	–0.34	0.03	y=12.2+0.57x
L-MPH	C18:3γ (γ-Linolenic acid)	–0.45	0.004	y=0.3–0.01x
L-MPH	C12:0	–0.33	0.04	y=0.1–0.005x
L-MPH	EPA	0.35	0.02	y=–0.7+0.08x
L-MPH	DHA	0.31	0.05	y=0.003+0.03x
T-MPI	Total protein	–0.30	0.05	y=24.8–0.03x
T-MPI	Crude fat	0.52	0.0005	y=0.5+0.02x
T-MPI	C14:0	–0.40	0.01	y=1.6–0.02x
T-MPI	C16:0	–0.51	0.0007	y=26.7–0.12x
T-MPI	C16:1	–0.38	0.02	y=4.1–0.03x
T-MPI	C18:0	–0.34	0.03	y=11.2–0.03x
T-MPI	SFA	–0.48	0.001	y=39.7–0.17x
T-MPI	OFA	–0.49	0.0008	y=28.5–0.13x
T-MPI	UFA/SFA	0.48	0.002	y=1.3+0.02x
T-MPI	DFA/OFA	0.51	0.0008	y=2.0+0.03x
T-MPI	PUFA/SFA	0.47	0.002	y=0.2+0.02x
T-MPH	C14:0	–0.33	0.04	y=1.9–0.07x
T-MPH	C16:1	–0.31	0.05	y=4.5–0.13x

Correlation analyses

Significant correlations between echotextural variables and chemical constituents of pectoralis major muscles in the chickens of the present study are given in Tables 3 and 4. When the present data were analyzed for correlations within each of the four experimental groups (Table 3), a total of 40 correlations out of possible 512 (2 scanning planes x 2 echotextural variables x 32 detectable chemical constituents or their groups/proportions × 4 groups) were recorded (7.8%). Twelve correlations were found in Group SO (L-MPI: 7; L-MPH: 4; and T-MPH: 1), five correlations in Group FO (L-MPI: 2; L-MPH: 2; and T-MPI: 1), fifteen correlations in Group SO+FO (L-MPI: 10; T-MPI: 4; and T-MPH: 1), and eight correlations in Group BF (L-MPI: 2; L-MPH: 1; and T-MPH: 5).With respect to the relative sensitivity of ultrasound imaging in animals allocated to the four groups, the strongest correlations (high correlations according to the Guilford's interpretation of the magnitude of significant correlations; Guilford and Fruchter, 1978) were as follows: Group SO: between L-MPI and C18:2 content (r=0.87, P=0.001); Group FO: between L-MPI and extractable (crude) fat (r=0.72, P=0.02); Group SO+FO: between L-MPI and PUFA (r=0.79, P=0.007); and Group BT: between L-MPI and C18:3γ (r=0.78, P=0.008). In Group SO, the proportion of significant correlations for scans obtained in the longitudinal plane was greater (P<0.001) compared with images in the transverse plane, whereas in Group SO+FO, a significantly greater proportion of correlations was observed for MPI than MPH values. No one correlation between the same echotextural variable and chemical constituent was recorded in all four fat supplementation group, but T-MPH was correlated with crude fat content of pectoral muscles, and L-MPI with PUFA and PUFA/SFA in Group SO and Group SO+FO; and L-MPI with C18:0 in Group FO and Group BT. Interestingly, L-MPH was also correlated with C18:2 and PUFA 6 content of chickens' pectoral muscles in

Groups SO and FO, but the correlations were in opposite directions (positive correlations in Group SO and negative in Group FO).

When data were pooled for all animals studied (Table 4), there were twenty-seven significant overall correlations out of a total of 128 possible correlations (21.2%; 2 scanning planes x 2 echotextural variables x 32 detectable chemical constituents or their groups/proportions). Nine correlations were recorded for L-MPI, five for L-MPH, eleven for T-MPI and two for T-MPH ($P<0.05$). Crude fat content, C16:0, SFA, OFA, UFA/SFA, DFA/OFA and PUFA/SFA were correlated with pixel intensity values of pectoral muscle ultrasonograms obtained in both planes (longitudinal and transverse). The strongest overall correlation was between crude fat content and T-MPI of pectoralis major muscles ($r=0.52$, $P=0.0005$; moderate correlation; Guilford and Fruchter, 1978).

Discussion

The fatty acid composition of pectoralis major muscles in broiler chickens of the present study resembled those of their respective fat supplements. For example, soybean oil and flaxseed oil are relatively high in PUFA and low in MUFA (Cortinas et al., 2004; Zambiazi et al., 2007; Daley et al., 2010; Smink et al., 2010), and this was reflected in the mean values for PUFA and MUFA content in Groups SO and FO. The lipid constituents of pectoralis major muscles in the mixed-oil group (Group SO+FO) were balanced between the two subsets of animals. These results are also consistent with earlier observations that supplementation with omega-3 rich oils, such as flaxseed oil, increases PUFA content in broiler chicken muscles (Anjum et al., 2013). Conversely, meat content of saturated fatty acids such as myristic acid (C14:0) and palmitic acid (C16:0) was significantly higher in Group BT.

Contrary to our initial expectations, there were no significant differences in echotextural characteristics of the left pectoral muscle between images obtained in the longitudinal or transverse plane. In the past, there were contrasting findings regarding skeletal muscle echotexture in relation to image acquisition planes. Nielsen et al. (2006) observed a noticeable decline in pixel intensity values of transverse scans compared with longitudinal scans of the human supraspinatus and vastus lateralis muscle. In contrast, Reimers et al. (1993) found an increase in echogenicity in transverse compared with longitudinal scans of various skeletal muscles in humans. The existence of measurable differences in muscle echogenicity between transverse and longitudinal planes may be attributed, at least partly, to the size and density of muscle fibers. In spite of tremendous variations in muscle size within (e.g., slow-twitch vs. fast-twitch vs. hybrid fibers) and between different skeletal muscles (Smith and Fletcher, 1988), the average diameter of human muscle fibers is 1.5- to 2-fold greater compared with that of chickens (Koomkrong et al., 2015; Meznaric and Cvetko, 2016). This relatively small size may explain a lack of inter-plane differences in muscle echotexture in the present study as well as a lack of correlations between quantitative echotextural attributes and pectoral muscle fiber diameters in a recent study in broiler chickens (Schwarz et al., 2019). Therefore, it is logical to infer that differences in echotextural attributes between the four groups of broiler chickens were predominantly due to the chemical composition of the muscle. In the present experiment, T-MPI values were lower in Group BT (characterized by the lowest crude fat content) and L-MPH was lower in Group SO (lowest protein content) compared with all other subsets of broiler chickens studied. Proteins are generally thought to attenuate ultrasound beams (Ghaedian et al., 1997) and the body of the muscle (parenchyma) in ultrasound images appears dark relative to more echogenic fat or some dense connective tissue components (Reimers et al., 1993; Nielsen et al., 2006).

No previous studies have investigated the relationship between echotextural attributes of skeletal muscles and their chemical composition including fatty acid profiles. In the present study, however, there was a lack of consistency in quantitative correlations among echotextural attributes and chemical composition of pectoralis major muscles. No echotextural variable determined in the longitudinal or transverse scanning plane was correlated with a chemical constituent or variable in all four fat supplementation groups of broiler chickens studied and only two echotextural parameters (T-MPH and L-MPI) were correlated with the same chemical constituent in two different subsets of birds. It is feasible that MPI and MPH values may only be used to predict the content of chemical constituents that comprise a sufficient percentage of the muscle tissue (i.e., equal to or greater than the "threshold" level). A correlation between crude fat and MPI or MPH was not recorded in Group BT, which had the lowest crude fat content. Alternatively, a significant correlation between MPH and total protein content was only observed in Group BT that had the highest amount of protein in pectoral muscles. Moreover, the only group of birds with a significant correlation between MPI and muscle lipids was Group FO characterized by the highest crude fat content; lipids significantly increase pixel intensity values (Walker et al., 2004; Tiidus, 2008).

The existence of quantitative correlations among echotextural variables and fatty acids is difficult to explain. Significant correlations were recorded between MPI/MPH and unsaturated fatty acids mainly in groups of birds fed diets supplemented with plant oils. But it is rather unlikely that the acoustic properties of individual fatty acids or their groups could be solely responsible for altering echotextural characteristics of pectoral muscles due mainly to their low relative content in the muscle mass. Moreover, nearly half of fatty acids or fatty acid groups for which significant correlations with echotextural variables had been recorded were not even the most abundant ones (represented only a small fraction of extractable lipids). One possible explanation of significant

correlations for fatty acid(s) would be collinearity. In statistics, collinearity describes a condition in which some of the input variables or data sets are correlated (Gujarati, 2009). Therefore, if the meat content of certain fatty acid(s) parallels the changes in crude fat or other major constituent recorded after computerized image assessment, the content of such fatty acid(s) could be estimated indirectly from echotextural parameters. More studies are needed to confirm this hypothesis.

The present experimental design using different dietary fat supplements allowed us to examine a variety in the degree of fatty acid saturation, mainly between the three plant oil groups (Groups SO, FO and SO+FO) and a non-plant oil (Group BT) treatment group. This model, however, was incapable of creating differences in fatty acid carbon chain lengths across the groups; eighteen-carbon fatty acids constituted ~70% of the present fatty acid profiles, and the remainder was mostly 16-carbon fatty acids (Tables 3 and 4). More extreme fat supplementation is required to thoroughly investigate the influence of fatty acid chain length on muscle echotexture, if any. In addition, different protein components may interact with ultrasound waves differently due to their size and solubility. Measuring separate protein components may yield more accurate results of correlation analyses.

The pectoral muscles of broiler chickens in this study were examined with a 7.5-MHz ultrasound transducer. Earlier ultrasonographic studies of skeletal muscles utilized ultrasound frequencies ranging from 5 to 8 MHz. High-frequency waves are suitable for imaging mainly superficial structures as they are more attenuated than lower-frequency waves for a given distance, but they offer images of higher resolution (Riley, 1996; Hoskins et al., 2010). The influence of scanning frequency on quantitative relationships between echotextural attributes and chemical composition of skeletal muscles has not been sufficiently examined.

To recapitulate, different dietary fats of plant and animal origin (soybean oil, flaxseed oil, soybean and flaxseed oil, and beef tallow) clearly induced significant differences in chemical composition and ultrasonographic characteristics of the pectoral muscles among the four fat-supplementation groups of broiler chickens. The present results indicate that chemical composition is an important factor contributing to the changes in muscle echotexture (first order ultrasonographic image attributes). Therefore, the present study demonstrates the potential applicability of computerized analysis of ultrasonograms for determining chemical composition of pectoralis major muscles in broiler chickens, including crude fat and protein content as well as select fatty acids. A lack of consistent correlations across the four dietary groups appears to be due mainly to the relative abundance of individual chemical constituents and warrants further studies.

Conflict of interests

There are no conflicts of interest to disclose. The authors of this study were not financially or otherwise associated with organizations that would bias the result of the present study.

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