

FACTORS AFFECTING ULTRASOUND INTRAMUSCULAR FAT CONTENT IN *MUSCULUS LONGISSIMUS DORSI* OF BEEF BULLS ESTIMATED WITH BIA PRO PLUS SOFTWARE*

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Abstract

The aim of the study was to determine the effect of ultrasound gain level, chemical composition and histological structure of *musculus longissimus dorsi* on pre-slaughter ultrasound evaluation of intramuscular fat content in *m.l.d.* of beef bulls. The study was conducted on 217 young bulls of six breeds: Red Angus, Charolais, Hereford, Limousin, Salers, and beef type Simmental. Measurements were conducted with an ultrasound system Aloka SSD-500 at five gain levels (90, 85, 80, 75, 70). After slaughter the content of intramuscular fat, total protein, connective tissue and muscle fibre type and diameter of *m.l.d.* were determined. Ultrasound evaluation of the intramuscular fat depended on the actual intramuscular fat and the total protein content ($P < 0.01$) in most of the measurements. The correlation between actual and ultrasound measurement of intramuscular fat content ranged from 0.2 to 0.36, and was highly significant for most of the ultrasound gain levels. There were highly significant ($P < 0.01$) and significant ($P < 0.05$) negative correlations between the total protein and ultrasound fat content ($r = -0.17$ to -0.31). The connective tissue percentage showed a significant effect on the ultrasound fat measurements at gains of 75 and 85. Ultrasound intramuscular fat content was positively correlated with the connective tissue in the *m.l.d.* at gain levels of 75 and 85 and the average ultrasound intramuscular fat measurement ($r = 0.16, 0.20, 0.16$). Highly significant and significant correlations between each ultrasound measurement were observed.

Key words: beef bulls, ultrasound, gain, intramuscular fat, connective tissue.

The increasing number of beef cattle and beef production in Poland caused the producers to take greater notice of the quality of beef. One of the most important features affecting the culinary value of beef is its marbling defined as the amount and distribution of intramuscular fat. It is an important factor of the beef grading system (Reddy et al., 1970; Cross et al., 1975). High content of intramuscular fat can

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disrupt connective tissue structure, which can lead to more tender meat (Nishimura et al., 1999). Marbling also affects the flavour of beef (Wulf and Page, 2000), because of the precursors of flavour substances contained in the fat that affect the sensory evaluation of meat (Scollan et al., 2005). Therefore, finding the possibilities of pre-slaughter assessment of marbling in beef cattle is important for both breeding and production purposes. Ultrasound evaluation of the intramuscular fat content is a method of low interference with animal welfare and enables making production decisions during fattening of animals (Wall et al., 2004). It can also be used in selection of breeding bulls. In Poland, ultrasound intramuscular fat evaluation studies of farm animals began more than ten years ago (Ślósarz et al., 2001). Thus far, studies were mainly conducted on sheep and goats using regression equations (Stanisz et al., 2004) and artificial neural networks (Ślósarz et al., 2011). Ultrasound evaluation of muscle marbling is based on the determination of intramuscular fat content and it uses the analysis of the size and intensity of gray speckles in ultrasound images (Brethour, 1994; Brethour, 2000; Ribeiro et al., 2008). The accuracy of ultrasound estimation of intramuscular fat is dependent on the quality of acquired images, the type and quality of the ultrasound system used, and the experience of the technician conducting the evaluation (Ślöniewski et al., 2000). The muscle structure can also influence the estimated amount of intramuscular fat, because of its effect on scattering of ultrasound waves and hence on number, size and intensity of speckles.

The aim of the study was to analyse factors affecting the ultrasound evaluation of intramuscular fat content in *longissimus dorsi* muscle of purebred slaughter bulls using specialized software.

Material and methods

The study was conducted on 217 bulls of six breeds: Red Angus (AR), Charolais (CHL), Hereford (HH), Limousin (LM), Salers (SL) and beef type Simmental (SM). Animals came from one company located in Western Pomerania and were slaughtered in one slaughterhouse. The feeding and maintenance were the same for all analysed bulls. The detailed structure of the study population is shown in Table 1.

Table 1. Population structure for all analysed traits

Traits	Selection based on	Breed						Total
		AR	CHL	HH	LM	SL	SM	
MARB 90 (%)	all	43	15	42	30	37	50	217
MARB 85 (%)								
MARB 80 (%) MARB 75 (%)								
MARB 70 (%) MARB (%)								
IMF (%)								
TP (%)	slaughter weight	30	15	30	30	30	30	165
CT (%)								
STO (%)		30	12	30	25	25	30	152
AMFD (μm)								

Aloka SSD-500 ultrasound system with 17 cm, 3.5 MHz linear transducer UST-5044 and BIA Pro Plus software (Designer Genes Technologies, Inc.) were used for evaluating intramuscular fat content of *m.l.d.*. The ultrasound examination was conducted the day before slaughter, according to the methodology of the Iowa State University Centralized Ultrasound Processing Lab. Intramuscular fat evaluation was conducted on the left side of the animal, based on longitudinal measurements of *musculus longissimus dorsi* at the junction of 11th-13th dorsal vertebrae. For each animal five ultrasound images were collected with five gain settings of the ultrasound system (90, 85, 80, 75 and 70, marked as MARB 90, MARB 85, MARB 80, MARB 75 and MARB 70) and saved using BIA Pro Plus software. On each image, measuring frame (Figure 3) was placed manually over the 12th thoracic vertebra to obtain the intramuscular fat content. The average intramuscular fat content for all measurements was determined (marked as MARB). After slaughter, from the left side of the carcass, section of the *m.l.d.* was collected at the height of the 12th thoracic vertebra. From the section samples were cut out for histological analysis (1×1×2 cm) and preserved in 4% buffered aqueous formalin solution. The fat content in the *musculus longissimus dorsi* (IMF, %) was determined according to PN - ISO 1444:2000 with the use of automatic extractor Buchi B-811. Total protein content (TP, %) was determined by the Kjeldahl method according to PN-75/A-04018, with Kjeldahl digestion unit DK 6 and automatic distillation unit UDK 129 (Velp Scientifica). The connective tissue content (CT, %) was determined based on the content of hydroxyproline and total protein according to Regulation of the Minister of Agriculture and Rural Development. The hydroxyproline content was determined spectrophotometrically using Hydroxyproline Assay Kit (BioVision). Material for histological analysis, fixed in 4% buffered formalin solution, was washed in running water for 24 hours and dehydrated in an alcohol series. After saturation in methyl benzoate material was embedded in paraffin. Sections 7 µm thick were stained with hematoxylin and eosin and methylene blue. The samples were cut longitudinally and perpendicularly to the course of the muscle fibres using a Microm HM 310 microtome. The samples were evaluated under a Nikon Eclipse 80i light microscope, in transmitted and polarized light. The morphometric analysis of sample images was performed using NisElements AR software. Average muscle fibre diameter (AMFD, µm) and red-type fibre content (STO, %) were determined. The diameter of muscle fibres was evaluated on the basis of three successive measurements for a single fibre cross-section and longitudinal section. For each sample the results were averaged from ten measurements. The fibre type content was determined in five consecutive fields of view.

In order to determine the influence of analysed factors on ultrasound measurements, the animals were divided into the following groups according to:

- intramuscular fat content (IMF): <1.00; 1–1.99; >2;
- total protein content (TP): <21; 21–21.99; 22–22.99; >23;
- connective tissue content (CT): <2.5; 2.5–4.99; >5;
- red fibre content (STO): 0–25; 25–49.99; 50–74.99; 75–100;
- average muscle fibre diameter (AMFD): <30; 30–39.99; 40–49.99; >50.

The data were analysed using the Statistica 10 software. A univariate analysis of variance was conducted. Significant differences between the groups were determined

using Duncan's test. Two-way analysis of variance was performed to determine the interaction between the analysed factors and breed of the animals. Correlation coefficients between the studied traits were determined.

Results

Ultrasound readings of intramuscular fat content obtained at various ultrasound gain levels, in relation to the actual intramuscular fat content, are shown in Figures 1–2. The mean values of MARB 90, MARB 85 and MARB 80 were 2.19, 2.2 and 2.17%, respectively, and did not differ statistically from each other. Ultrasound measurements of intramuscular fat content at gains of 75 (1.90%) and 70 (1.53%) were highly significantly lower than the values obtained using the other gain settings and also differed between each other ($P > 0.01$). Results indicate that BIA Pro Plus software may overestimate the values of intramuscular fat content for all gain levels.

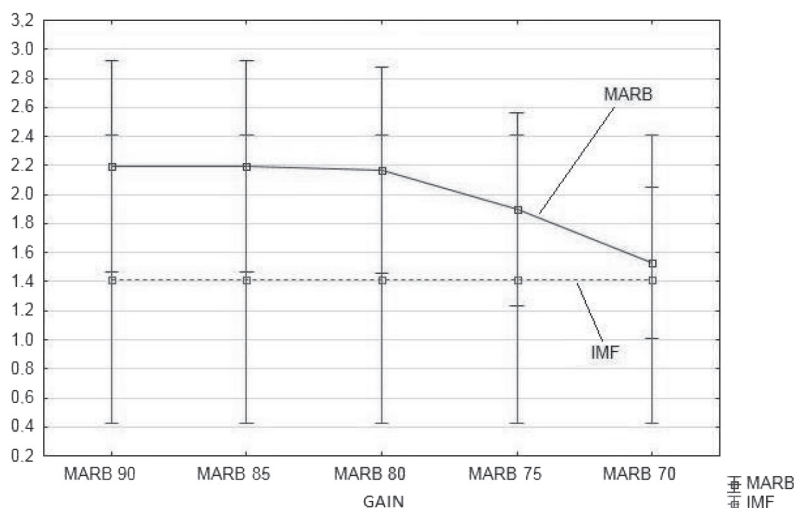


Figure 1. Mean values and standard deviations of ultrasound (MARB, %) and actual (IMF, %) intramuscular fat content in *m.l.d.* of all studied bulls

The ultrasound intramuscular fat content shows a downward trend with decreasing gain level in the AR breed; however, for gains of 75 and 70 the ultrasound measurements seem to be underestimated. Closest ultrasound readings to the actual intramuscular fat content were observed at gain of 75. Similarly, in the HH breed almost equal ultrasound and actual intramuscular fat contents were observed at 75, but also at maximum gain level of 90. It is interesting that in the HH bulls ultrasound measurement is overestimated at gains of 85 and 80, and underestimated for the other settings. MARB values in other breeds were overstated regardless of the gain level.

The highest MARB values were observed at gains of 80 in the CHL and SM, 90 in the LM and 85 in the SL bulls. The lowest ultrasound intramuscular fat content was always observed at gain of 70 (Figure 3). Mean values of the differences between ultrasound and actual intramuscular fat content of *musculus longissimus dorsi* are shown in Table 2.

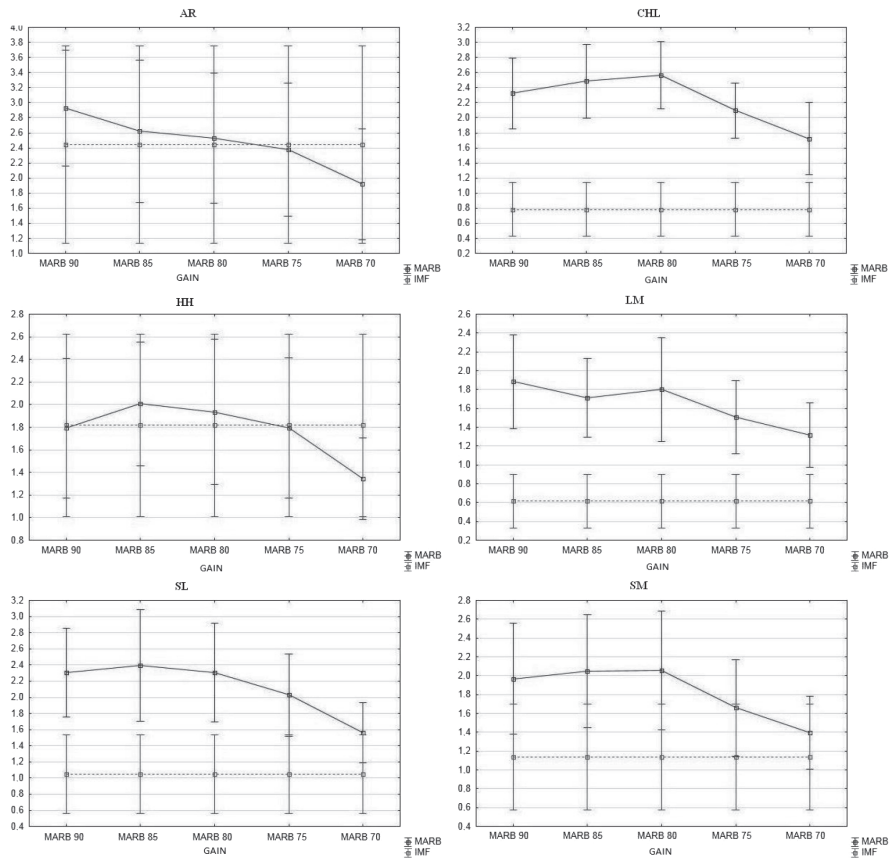


Figure 2. Mean values and standard deviations of ultrasound (MARB, %) and actual (IMF, %) intramuscular fat content in *m.l.d.* of bulls according to breed

The higher the actual intramuscular fat content was in *m.l.d.* of analysed bulls, the higher the ultrasound MARB readings were observed, but the differences between the group of <1% IMF and the group of 1–1.99% are not statistically significant (Table 3). The differences between the highest IMF group (>2%) and the other groups were observed at all gain levels as well as the average MARB value, and are highly significant ($P < 0.01$) or significant ($P < 0.05$).

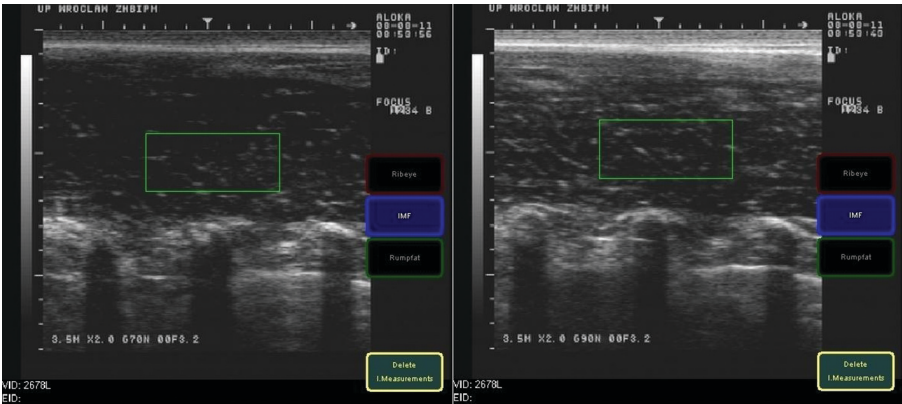


Figure 3. Ultrasound images collected at gains of 70 (left) and 90 (right), frame marks the intramuscular fat content measuring area

Table 2. Main differences between ultrasound and actual intramuscular fat content (%) according to breed and gain level

Breed	Gain					Average
	MARB 90	MARB 85	MARB 80	MARB 75	MARB 70	
AR	0.48±1.28	0.17±1.45	0.08±1.53	-0.07±1.52	-0.53±1.39	0.03±1.46
CHL	1.54±0.49	1.70±0.60	1.78±0.48	1.31±0.40	0.94±0.58	1.46±0.59
HH	-0.02±0.85	0.19±0.90	0.12±0.96	-0.02±0.94	-0.47±0.84	-0.04±0.92
LM	1.27±0.62	1.10±0.53	1.18±0.67	0.89±0.52	0.70±0.47	1.03±0.60
SL	1.26±0.72	1.34±0.73	1.26±0.64	0.98±0.63	0.51±0.51	1.07±0.71
SM	0.83±0.77	0.91±0.79	0.92±0.76	0.52±0.60	0.26±0.57	0.69±0.75
Total	0.78±1.00	0.78±1.06	0.75±1.11	0.48±1.02	0.11±0.98	0.58±1.06

Table 3. IMF group effect on ultrasound measurement of intramuscular fat content in *m.l.d* at various gain levels

Trait	IMF group			Total N = 217
	<1.00 N = 96	1.00–1.99 N = 78	>2.00 N = 43	
MARB 90 (%)	2.08±0.61 B	2.08±0.68 B	2.64±0.88 A	2.19±0.73
MARB 85 (%)	2.07±0.62 B	2.17±0.68 B	2.54±0.90 A	2.20±0.72
MARB 80 (%)	2.10±0.63 b	2.12±0.69 b	2.42±0.85 a	2.17±0.71
MARB 75 (%)	1.73±0.52 B	1.93±0.63 B	2.22±0.87 A	1.90±0.67
MARB 70 (%)	1.42±0.39 B	1.53±0.46 B	1.77±0.74 A	1.53±0.52
MARB (%)	1.88±0.49 B	1.95±0.53 B	2.33±0.74 A	2.00±0.58
IMF (%)	0.66±0.21 A	1.46±0.30 B	3.02±0.94 C	1.42±0.99

a, b – values in rows with different letters differ significantly ($P \leq 0.05$); A, B – as above for $P \leq 0.01$.

Table 4. TP group effect on ultrasound and actual measurement of intramuscular fat content in *m.l.d* at various gain levels

Trait	TP group				Total N = 165
	<21 N = 34	21–21.99 N = 68	22–22.99 N = 42	>23 N = 21	
MARB 90 (%)	2.28±0.72 b	2.23±0.69 b	2.17±0.76	1.86±0.72 a	2.18±0.73
MARB 85 (%)	2.41±0.72 Bb	2.32±0.80 B	2.05±0.64 a	1.81±0.50 A	2.20±0.73
MARB 80 (%)	2.28±0.73 b	2.28±0.75 b	2.09±0.63	1.86±0.55 a	2.18±0.70
MARB 75 (%)	2.10±0.67 B	1.98±0.72 b	1.86±0.63	1.61±0.41 Aa	1.93±0.67
MARB 70 (%)	1.71±0.61 B	1.57±0.56 b	1.51±0.43 b	1.25±0.29 Aa	1.55±0.53
MARB (%)	2.17±0.63 B	2.07±0.62 B	1.94±0.50	1.67±0.40 A	2.01±0.59
IMF (%)	1.59±1.25	1.36±0.87	1.35±1.11	1.66±0.88	1.44±1.02

a, b – values in rows with different letters differ significantly ($P \leq 0.05$); A, B – as above for $P \leq 0.01$.

It was found that the total protein content in the *musculus longissimus dorsi* also has a significant impact on the ultrasound measurements of intramuscular fat (Table 4). For MARB 90 and MARB 80 there was a significant difference between the group of animals with total protein content above 23%, and the two groups with the lowest TP, whilst the difference was highly significant for gain level of 85. Bulls with TP of 22–22.99% had significantly lower ultrasound intramuscular fat readings than animals from the group with TP below 21%. MARB 75 measurements were highly significantly lower in bulls with the highest total protein content than the group with the lowest protein content, and significantly lower than animals in the group of 21–21.99% TP. Analogous differences occurred at the gain level of 70, but with an additional significant difference in the ultrasound fat content found between the groups with TP >23% and 22–22.99%. The MARB value is highly significantly lower in animals from the >23% TP group than in the two groups with the lowest protein content. The influence of TP group on the actual intramuscular fat content in *m.l.d.* was not observed.

Relationships between connective tissue content and ultrasound intramuscular fat measurements were observed only at gain levels of 85 and 75. Bulls with the highest connective tissue levels (CT >5%) had significantly higher MARB 75 and MARB 85 than the animals with the lowest CT (<2.5%). An increased value of ultrasound intramuscular fat was observed with the increase of the connective tissue content for all gain levels (Table 5). There was no relationship between the IMF and the connective tissue in *m.l.d.*

Influence of muscle fibre diameter on ultrasound intramuscular fat measurement was observed only at gain level of 85. In animals with muscle fibre diameter greater than 50 μm MARB 85 was significantly lower than in other animals. There was no effect of the red fibre content on ultrasound readings of intramuscular fat in the studied population.

It was found that there were highly significant positive correlations between ultrasound measurements and actual intramuscular fat content (Table 6), except MARB 80, for which the correlation was significant. The highest correlation coefficient

($r = 0.42$) between ultrasound and the actual proportion of intramuscular fat occurred at gain of 90 in the group of animals with the highest fat content (IMF $>2\%$). Significant and highly significant negative correlations between TP in *musculus longissimus dorsi* and ultrasound measurements of intramuscular fat were observed, regardless of the gain. The connective tissue content showed positive correlations with MARB 85, MARB 75 and MARB. In the AR breed correlations between ultrasound measurements of intramuscular fat and CT were highly significant (MARB 85 $r = -0.53$; MARB 80 $r = -0.51$; MARB 75 $r = -0.47$; MARB 70 $r = -0.47$; MARB $r = -0.56$).

Table 5. CT group effect on ultrasound and actual measurement of intramuscular fat content in *m.l.d.* at various gain levels

Trait	CT group		
	<2.5 N = 27	2.5–4.99 N = 98	>5 N = 40
MARB 90 (%)	2.20±0.79	2.15±0.72	2.23±0.71
MARB 85 (%)	2.04±0.68 a	2.16±0.74	2.42±2.42 b
MARB 80 (%)	2.11±0.80	2.14±0.72	2.33±0.59
MARB 75 (%)	1.75±0.65 a	1.90±0.70	2.11±0.55 b
MARB 70 (%)	1.46±0.46	1.52±0.57	1.66±0.43
MARB (%)	1.91±0.59	1.98±0.61	2.14±0.52
IMF (%)	1.34±0.99	1.54±1.09	1.27±0.83

a, b – values in rows with different letters differ significantly ($P \leq 0.05$).

Table 6. Correlation coefficients for all analysed traits (*P<0.05; **P<0.01)

[illegible]

There was a significant interaction between the IMF group and the breed of analysed animals for the measurement of MARB 80 and MARB ($P < 0.05$).

Discussion

The results of the impact of gain level on the ultrasound intramuscular fat values correspond with the results of other authors. Tomka et al. (2006) reported that ultrasound intramuscular fat content for gain set at 75 ranged from 0.572 to 0.829% depending on the breed, whereas at gain of 90 the values obtained were much higher (3.053–5.561%). Polák et al. (2008) also noted that the higher the gain level, the higher the gray speckle value of ultrasound images. In our study a smaller variation of MARB was observed between the values obtained at extreme ultrasound gain settings (MARB 90–2.19% and MARB 70–1.53%), but analyses were made with the use of a different method of ultrasound image evaluation. Tomka et al. (2006 and 2007) reported that intramuscular fat content determined using ultrasound method was overestimated in the CHL and Slovak Simmental breeds at gains of 90 and 85, respectively. The differences between MARB and IMF fat, including the highest values of ultrasound fat content obtained at gains of 80 and 85 in the CHL, HH, LM, SL and SM breeds may be due to the use of specific software. High gain levels may generate noise artifacts in ultrasound image and influence measurement accuracy. Lower ultrasound intramuscular fat content observed at gain of 90 compared to other gain settings (80 and 85) in four of the six breeds in our study may suggest that BIA Pro Plus software is capable of adjusting potentially overestimated results that may occur due to high gain levels. However, the adjustment method is not known. It is noteworthy, however, that at gain levels of 80 and 85 lower correlation coefficients occur between the IMF and the MARB.

According to Nielsen et al. (2006) the impact of connective tissue on ultrasound images is caused by increased ultrasound echo while ultrasound waves pass through the non-contractile tissues, located within the muscle. This phenomenon is rarely taken into consideration during ultrasound evaluation of intramuscular fat content of meat. Brethour (1990) draws attention to the potentially higher readings of marbling from the images obtained from animals with a higher content of connective tissue, which is probably due to greater scattering of ultrasonic waves in tissues with high collagen content, resulting in increased size and intensity of gray speckles on assessed images. Similarly Sehgal (1993) observed the impact of even a small amount of collagen in the tissue on the dispersion of ultrasonic waves.

Total protein content, and the diameter of muscle fibres were not analysed in previous studies. The correlation observed in our study may be due to different density of the muscle fibres, depending on the total protein content and morphological characteristics. According to Whittaker et al. (1992) density of the analysed tissue affects the acoustic resistance, and thus the image.

Tomka et al. (2007) found that the correlation coefficients between ultrasound measurements at various gain levels varied between 0.46 and 0.74, whereas the co-

efficients obtained in our study were higher ($r = 0.52\text{--}0.91$). The high correlation coefficient between ultrasound and actual intramuscular fat content in animals with $>2.0\%$ IMF at the highest gain level corresponds with the results of other authors. Polák et al. (2008) report higher correlation coefficients for images obtained with a higher gain level ($r = 0.60\text{--}0.79$) depending on the breed. The authors also suggest that the higher the actual intramuscular fat content, the higher the observed correlation coefficients are between IMF and MARB in *musculus longissimus dorsi*. This may be the cause of the relatively low correlation coefficients obtained in the entire study population.

Ultrasound gain level is an important factor affecting the accuracy of the ultrasound intramuscular fat measurement. The present results suggest that the evaluation of intramuscular fat using BIA Pro Plus software is also affected by a number of features associated with the internal structure of muscle, including the intramuscular fat, total protein and connective tissue content of the *m.l.d.* The impact of these traits on the ultrasound image requires further study to obtain more accurate evaluation results and for practical application of this method. Of note are the higher correlation coefficients between ultrasound and the actual muscle fat content in animals with higher IMF. The results suggest that this method can be applied only for evaluating breeds which tend to have higher intramuscular fat deposition.

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IRMINA NEWLACIL, MONIKA DEMKOWICZ, KATARZYNA PACZYŃSKA,
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Czynniki wpływające na ultrasonograficzną ocenę udziału tłuszczu śródmięśniowego mięśnia najdłuższego grzbietu buhajów mięsnych

STRESZCZENIE

Celem badań było określenie wpływu składu chemicznego i struktury histologicznej *musculus longissimus dorsi* oraz poziomu wzmocnienia ultrasonografu na przyżyciową ocenę udziału tłuszczu śródmięśniowego w mięśni najdłuższym grzbietu buhajów ras mięsnych. Badania przeprowadzono na 217 buhajkach ras: AR, CHL, HH, LM, SL oraz SM. Pomiar ultrasonograficzny *m.l.d.* wykonano aparatem Aloka SSD-500 przy pięciu wzmocnieniach ultrasonografu (90, 85, 80, 75, 70). Chemicznie określono udział tłuszczu śródmięśniowego, białka ogólnego oraz tkanki łącznej. Oceniono średnicę włókien mięśniowych oraz udział typów włókien mięśniowych w *m.l.d.* Ultrasonograficzny pomiar udziału tłuszczu śródmięśniowego był wysoko istotnie zależny od udziału tłuszczu śródmięśniowego oraz udziału białka ogólnego ($P < 0,01$) w większości dokonanych pomiarów. Korelacja pomiędzy rzeczywistym, a ultrasonograficznym pomiarem udziału tłuszczu śródmięśniowego wynosiła od 0,2 do 0,36 i była dla większości ustawień USG wysoko istotna. Zanotowano wysoko istotne ($P < 0,01$) oraz istotne ($P < 0,05$) ujemne korelacje pomiędzy udziałem białka ogólnego, a ultrasonograficznym udziałem tłuszczu ($r = -0,17$ – $-0,31$). Udział tkanki łącznej wykazywał istotny wpływ na pomiary przy ustawie-

niach 75 i 85. Ultrasonograficzny pomiar udziału tłuszczu śródmięśniowego skorelowany był dodatnio z udziałem tkanki łącznej w mięśniu najdłuższym grzbietu przy ustawieniach USG 75, 85 oraz średniego pomiaru MARB ($r=0,16; 0,20; 0,16$). Zanotowano wysoko istotne oraz istotne korelacje pomiędzy poszczególnymi pomiarami ultrasonograficznymi.