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Validation of new proprietary software (r-Algo) for predicting meat chemical composition from ultrasound images of skeletal muscles in live animals: Pectoralis major muscles of broiler chickens

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ABSTRACT. The objective of this study was to validate a novel computerized method of ultrasound image analysis to determine chemical composition of pectoralis major muscles in broiler chickens. Ultrasonograms of pectoral muscles in the longitudinal and transverse planes were obtained from 40 birds just before slaughter. All chemical constituents of muscle samples were determined with the validated laboratory techniques, and the results served as a benchmark for developing the present algorithmic estimates of chicken meat composition. An in-house developed algorithm (r-Algo) was used to normalize the ultrasonograms and to identify pixel intensity ranges for which linear correlations between mean numerical pixel values and the content of various chemical constituents were the strongest (based on the values of correlation coefficients), using a stepwise sequestration of ultrasound bitmaps. Percentages of chemical constituents were the dependent (accepted) variables and the results of echotextural analyses (luminance or pixel intensity), carried out with a commercially available image analysis software (ImageProPlus®), were the explanatory variables. The predictive regression equations were determined in 30 randomly selected algorithm-training experimental units, and their accuracy was tested in a subset of 10 birds allocated to the algorithm-validation group. Significant determination coefficients were found for all chemical constituents studied, with the accuracy ranging from 62.70% (linoleic acid, transverse plane, pixel range of 141–142) to 96.65% (total hypocholesterolemic acids, longitudinal plane, pixel range of 136–150). The present validation results indicate that accurate prediction of muscle chemical composition using echotextural image analyses is feasible after identifying specific pixel intensity ranges.

Introduction

In 1999, Chanamai and McClements demonstrated the existence of the quantitative relationship between ultrasonic velocity and chemical composition of chicken meat using liquid 'chicken analogues' prepared from dried chicken powder, corn oil and distilled water. Although their experiment did not involve live animals or intact muscles, it provided the first evidence that ultrasound technology could be used to characterize meat proximate composition. Over the last three decades, however, studies utilizing real-time B-mode ultrasonography of skeletal muscles in food-producing animals have focused primarily on carcass composition to identify the finest breeding animals in genetic improvement programs (McLaren et al., 1989; Houghton and Turlington 1992; Stanford et al., 1998;

Oviedo-Rondón et al., 2007; Teixeira et al., 2008; Kleczek et al., 2009; Silva et al., 2009). There were, however, only few attempts to apply computerized analysis of muscle ultrasonograms for predicting meat composition and physicochemical properties (e.g., textural properties, chemical composition, and fatty acid profiles of pectoralis major muscles in broiler chickens (Schwarz et al., 2019) or intramuscular fat content in beef (Izquierdo et al., 1996), American feedlot steers and heifers (Amin et al., 1997), lambs (Ślósarz et al., 2001), and swine (Newcom et al., 2002)).

Echointensity (EI)/image texture analysis has long been used in engineering, botany, agriculture, and biomedical applications to classify various types of imagery (Han and Hayes, 1990; Layer et al., 1990; Shearer and Holmes, 1990). Computerized image analysis is used to extract information from the pixel intensity patterns formed in ultrasound B-scan images that are generated and displayed because of backscattered ultrasound beams (McCauley et al., 1994). The development of EI measurements using co-occurrence pixel matrices is credited to Haralick et al. (1973), who compiled a list of 14 different echotextural characteristics or features for image classification (e.g., angular second moment, contrast, entropy, etc.). The means and standard deviations of these attributes yielded a total of 28 textural attributes. In a recent study, Fabbri et al. (2021) performed texture analysis of beef muscles utilizing approximately 300 metrics falling into 6 main categories (i.e., histogram features, autoregressive models, co-occurrence matrix, gradient features, run-length matrix, and wavelet transform); their detailed descriptions can be found in Szczypiński et al. (2009), Wu et al. (2015), and Banzato et al. (2016).

Mean pixel intensity or brightness, traditionally expressed as an index of numerals ranging from 0 to 255 (0 – absolute black; 255 – absolute white), and its standard deviation (pixel heterogeneity) in a defined region of interest have been referred to as first-order EI variables. Even though some earlier and most recent studies suggested that an application of complex models containing multiple ultrasonographic attributes might be necessary for determining intramuscular fat content (McCauley et al., 1994; Fabbri et al., 2021), the use of first-order image characteristics would be the most practical and hence the most appealing solution (Ślósarz et al., 2001; Newcom et al., 2002; Schwarz et al., 2019). However, most prior studies in which echotextural analyses of grey-scale B-mode images were used to estimate the chemical composition and physicochemical properties of muscles in animals, including experiments conducted in our laboratories, were not completely successful (Thane, 1992; McCauley et al., 1994). The accuracy of Izquierdo's method to determine the percentage of intramuscular fat declines with an increasing percentage of lipids (Izquierdo et al., 1996). The USOFT software developed by Amin et al. (1997) only detects the changes in intramuscular fat content in the range of 2–8 percent, and only in American feedlot steers and heifers. Results of a study by Newcom et al. (2002) indicated that their method of predicting intramuscular fat content in swine significantly underestimated or overestimated the percentage of extractable fat in animals with greater or less than 5% of intramuscular fat content, respectively.

Ślósarz et al. (2001) used grey-level pixel luminance analyses to predict extractable fat content in the loin (*longissimus dorsi* muscle) from meat-type lambs. All analyses were first done on normalized but non-transformed images and then on binary-transformed images at three threshold values for image pixel luminance (134, 139 and 144). The 139-pixel intensity threshold value was determined during the preliminary analysis of unprocessed images of the back fat layer detected ultrasonographically above the loin, and the threshold values higher and lower by 5 were added for comparison. An application of higher threshold values (i.e., 139 and 144) resulted in elimination of a greater proportion of luminous (bright) pixels in the images compared with that at the 134-threshold. The correlations between mean numerical pixel values and crude fat content of *longissimus dorsi* muscles estimated for non-transformed images were weaker compared with those for binary-transformed images at the threshold level of 139, but stronger when threshold values of 134 and 144 were applied. Those results imply that pre-determining pixel intensity ranges may enhance the efficacy of computerized image analyses for quantifying various tissue components or chemical constituents using EI parameters. Such a pre-determination, however, must be based on objective criteria and standards to ensure the accuracy and repeatability of results.

In evaluating intramuscular fat content of beef meat using texture analysis of ultra-sonograms, Fabbri et al. (2021) employed a stepwise linear regression (discriminant analysis) of various ultrasonographic parameters and related the outcomes directly to the ex-tractable fat measurements obtained with the petrol ether (Randall) extraction method. Hence, the lipid content of the muscle samples determined with a standardized laboratory technique (dependent variable) served as the reference to validate the sensitivity and specificity of the combined ultrasound texture parameters (explanatory variables). Using

a similar conceptual framework, we designed a computer algorithm called r-Algo to examine the usefulness of first-order EI characteristics for predicting meat proximate composition and fatty acid content. The r-Algo is a Python application that progressively screens all pixel intensity values within a normalized and bitmapped ultrasonographic region of interest, and then calculates the coefficients of correlation among all possible combinations of pixel intensities and any set of numerical output variables (e.g., chemical constituents of meat samples). We hypothesized that chemical composition of pectoralis major muscles from broiler chickens would be strongly correlated with luminance values for specific clusters or ranges of pixel intensity values within the histograms of B-mode scans.

Material and methods

Animals and experimental procedures

The experiment described in this article utilized humane slaughter practices compliant with the EC Council Regulation no. 1099/2009 of 24 September 2009 on the protection of animals at the time of killing (Document 32009R1099; https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32009R1099). The present study used the original data set (laboratory analyses) and ultrasound images obtained and analysed by Schwarz et al. (2019) and Ahmadi et al. (2022) but subjected to more discriminating analyses with the new image analysis software r-Algo. The initial study was carried out at the National Research Institute of Animal Production situated in Aleksandrowice near Kraków, Poland. Forty fast-growing, male broiler chicks (Ross 308) were hatched in a commercial poultry hatchery and transported to an experimental farm equipped with the automated control of environmental conditions (light, temperature, and ventilation). Birds were fed *ad libitum* and had unlimited access to drinking water. Feed mixes were prepared according to the dietary requirements for broiler chickens (Smulikowska and Rutkowski, 2005) and contained 22, 20.5 and 20.5 crude protein and 2990, 3130 and 3130 kcal metabolizable energy per kg for starter, grower and finisher diets, respectively. The proportions of fat supplements added to all mixtures were 2.9% in starter, 4.8% in grower, and 4.8% in finisher. Group SO received soybean oil, which contains a majority of polyunsaturated fatty acids (PUFA) n-6 fatty acids; group FO received flaxseed oil, which contains polyunsaturated fatty acids (PUFA) n-3 fatty acids; group SO+FO received a mixture of both oils in a proportion of 44 percent:56 percent, which corresponds to an equal amount of both PUFA in the diet; and group BT received mixes supplemented with beef tallow, which mainly contains saturated fatty acids and the minimal amount of PUFA from the PUFA n-3 and PUFA n-6 families. After the 6-week rearing period, on the day of slaughter, the birds' pectoralis major muscles (left) were scanned in the longitudinal and transverse plane using a 7.5-MHz transducer connected to a portable Aloka ProSound 2 scanner (Hitachi Aloka Medical Ltd., Tokyo, Japan), with constant settings for main and near/far gain, and focal points (Figure 1). All birds were shackled, subjected to electrical water bath stunning at 20 mA per bird for 12 s, and then sacrificed by decapitation (Schwarz et al., 2019). The birds were allowed to bleed for a minimum of 90 s.

Laboratory analyses of meat samples

Dry matter (oven-drying technique; PN-ISO 1442: 2000, 2013), crude protein (Kjeldahl method; nitrogen to the protein conversion rate of 6.25; PN-75/A-04018/Az3, 2002), and crude fat content (Soxhlet extraction; PN-ISO 1444: 2000, 2013) were estimated after the slaughter. The composition of fatty acid methyl esters was determined with gas chromatography (Varian 450-GC, Varian BV, Middelburg, The Netherlands; ISO 12966-2). Schwarz et al. (2019) have described in detail sample collection and all the laboratory tests that presaged this retrospective study.

Image normalization and echotextural analyses, technique validation and statistical comparisons

The validation of the present method to determine chemical constituents of the pectoralis major muscles in broiler chickens utilized the automated progressive discriminant analysis (Ślósarz et al., 2001; Fabbri et al., 2021; Kim, 2021) (Figure 2). Images containing the largest cross-sectional area of the left pectoralis major muscle were saved as Digital Imaging and Communications in Medicine (DICOM) images with a size/resolution of 640 x 480 pixels. Echotextural analyses conducted at the University of Guelph, ON, Canada, utilized image bitmapping with commercially available ImageProPlus® 7.0 analytical software (Media Cybernetics Inc., Rockville, MD, USA) and ensuing bitmap processing with new proprietary software provisionally called r-Algo. The original red, green and blue (RGB) images were converted to grey-scale (8 bites) images and normalized (histogram stretching; $G_i=T(f_i-f_{min})/(f_{max}-f_{min})$, where f_i was the original intensity in the range (f_{min}, f_{max}) and Gi was the corresponding scaled intensity in the (0,T) range; T value was equal to 255). The images

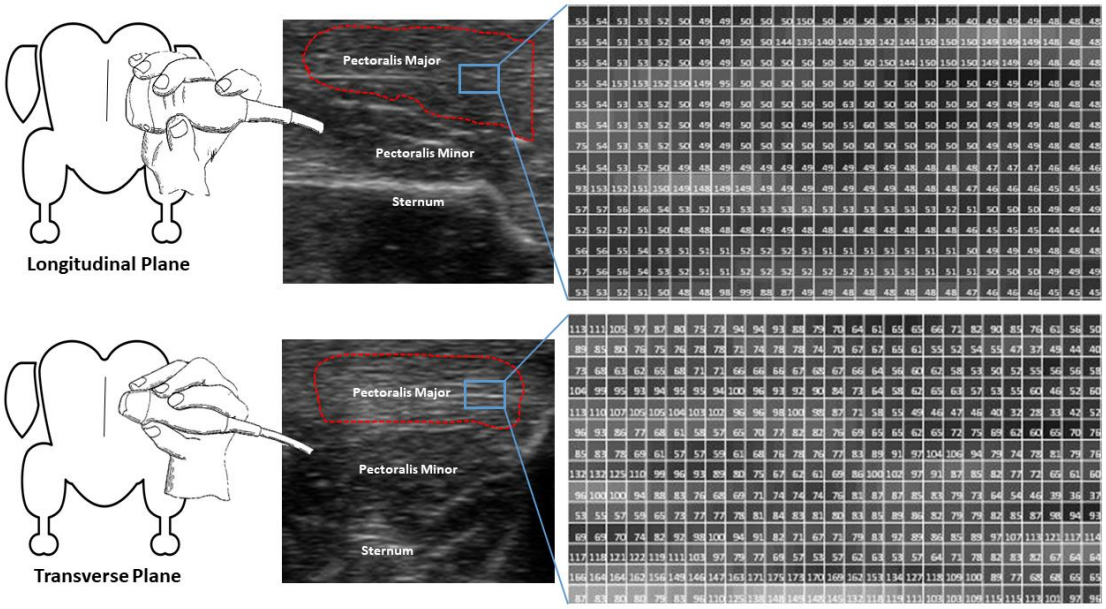

Figure 1. Ultrasonographic images of pectoral muscles in broiler chickens (left side) obtained in the longitudinal and transverse scanning plane (relative to the sternum). The sternum (bone and cartilage) is plainly seen in the lower portion of both images. Rectangular regions of interest placed within the artifact free cross-section of the pectoralis major muscle, with corresponding bitmaps, are also shown

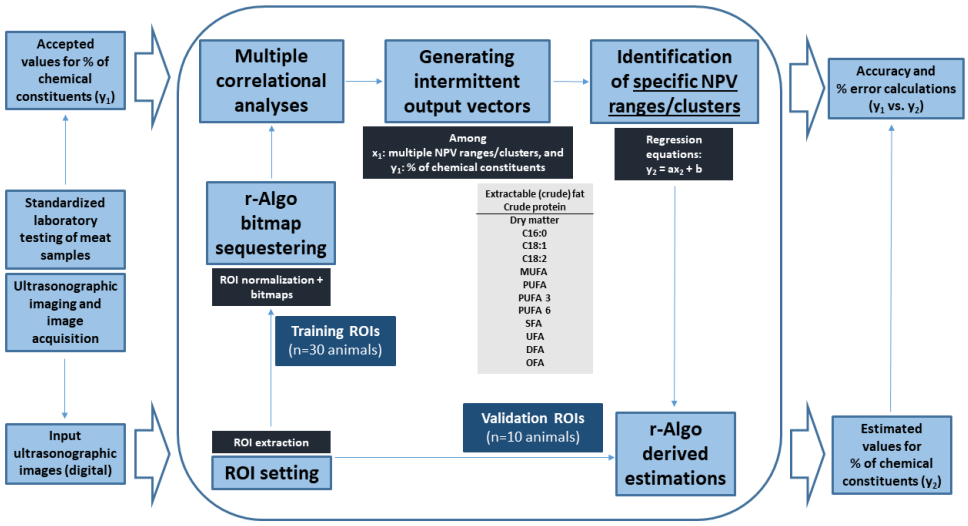

Figure 2. A block diagram of the processes to compute the percentages of various chemical constituents using ultrasound image processing by the r-Algo computer program

were randomly divided into two subsets: a training group of 30 images and a validation group of 10 images. From each training set of ultrasonograms, the r-Algo first computed an image bitmap, which is a grid or numerical 'roadmap' of pixels with a specific intensity value assigned to each pixel, and then calculated the mean value of intensities for each possible combination of individual pixel values (0–255) in the bitmap (2^{256} = 1099511627776 possible combinations). Subsequently, it used the Pearson Product Moment analysis to compute the coefficients of correlation between mean pixel values for all possible pixel ranges/clusters (input variables) and chemical composition data (output variables, accepted values). Then the r-Algo identified the specific clusters of pixels for which the linear correlation between mean NPV and chemical composition values were strongest. The algorithm also computed the linear regression equations for the significant correlations. Finally, the mean pixel intensity of specific ranges was calculated for each image using the bitmaps of the validation set images, and the content of chemical constituents was calculated using the linear regression equation (experimental values). The soundness of the proposed method to determine the chemical composition of the muscles was evaluated on the individual animal basis by calculating the accuracy and percent error using the following formulas:

Percent error = (estimated value – accepted value)/accepted value × 100%

Accuracy = 100 – |percent error|%

Mean accepted and estimated chemical contents within each scanning plane as well as mean accuracy and percent errors of estimated contents of all chemical constituents studied (Tables 1 and 2), for the images in the longitudinal and transverse plane, were compared with a paired Student's-t test using a SigmaPlot® (ver. 11.0; Systat Software Inc., San Jose, CA, USA). Proximate chemical constituents, major fatty acid groups and the most abundant saturated, monounsaturated, and PUFAs were assessed in the present study.

Results and discussion

Proximate chemical composition

Specific pixel intensity ranges identified by the r-Algo computer program in digital image bitmaps and correlation coefficients/regression equations for quantitative correlations between mean numerical pixel intensities for the pixel ranges and percentages of chemical constituents studied are given in Table 1 (longitudinal plane) and Table 2 (transverse plane). The identified ranges specific for individual chemical constituents were different and did not overlap between the images obtained in the longitudinal and transverse plane. Ślósarz et al. (2001) reported that pixel intensity measurements taken on transverse-plane images of the *longissimus dorsi* muscle in lambs were generally greater compared with those in images obtained in the longitudinal plane. Due mainly to differences in the spatial orientation of muscle fibres, both types of images are distinctive images, and they are capturing 'a similar, although not identical, fragment of the muscle' (Ślósarz et al., 2001). However, there were no differences in mean pixel intensity (numerical pixel values) and heterogeneity (standard deviation of numerical pixel values) of the left pectoralis major muscle between the longitudinal and transverse scanning plane in the broiler chickens of the present study (Ahmadi et al., 2022). Therefore, the reason(s) for the differences in specific ranges between the two scanning planes (non-overlapping ranges for all proximal chemical constituents in the longitudinal and transverse planes) are difficult to explain. Nevertheless, the scanning plane must be taken into consideration during the development of an auto-mated algorithmic system for the ultrasonographic determination of muscle chemical composition.

In our previous study using the same data set (Ahmadi et al, 2022), only the percentage of crude

Table 1. A summary of pixel intensity ranges identified with the computer algorithm r-Algo as well as corresponding coefficients of correlations and regression equations (Pearson Product Moment) for quantitative relationships between mean numerical pixel values for specific ranges (x) (ultrasonograms of pectoralis major muscles in the randomly selected training-group broiler chickens (n=30) scanned in the longitudinal plane) and the content of chemical constituents analysed (y)

Constituent	r-Algo specific pixel range	r	Regression equation
DM	76–83	0.54	y = 1.27x − 76.10
Crude protein	59–76	−0.54	y = −0.81x + 78.54
IMF	74–91	0.60	y = 0.36x − 27.30
SFA	133–150	−0.57	y = −1.68x + 264.40
C16:0	136–150	−0.52	y = −0.97x + 156.51
UFA	133–150	0.57	y = 1.68x − 164.40
MUFA	85–91	−0.51	y = −9.86x + 898.77
C18:1	85–91	−0.51	y = −8.32x + 761.32
PUFA	84–95	0.57	y = 8.77x − 742.04
C18:2	110–116	−0.58	y = −15.90x + 1818.30
DFA	136–150	0.55	y = 1.19x − 88.24
OFA	136–150	−0.55	y = −1.19x + 188.24

DM – dry matter, IMF – intramuscular (crude) fat, SFA – saturated fatty acids, C16:0 – palmitic acid, UFA – unsaturated fatty acids, MUFA – monounsaturated fatty acids, C18:1 – oleic acid, PUFA – polyunsaturated fatty acids, C18:2 – linoleic acid, DFA – total hypocholesterolemic acids, OFA – total hypercholesterolemic acids, r – Pearson correlation coefficients. The content of major chemical constituents was calculated as a percentage of pectoral muscle sample whereas select fatty acids and fatty acid groups were quantified as the percentage of total fatty acids methyl esters (extractable fatty acids)

Table 2. A summary of pixel intensity ranges identified with the computer algorithm r-Algo as well as corresponding coefficients of correlations and regression equations (Pearson Product Moment) for quantitative relationships between mean numerical pixel values for specific ranges (x) (ultrasonograms of pectoralis major muscles in muscles in the randomly selected training-group broiler chickens (n=30) scanned in the transverse plane) and the content of chemical constituents analyzed (y)

Constituent	r-Algo specific pixel range	r	Regression equation
DM	131–134	−0.62	y = −1.06x + 164.98
Crude protein	128–131	0.58	y = 1.18x − 129.91
IMF	101–123	0.59	y = 0.23x − 23.63
SFA	66–74	−0.74	y = −6.93x + 514.29
C16:0	66–74	−0.75	y = −4.63x + 343.12
UFA	66–74	0.74	y = 6.93x − 414.29
MUFA	52–59	0.59	y = 3.87x − 183.75
C18:1	141–142	0.58	y = 6.67x − 913.33
PUFA	66–74	0.63	y = 3.95x − 248.69
C18:2	141–142	−0.68	y = −10.58 + 1519.19
DFA	66–74	0.76	y = 5.40x − 298.03
OFA	66–74	−0.76	y = −5.40x + 398.03

DM – dry matter, IMF – intramuscular (crude) fat, SFA – saturated fatty acids, C16:0 – palmitic acid, UFA – unsaturated fatty acids, MUFA – monounsaturated fatty acids, C18:1 – oleic acid, PUFA – polyunsaturated fatty acids, C18:2 – linoleic acid, DFA – total hypocholesterolemic acids, OFA – total hypercholesterolemic acids, r – Pearson correlation coefficients. The content of major chemical constituents was calculated as a percentage of pectoral muscle sample whereas select fatty acids and fatty acid groups were quantified as the percentage of total fatty acids methyl esters (extractable fatty acids)

fat (r = 0.46, $P = 0.003$ and r = 0.53, $P = 0.0005$ for the longitudinal and transverse scanning plane, respectively) and of total protein (r = –0.30, $P = 0.05$ for the transverse plane) was correlated with the mean pixel intensity in the range of 0–255 pixels in all birds studied. However, using the r-Algo algorithm to sequester pixel intensity ranges in the ultrasound bitmaps, the content of each chemical component of the pectoralis major muscles studied was correlated with mean pixel intensity values in both scanning planes (Tables 1 and 2). The corresponding values for the coefficients of correlation obtained with the aid of the r-Algo algorithm were: 0.60 and 0.59 (crude fat or intramuscular (crude) fat (IMF)) and –0.54 and 0.58 (crude protein) in the longitudinal and transverse plane, respectively. All correlations for proximate chemical composition of chicken pectoralis major muscles in this study were moderate (indicative of a substantial relationship), according to Guilford's interpretation of the magnitude of linear correlations (Guilford et al., 1978). The sample size for this study, albeit sufficient for correlational analyses, was relatively small and an increase in the number of samples would further improve the already satisfactory accuracy. Interestingly, the correlations for dry matter (DM) and crude protein content detected in longitudinal and transverse plane images were in the opposite direction (i.e., negative vs. positive). The reason for this reversal in the linear relationship remains unknown.

A complete list of the accepted (obtained after slaughter by validated laboratory techniques) and estimated values for the percentages of intramuscular fat, protein, and DM, as well as the accuracy/percent error of algorithmic estimates in 10 birds (validation subset), are presented in Tables 3 and 4.

Table 3. Accepted (laboratory) and algorithmically estimated values for the percentage of intramuscular fat (IMF), crude protein and dry matter (DM) in pectoralis major muscles of broiler chickens (n = 10 algorithm-validation subset of birds) scanned in the longitudinal plane as well as accuracy and percent error of the estimated values

Animal no.	Accepted values			Estimated values			Accuracy/Percent error		
	% IMF	% protein	% DM	% IMF	% protein	% DM	IMF	protein	DM
1	1.65	18.91	20.77	1.83	23.33	24.47	88.96/11.04	76.63/23.37	82.19/17.81
2	2.24	21.98	24.12	2.11	22.67	24.31	94.33/−5.67	96.85/3.15	99.22/0.78
3	2.13	23.07	25.13	1.52	24.78	24.38	70.98/−29.02	92.60/7.40	97.03/−2.97
4	1.47	22.18	23.93	1.75	24.28	25.50	80.94/19.06	90.55/9.45	93.43/6.57
5	2.50	23.09	25.78	1.96	22.61	24.51	78.37/−21.63	97.94/−2.06	95.09/−4.91
6	2.39	22.10	24.39	1.71	23.34	25.06	71.41/−28.59	94.38/5.62	97.25/2.75
7	2.21	23.30	25.64	1.91	22.01	24.65	86.62/−13.38	94.48/−5.52	96.15/−3.85
8	1.28	24.21	25.29	1.48	23.10	24.61	84.25/15.75	95.43/−4.57	97.33/−2.67
9	1.44	23.45	24.71	1.65	22.84	24.29	85.11/14.89	97.39/−2.61	98.30/−1.70
10	1.46	24.18	25.17	1.66	23.78	23.94	86.04/13.96	98.35/−1.65	95.11/−4.89
Overall	1.88 ± 0.14	22.65 ± 0.48	24.49 ± 0.46	1.76 ± 0.06	23.27 ± 0.26	24.57 ± 0.14	82.70 ± 2.35/ −2.36 ± 6.18	93.46 ± 2.03/ 3.26 ± 2.77	95.11 ± 1.53/ 0.69 ± 2.22

Overall values are mean ± standard error of mean (SEM)

Table 4. Accepted (laboratory) and algorithmically estimated values for the percentage of intramuscular fat (IMF), crude protein and dry matter (DM) in pectoralis major muscles of broiler chickens (n = 10 algorithm-validation subset of birds) scanned in the transverse plane as well as accuracy and percent error of the estimated values

Animal no.	Accepted values			Estimated values			Accuracy/Percent error		
	% IMF	% protein	% DM	% IMF	% protein	% DM	IMF	protein	DM
1	1.65	18.91	20.77	1.49	23.31	24.62	90.18/−9.82	76.75/23.25	81.48/18.52
2	2.37	19.47	21.95	2.27	22.55	24.76	95.78/−4.22	84.18/15.82	87.18/12.82
3	2.22	21.88	23.97	1.97	22.75	24.71	88.93/−11.07	96.02/3.98	96.89/3.11
4	2.45	22.36	24.94	2.20	22.48	24.92	89.79/−10.21	99.48/0.52	99.93/−0.07
5	1.47	24.11	25.36	1.81	23.24	25.32	76.73/23.27	96.40/−3.6	99.85/−0.15
6	1.69	23.66	25.18	2.02	22.33	23.21	80.69/19.31	94.38/−5.62	92.16/−7.84
7	1.88	22.81	24.67	1.93	22.98	24.92	97.35/2.65	99.23/0.77	98.98/1.02
8	1.96	22.99	24.65	2.40	23.88	24.95	77.68/22.32	96.11/3.89	98.78/1.22
9	1.38	22.93	24.21	1.74	22.25	24.42	73.77/26.23	97.05/−2.95	99.15/0.85
10	1.50	24.20	24.97	1.75	22.25	24.48	83.32/16.68	91.92/-8.08	98.02/−1.98
Overall	1.86 ± 0.12	22.33 ± 0.57	24.07 ± 0.48	1.96 ± 0.09	22.80 ± 0.17	24.63 ± 0.18	85.42 ± 2.59/ 7.51 ± 4.9	93.15 ± 2.29/ 2.80 ± 3.10	95.24 ± 2.00/ 2.75 ± 2.38

Overall values are mean±standard error of mean (SEM)

There were no differences ($P > 0.05$) between the mean accepted and estimated values within each scanning plane, and no differences ($P > 0.05$) in mean accuracy/percent error for each chemical constituent between the two scanning planes. Based on the mean percent errors, algorithmic prediction of proximal composition was associated with non-significant underestimation of IMF and overestimation of crude protein and DM content in the longitudinal plane images, whereas in the transverse plane images of the pectoralis major muscles, all constituents were non-significantly overestimated. Mean estimation accuracies for the proximate chemical composition of chickens' muscles ranged from 82.70% for IMF (longitudinal plane) to 95.24% (DM, transverse plane). In a variety of machine learning models, the accuracy range (probability that the algorithm can correctly predict the outcome) between 100–87.5% is considered very good, 87.5–75% good, 75–62.5% is classified as satisfactory, and 62.5–50% is regarded unsatisfactory (Barkved, 2023).

Fatty acid profile

The consumption of chicken meat is increasing worldwide (Scanes, 2007) as it is healthier compared with red meat and perfectly fits modern culinary habits-it is easy and fast to prepare a meal from it. Culinary use of chicken breast meat offers a variety of nutritional benefits, including a high protein and low lipid content of the meals (Gallardo et al., 2012; Nkukwana et al, 2014), with a high proportion of PUFAs (Berzaghi et al., 2005). Fat in chicken breast meat is comprised of approximately 33.5% of saturated fatty acids, 30.5% of unsaturated fatty acids, and 32.0% of PUFA (Morales-Barrera et al., 2013). Variations in the fatty acid composition of the diet not only can increase the risk of developing multiple health problems such as heart diseases, stroke, diabetes, and some cancers, but also strongly affects several technological properties of meat such as intramuscular fat firmness, shelf life of the product and flavour development during cooking (Wood et al., 2004).

Table 5. Accepted (laboratory) and algorithmically estimated values for the percentage of fatty acids and fatty acid groups in pectoralis major muscles of broiler chickens (n = 10 algorithm-validation subset of birds) scanned in the longitudinal plane

Animal	Accepted values								
no.	SFA	C16:0	UFA	MUFA	C18:1	PUFA	C18:2	DFA	OFA
1	30.78	20.14	69.22	24.88	23.63	44.34	31.54	79.52	20.48
2	26.03	16.68	73.97	27.72	26.54	46.24	33.06	82.93	17.07
3	27.94	18.06	72.06	31.28	29.12	40.78	29.10	80.96	19.04
4	29.51	19.77	70.49	29.31	27.47	41.17	20.09	79.60	20.40
5	22.78	14.50	77.23	29.72	28.42	47.50	19.31	85.18	14.82
6	26.08	17.75	73.92	30.48	28.50	43.44	25.56	81.64	18.36
7	25.38	17.00	74.62	30.16	28.41	44.47	23.75	82.71	17.29
8	34.99	23.22	65.01	37.06	33.57	27.95	16.24	75.75	24.25
9	34.30	22.55	65.70	40.76	37.28	24.94	15.64	76.32	23.68
10	34.42	22.30	65.58	43.86	40.54	21.72	14.51	75.97	24.03
Overall	29.22 ± 1.36	19.20 ± 0.91	70.78 ± 1.36	32.52 ± 1.91	30.35 ± 1.64	38.26 ± 3.03	22.88 ± 2.14	80.06 ± 1.03	19.94 ± 1.03
Animal	Estimated values								
no.	SFA	C16:0	UFA	MUFA	C18:1	PUFA	C18:2	DFA	OFA
1	30.07	18.98	69.93	28.41	26.79	36.70	24.53	80.37	19.63
2	28.04	18.62	71.96	32.44	30.19	44.74	27.75	80.82	19.18
3	31.28	20.32	68.72	34.31	31.77	31.34	14.97	78.73	21.27
4	30.29	19.22	69.71	36.82	33.88	34.79	32.79	80.07	19.93
5	28.24	18.61	71.76	27.65	26.14	42.09	21.41	80.83	19.17
6	29.02	19.07	70.98	37.23	34.23	41.57	30.72	80.26	19.74
7	29.48	19.08	70.52	31.95	29.77	40.03	24.97	80.25	19.75
8	28.88	19.15	71.12	33.71	31.26	40.29	22.79	80.16	19.84
9	27.78	18.44	72.22	28.92	27.21	39.58	23.76	81.03	18.97
10	30.41	19.63	69.59	33.67	31.22	21.63	23.23	79.57	20.43
Overall	29.35 ± 0.37	19.11 ± 0.17	70.65 ± 0.37	32.51 ± 1.06	30.25 ± 0.89	37.27 ± 2.12	24.69 ± 1.58	80.21 ± 0.21	19.79 ± 0.21

SFA – saturated fatty acids, C16:0 – palmitic acid, UFA – unsaturated fatty acids, MUFA – monounsaturated fatty acids, C18:1 – oleic acid, PUFA – polyunsaturated fatty acids, C18:2 – linoleic acid, DFA – total hypocholesterolemic acids, OFA – total hypercholesterolemic acids. Overall values are mean ± standard error of mean (SEM). The content of fatty acids and fatty acid groups was originally calculated as the percentage of total fatty acids methyl esters (extractable fatty acids)

For example, PUFA that contain important essential fatty acids are characterized by the low melting point temperature. The effects that fatty acids can exert on human health and meat quality traits encourage poultry industry to monitor the fatty acid profile of chicken meat in such a way that the product can be devolved to its optimal use (e.g., direct consumption or further transformation/ pre-processing treatments). As the poultry industry is known for its high throughput and productivity, traditional analytical techniques used to determine the fatty acid profile of chicken meat are not suitable for regular commercial operations and data collection. Since these techniques involve fat extraction, fatty acid conversion to methyl esters, and subsequent analysis by capillary gas chromatography, they are costly and labour-intensive, and they generate hazardous waste due to the use of toxic chemical compounds (Fernández-Cabanás et al., 2011).

The accepted and estimated values for the percentages of different fatty acids/fatty acid groups as well as the accuracy/percent error of their algorithmic estimates in ten birds (validation subset) are presented in Tables 5, 6, 7 and 8. There were no differences ($P > 0.05$) between the mean accepted and estimated values within each scanning plane, and no differences ($P > 0.05$) in mean accuracy/percent error for each fatty acid constituent between the two scanning planes. The algorithmic prediction of fatty acid composition was associated with a non-significant overestimation of all fatty acids for the images of the pectoralis major muscle obtained in the longitudinal plane. In the transverse plane, the content of fatty acid/fatty acid groups was mainly non-significantly overestimated, apart from that for saturated fatty acids (SFA), palmitic acid (C16:0), PUFA and total hypercholesterolemic acids (OFA), which was non-significantly underestimated. The mean accuracy of the estimated (r-Algo) contents of fatty acids/fatty acid groups, using images in the longitudinal plane, were >80% except that for linoleic fatty acid (C18:2; 66.12 ± 6.78%), >81% for

Table 6. Accepted (laboratory) and algorithmically estimated values for the percentage of fatty acids and fatty acid groups in pectoralis major muscles of broiler chickens (n = 10 algorithm-validation subset of birds) scanned in transverse plane

Animal no.	Accepted values								
	SFA	C16:0	UFA	MUFA	C18:1	PUFA	C18:2	DFA	OFA
1	30.78	20.14	69.22	24.88	23.63	44.34	31.54	79.52	20.48
2	26.81	17.24	73.19	26.50	25.25	46.69	32.13	82.41	17.60
3	29.61	20.48	70.39	30.15	27.89	40.25	30.69	78.91	21.09
4	25.21	16.42	74.79	31.27	29.78	43.51	18.32	83.28	16.72
5	27.03	17.26	72.97	30.32	28.45	42.66	18.47	82.25	17.75
6	28.93	19.83	71.07	30.32	28.16	40.75	23.31	79.73	20.27
7	27.79	17.99	72.22	27.89	26.46	44.33	26.07	81.62	18.38
8	28.34	19.68	71.66	30.83	28.14	40.83	24.50	79.77	20.23
9	33.79	22.30	66.21	40.19	36.82	26.02	15.74	76.44	23.56
10	35.04	23.10	64.96	42.62	39.07	22.34	14.25	75.23	24.77
Overall	29.33 ± 0.98	19.44 ± 0.70	70.67 ± 0.98	31.50 ± 1.78	29.36 ± 1.54	39.17 ± 2.59	23.50 ± 2.10	79.92 ± 0.82	20.08 ± 0.82
Animal no.	Estimated values								
	SFA	C16:0	UFA	MUFA	C18:1	PUFA	C18:2	DFA	OFA
1	27.60	18.17	72.40	33.69	30.23	28.81	22.42	81.24	18.76
2	19.01	12.44	80.99	27.65	33.56	33.71	17.13	87.94	12.06
3	17.75	11.60	82.25	31.67	33.56	34.43	17.13	88.92	11.08
4	26.64	17.53	73.36	32.31	28.74	29.36	24.77	81.99	18.01
5	29.98	19.77	70.02	28.48	29.70	27.45	23.25	79.38	20.62
6	27.37	18.02	72.63	32.33	31.34	28.94	20.65	81.42	18.58
7	31.81	20.99	68.19	32.59	33.56	26.41	17.13	77.96	22.04
8	32.28	21.30	67.72	37.42	26.89	26.14	27.70	77.59	22.41
9	29.83	19.66	70.17	33.21	26.89	27.54	27.70	79.50	20.50
10	35.11	23.19	64.89	33.59	30.23	24.53	22.42	75.39	24.61
Overall	27.74 ± 1.76	18.27 ± 1.17	72.26 ± 1.76	32.29 ± 0.87	30.47 ± 0.81	28.73 ± 1.00	22.03 ± 1.28	81.13 ± 1.37	18.87 ± 1.37

SFA – saturated fatty acids, C16:0 – palmitic acid, UFA – unsaturated fatty acids, MUFA – monounsaturated fatty acids, C18:1 – oleic acid, PUFA – polyunsaturated fatty acids, C18:2 – linoleic acid, DFA – total hypocholesterolemic acids, OFA – total hypercholesterolemic acids. Overall values are mean ± standard error of mean (SEM). The content of fatty acids and fatty acid groups was originally calculated as the percentage of total fatty acids methyl esters (extractable fatty acids)

Table 7. Accuracy and percent error of the estimated values for the percentage of fatty acids and fatty acid groups in pectoralis major muscles of broiler chickens (n = 10 algorithm-validation subset of birds) scanned in the longitudinal plane

Animal no.	Accuracy								
	SFA	C16:0	UFA	MUFA	C18:1	PUFA	C18:2	DFA	OFA
1	97.70	94.27	98.98	85.82	86.64	82.75	77.77	98.93	95.85
2	92.29	88.40	97.29	82.97	86.25	96.74	83.93	97.45	87.60
3	88.03	87.46	95.36	90.32	90.89	76.85	51.42	97.25	88.30
4	97.37	97.23	98.90	74.39	76.64	84.49	36.77	99.40	97.67
5	75.99	71.65	92.92	93.01	92.00	88.62	89.16	94.88	70.58
6	88.73	92.59	96.02	77.85	79.89	95.71	79.79	98.31	92.50
7	83.82	87.80	94.50	94.05	95.19	90.01	94.85	97.03	85.77
8	82.54	82.48	90.60	90.96	93.11	55.82	59.63	94.18	81.82
9	80.99	81.79	90.08	70.94	73.00	41.32	48.01	93.83	80.11
10	88.35	88.01	93.88	76.76	77.01	99.58	39.85	95.26	85.01
Overall	87.58 ± 2.21	87.17 ± 2.30	94.85 ± 0.98	83.71 ± 2.64	85.06 ± 2.50	81.19 ± 5.95	66.12 ± 6.78	96.65 ± 0.63	86.52 ± 2.52
Animal no.	Percent error								
	SFA	C16:0	UFA	MUFA	C18:1	PUFA	C18:2	DFA	OFA
1	–2.30	–5.73	1.02	14.18	13.36	–17.25	–22.23	1.07	–4.15
2	7.71	11.60	–2.71	17.03	13.75	–3.25	–16.07	–2.55	12.40
3	11.97	12.54	–4.64	[b]9.68	9.11	–23.15	–48.58	–2.75	11.70
4	2.63	–2.77	–1.10	25.61	23.36	–15.51	63.23	0.60	–2.33
5	24.01	28.35	–7.08	–6.99	–8.00	–11.38	10.84	–5.12	29.42
6	11.27	7.41	–3.98	22.15	20.11	–4.30	20.21	–1.69	7.50
7	16.18	12.20	–5.50	5.95	4.81	–9.99	5.15	–2.97	14.23
8	–17.46	–17.52	9.40	–9.04	–6.89	44.17	40.37	5.82	–18.18
9	–19.01	–18.21	9.92	–29.06	–27.00	58.68	51.99	6.17	–19.89
10	–11.65	–11.99	6.12	–23.24	–22.99	–0.41	60.15	4.74	–14.99
Overall	2.36 ± 4.63	1.59 ± 4.83	0.14 ± 1.98	2.63 ± 5.96	1.96 ± 5.53	1.76 ± 8.63	16.51 ± 11.97	0.33 ± 1.28	1.57 ± 5.13

SFA – saturated fatty acids, C16:0 – palmitic acid, UFA – unsaturated fatty acids, MUFA – monounsaturated fatty acids, C18:1 – oleic acid, PUFA – polyunsaturated fatty acids, C18:2 – linoleic acid, DFA – total hypocholesterolemic acids, OFA – total hypercholesterolemic acids. Overall values are mean ± standard error of mean (SEM)

the scans in the transverse plane, except those for PUFA (73.35 ± 3.85%) and C18:2 (62.70 ± 6.24%) (Tables 7 and 8). In both scanning planes, the total hypocholesterolemic fatty acids (DFA) content had the numerically greatest mean prediction accuracy (longitudinal: 96.65 ± 0.63%, and transverse: 95.99 ± 3.53%), while C18:2 had the lowest mean prediction accuracy (longitudinal: 66.12 ± 6.78% and transverse: 62.70 ± 19.72%).

Interestingly, in both scanning planes, the algorithmically identified pixel intensity ranges corresponding to SFA and palmitic fatty acid (C16:0), SFA and unsaturated fatty acids (UFA), monounsaturated fatty acids (MUFA) and oleic acid (C18:1) or DFA and OFA contents were identical or were the superset/subset ranges, excepting the ranges for MUFA and C18:1 from the images in the transverse plane (Tables 1 and 2). Such a phenomenon was not observed for PUFA and C18:2, for which non-overlapping ranges were identified by the r-Algo computer program using ultrasonograms recorded in the longitudinal and transverse plane. Moreover, the r values for correlations between mean numerical pixel values (NPV) values and fatty acid content were identical for SFA and UFA and for DFA and OFA in both scanning planes, but the correlations were negative for SFA and OFA and positive for UFA and DFA. Based on these observations, it is attractive to speculate that the most abundant fatty acids typically 'share' their specific pixel detection ranges with the family of fatty acid they belong to and that differences in the direction of correlations between dissimilar fatty acid groups/families is indicative of their chemical properties. More studies are needed to confirm or refute this hypothesis.

Table 8. Accuracy and percent error of the estimated values for the percentage of fatty acids and fatty acid groups in pectoralis major muscles of broiler chickens (n = 10 algorithm-validation subset of birds) scanned in the transverse plane

Animal no.	Accuracy								
	SFA	C16:0	UFA	MUFA	C18:1	PUFA	C18:2	DFA	OFA
1	89.68	90.26	95.41	64.59	72.08	64.97	71.07	97.84	91.60
2	70.89	72.14	89.34	95.69	67.11	72.21	53.30	93.29	68.56
3	59.95	56.64	83.16	94.94	79.67	85.54	55.80	87.33	52.57
4	94.36	93.23	98.10	96.70	96.51	67.47	64.82	98.45	92.28
5	89.06	85.45	95.95	93.95	95.60	64.36	74.09	96.51	83.84
6	94.62	90.88	97.81	93.39	88.70	71.02	88.58	97.88	91.68
7	85.50	83.33	94.42	83.17	73.15	59.58	65.68	95.51	80.06
8	86.09	91.76	94.50	78.61	95.57	64.02	86.90	97.27	89.22
9	88.26	88.15	94.01	82.63	73.04	94.15	24.02	96.00	87.01
10	99.82	99.63	99.90	78.80	77.36	90.18	42.70	99.78	99.35
Overall	85.82 ± 3.76	85.15 ± 3.90	94.26 ± 1.53	86.25 ± 3.32	81.88 ± 3.54	73.35 ± 3.85	62.70 ± 6.24	95.99 ± 1.12	83.62 ± 4.34
Animal no.	Percent error								
	SFA	C16:0	UFA	MUFA	C18:1	PUFA	C18:2	DFA	OFA
1	–10.32	–9.74	4.59	35.41	27.92	–35.03	–28.93	2.16	–8.40
2	–29.11	–27.86	10.66	4.31	32.89	–27.79	–46.70	6.71	–31.44
3	–40.05	–43.36	16.84	5.06	20.33	–14.46	–44.20	12.67	–47.43
4	5.64	6.77	–1.90	3.30	−3.49	−32.53	35.18	−1.55	7.72
5	10.94	14.55	–4.05	–6.05	4.40	−35.64	25.91	−3.49	16.16
6	–5.38	–9.12	2.19	6.61	11.30	–28.98	–11.42	2.12	–8.32
7	14.50	16.67	–5.58	16.83	26.85	–40.42	–34.32	–4.49	19.94
8	13.91	8.24	–5.50	21.39	–4.43	–35.98	13.10	–2.73	10.78
9	–11.74	–11.85	5.99	–17.37	–26.96	5.85	75.98	4.00	–12.99
10	0.18	0.37	–0.10	–21.20	–22.64	9.82	57.30	0.22	–0.65
Overall	–5.14 ± 5.79	–5.53 ± 6.03	2.31 ± 2.33	4.83 ± 5.42	6.62 ± 6.65	–23.52 ± 5.68	4.19 ± 13.84	1.56 ± 1.66	–5.46 ± 6.74

SFA – saturated fatty acids, C16:0 – palmitic acid, UFA – unsaturated fatty acids, MUFA – monounsaturated fatty acids, C18:1 – oleic acid, PUFA – polyunsaturated fatty acids, C18:2 – linoleic acid, DFA – total hypocholesterolemic acids, OFA – total hypercholesterolemic acids. Overall values are mean ± standard error of mean (SEM)

Conclusions

Results of this study confirm that changes in the chemical composition of chicken breast may not have an equal impact on all ranges of pixel intensity values since different chemical constituents possess distinctive physicochemical and hence echotextural attributes. Moreover, our findings confirm that measuring the entire echointensity (pixel intensity range from 0 to 255) of muscle ultrasonograms provides insufficient information on its proximate chemical composition and fatty acid content. Therefore, instead of using 'traditional' (total) pixel intensity analysis, we identified specific pixel intensity ranges in ultrasonograms obtained in both scanning planes. All chemical constituents of chicken pectoralis major muscles studied were found to be moderately (indicative of substantial relationship) correlated with the mean echointensity of specific pixel ranges (Table 9). The new method of image analysis (r-Algo) was validated by comparing estimated values obtained from regression equations with those obtained using validated laboratory techniques. All prediction accuracies and percentage errors were satisfactory. To the best of the authors' knowledge, this is the first demonstration of significant and accurate ultrasonographic estimates of all proximate chemical constituents and major fatty acids/fatty acid groups in skeletal muscles of animals. However, more studies are necessary to elucidate the biological basis for correlations between first-order echotextural characteristics for specific pixel intensity ranges and various chemical constituents of skeletal muscles, and to improve the accuracy of the estimates obtained in both scanning planes.

Table 9. A comparison of correlational analyses among chemical constituents and mean pixel intensities of pectoralis major muscles (images obtained in transverse or longitudinal planes) in broiler chickens. Digital images were analysed using a traditional, 'total' intensity range approach (Ahmadi et al., 2022) or following the identification of specific pixel ranges for which echointensity values were correlated with the content of chemical constituents studied (present study)

Constituent/Scanning plane	Total echointensity (Ahmadi et al., 2022)				Echointensity of specific (r-Algo-identified) pixel ranges (present study)			
	transverase		*longitudinal*		*transverase*		*longitudinal*	
	P-value	*r*	*P*-value	*r*	*P*-value	*r*	*P*-value	*r*
Dry matter	NS		NS		0.0006	−0.62	0.003	0.54
Crude protein	0.05	−0.30	NS		0.001	0.58	0.003	−0.54
Crude fat	0.0005	0.53	0.003	0.46	0.0006	0.59	0.0007	0.60
SFA	0.001	−0.48	0.01	−0.39	0.000003	−0.74	0.002	−0.57
C16:0	0.0007	−0.51	0.01	−0.39	0.000002	−0.75	0.004	−0.52
UFA	NS		NS		0.000003	0.74	0.002	0.57
MUFA	NS		NS		0.0006	0.59	0.006	−0.51
C18:1	NS		NS		0.001	0.58	0.005	−0.51
PUFA	NS		NS		0.0001	0.63	0.002	0.57
C18:2	NS		NS		0.00006	−0.68	0.001	−0.58
DFA	NS		NS		0.000001	0.76	0.003	0.55
OFA	0.0008	−0.59	0.01	−0.39	0.000001	−0.76	0.003	−0.55

r – correlation coefficient, NS – non-significant, SFA – saturated fatty acids, C16:0 – palmitic acid, UFA – unsaturated fatty acids, MUFA – monounsaturated fatty acids, C18:1 – oleic acid, PUFA – polyunsaturated fatty acids, C18:2 – linoleic acid, DFA – total hypocholesterolemic acids, OFA – total hypercholesterolemic acids

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Data availability statement

All data are available from corresponding authors upon reasonable requests. The r-Algo is a proprietary software developed by BA and PMB, and subject to patent regulations.

Conflict of interest

The Authors declare that there is no conflict of interest.

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