Animal (2008), **2:9**, pp 1340–1345 © The Animal Consortium 2008
doi:10.1017/S1751731108002644

Bioelectrical impedance analysis for the prediction of fat-free mass in buffalo calf

F. Sarubbi†, R. Bàculo and D. Balzarano

National Research Council (CNR), Institute for Animal Production System in Mediterranean Environment, Via Argine 1085, 80147 Naples, Italy

(Received 11 May 2007; Accepted 28 March 2008; First published online 8 July 2008)

The objective of this study has been to develop a prediction equation of fat-free mass (FFM) from buffalo calves. Twenty buffaloes were fed ad libitum *access at* unifeed*, with vitamin–mineral integration, for 14 months. Seven days before slaughtering, the animals were weighed and bioelectrical impedance measurements were collected. The data were analyzed by multiple linear regressions to evaluate the relationship between FFM and various predictor variables. Stepwise regression was used to eliminate variables that did not influence variation in the model. The value of resistance collected showed a decrease when the electrical frequency increases, while the values of reactance (Xc) increase. When using live weight (LW) and reactance at 500 and at 1000 kHz as independent variables, we obtained the best* R^2 *Adj (0.967) and Durbin Watson statistic (2.596) that explain the prediction model (*$FFM = -30.59 + 0.993LW + 0.150Xc_{500} - 0.123Xc_{1000} + 9.11$*). These results indicate that the use of bioelectrical impedance analysis has excellent potential as a rapid method, with minimal perturbation for the animal, to predict FFM in buffalo.*

Keywords: buffalo calf, bioelectrical impedance analysis, fat-free mass prediction equations

Introduction

Buffalo meat is acquiring more interest regarding the past both for motivations of economic order and for improving knowledge to obtain products of good quality (Ferrara *et al.*, 1994; Gigli *et al.*, 2001).

The main characteristics of buffalo meat are small contents of intramuscular fat (Gigli *et al.*, 2001), a low level of cholesterol and high contents of micro- and macro-elements. Moreover, buffalo meat is adapted to endure various processes of transformation and, in this way, its products are able to find a market for human nutrition utilization.

Ferrell and Cornelius (1984) stated that the ideal technique for measurement of body composition should be accurate, easily accomplished, inexpensive, applicable to a wide range of ages and compositions, and capable of being applied to the live animal with minimal perturbation of subsequent performance. Various procedures such as the Fat-O-Meater (Kempster *et al.*, 1985; Sather *et al.*, 1989), the electrical meat measuring equipment (Domermuth *et al.*, 1976; Fredeen *et al.*, 1979) or total body electrical conductivity (Keim *et al.*, 1988; Forrest *et al.*, 1989) have found acceptable results in the estimation of composition of pork carcasses, with similar or better predictive capability compared with direct measurements ($R^2 = 0.81$) of the *longissimus dorsi* muscle area, fat depth and carcass weight (Forrest *et al.*, 1989).

An alternative procedure using the bioelectrical impedance analyzer (BIA) has been proposed for humans (Miyatani *et al.*, 2001; Salinari *et al.*, 2003; Stahn *et al.*, 2007). This technology, because of its accuracy, simplicity and portability, has been conveniently applied for pig (Swantek *et al.*, 1991 and 1992), sheep (Jenkins *et al.*, 1988; Berg *et al.*, 1996), beef cattle (Marchello and Slanger, 1992; Marchello *et al.*, 1999; Velazco *et al.*, 1999) and horse (Forro *et al.*, 2000).

These initial findings indicate that BIA has excellent potential as a means of predicting lean and fat-free mass (FFM) and body mass in commercial situations, given its precision, simplicity and portability (Kushner and Schoeller, 1986; Berg *et al.*, 1996). FFM, also called lean body mass, is the total amount of non-fat (lean) corresponding also to skeletal muscle; therefore an increase in FFM equals an increase in skeletal muscle.

Impedance of a geometric system depends on the conductor length, cross-sectional area and signal frequency. The repeatability of impedance measurements can be improved by using a fixed signal frequency and a constant

---

† E-mail: f.sarubbi@iabbam.na.cnr.it

conductor configuration. The impedance of a biological tissue comprises two components, resistance and reactance. The conductive characteristics of body fluids provide the resistive component, whereas the cell membranes, acting as imperfect capacitors, contribute a frequency-dependent reactive component. Impedance measurements made over a range of low to high (1 MHz) frequencies allow the development of prediction equations; this is known as multi-frequency bioelectrical impedance analysis.

Several authors reported results at 50 kHz in farm animals (Berg *et al.*, 1996; Swantek *et al.*, 1999; Velazco *et al.*, 1999). Schoeller (2000) assumed that the impedance at 50 kHz is overwhelmed by the extracellular water, and the high correlation between impedance and total body water, as well as the FFM, was due to the close relation of these compartments to the extracellular water.

Bioelectrical impedance methodology utilizing transdermal attachment of electrodes at various frequencies has not been reported in live buffaloes. Because the impedance is frequency dependent, it is favorable to extend the frequency range for greater accuracy. Research on humans at various frequencies suggested confounding frequencies for the best prediction of body composition (Pietrobelli *et al.*, 1998; Tagliabue *et al.*, 2001). Considering that an increase in FFM corresponds to an increase in skeletal muscle, we have placed attention on the study for the development of a procedure to determine the FFM in buffalo via the measurement of BIA at various frequencies in live animals.

Material and methods

Twenty young buffalo bulls, born and bred in a farm located in the province of Salerno (Italy), after colostrum administration, received a milk replace until weaning. Animals had *ad libitum* access to a unifeed (49% mais silage, 32% lolium, 7% alfalfa hay, 6% barley flakes and 5% soybean meal). The ration was calculated assigning 0.87 milk forage unit, 15% crude protein (CP) and 50% NDF, supplemented with vitamin–mineral integration. The animals were kept in experimental conditions for 14 months.

For BIA measurements the buffaloes were sedated using Rompum® with a dose of 0.25 mg/100 kg live weight (LW), enough to immobilize the animals (Figure 1). Two electrodes were placed approximately 1 cm from the dorsal midline, 10–15 cm caudal from the last cervical vertebra and approximately 5 and 10 cm cranial from the first sacral vertebra. The impedenzometric measurements of resistance (Rs) and reactance (Xc) were expressed in kHz (5 to 1000) and was made using electrodes, composed of 20-gauge Vacuteiner needles (Becton Dickinson, Rutherford, NJ), placed in specific locations on the trunk (Figure 2).

The impedenzometric method has been carried out by the Human Im Plus II® instrument. Buffaloes were weighed and BIA measurements were collected 7 days before slaughtering.

The measurements were collected by the same operator in the same environmental conditions. Slaughtering and dissection were according to the ASPA Commission (1991). The sample cut was taken from the left side of sides at the 10th thoracic vertebra level, according to Lanari's indication (1973). On the *longissimus dorsi* section, located between the skull margin of the 9th vertebra and the caudal margin of the last thoracic vertebra of the right side, the chemical–nutritional characteristics of meat were determined according to the ASPA Commission (1991). These measures have not been taken into consideration in the present study, except for fat content, but they will be studied in future work.

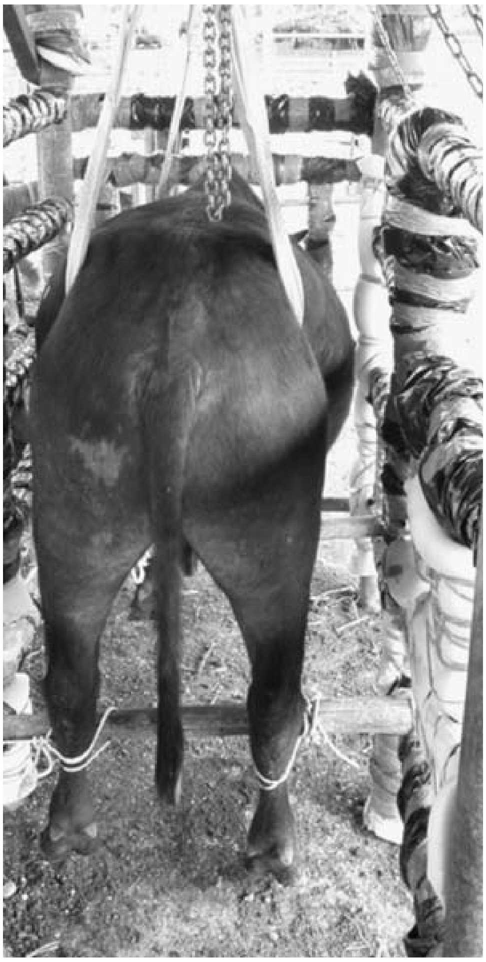

Figure 1 Immobilization of the animal.

The FFM of the subject was calculated by subtracting the product of live body mass and percentage of fat from the live body mass (FFM = live weight − [live weight × % fat/100]) (Lukaski *et al.*, 1985). Jenkins *et al.* (1988) defined the non-fat component of carcass composition as the sum of a carcass's water and chemical CP.

The data analyzed were LW, live volume (Lvol), resistance (Rs) and reactance (Xc) at any frequency.

The data were analyzed by multiple linear regressions (SPSS 12.0, 2003) to evaluate the relationship between FFM and various predictor variables.

Stepwise regression was used to eliminate variables that did not influence variation in the model. The adjusted R^2 selection method, Durbin Watson statistics and Akaike

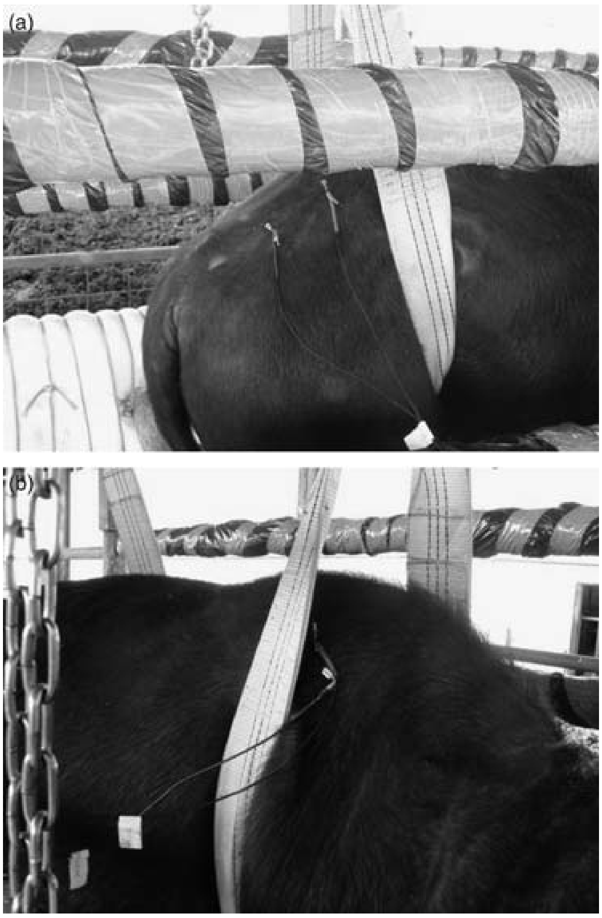

Figure 2 Specific locations for impedance measurements of Rs and Xc (a) and (b).

Information Criterion (AIC) statistic were used to make the final decision about the best models. Simple coefficients of correlation were examined for data obtained in live buffaloes.

The descriptive statistics of the data analyzed are listed in Table 1.

Table 1 *Descriptive statistics of the data analyzed*

	Minimum	Maximum	Mean ± s.d.
PV	337	533	454.3 ± 55.4
Lvol	54	122	88.3 ± 17.4
Rs_{50}	21	42	25.6 ± 4.62
Xc_{50}	2	5	3.1 ± 0.788
Rs_{10}	26	34.6	29.46 ± 2.76
Rs_{100}	8.9	37.6	21.72 ± 5.90
Rs_{500}	3.9	33.3	16.99 ± 5.64
Rs_{1000}	1.8	29.7	13.48 ± 5.42
Xc_{5}	1.1	1.9	1.56 ± 0.244
Xc_{10}	1.4	3.4	2.48 ± 0.450
Xc_{100}	1.8	9.2	3.12 ± 1.72
Xc_{500}	6.3	8.1	7.20 ± 0.567
Xc_{1000}	10	21.8	18.01 ± 2.43

Lvol = length^2/resistance.

Table 2 *Correlation between LW, Lvol and values of Rs and Xc at any frequency*

	LW	Lvol
LW		
Lvol	0.535*	
Rs_{50}	−0.168	−0.792**
Xc_{50}	0.087	−0.167
Rs_{5}	0.030	−0.561*
Rs_{10}	−0.131	−0.785**
Rs_{100}	−0.370	−0.855**
Rs_{500}	−0.391	−0.802**
Rs_{1000}	−0.389	−0.715**
Xc_{5}	0.282	0.282
Xc_{10}	0.172	0.122
Xc_{100}	20	20
Xc_{500}	−0.105	0.306
Xc_{1000}	0.048	0.009

*Correlation is significant at 0.05.
**Correlation is significant at 0.01.

Results and discussion

Table 1 shows the variability of Rs values obtained. Perhaps the increase of kHz induces trouble on signal transmission inside the cellular membrane, which in this case has generated variability of Rs measurements. The table shows also that the value of Rs decreases when the frequency increases, while the values of reactance increase. This is caused, probably, by the fact that electrical impedance is a measure of the resistance of an electrical current through muscle tissue, which is related to the amount of free water in the muscle. This free water, at higher frequency, caused the current dispersion.

Subcutaneous fat content measurement in the buffalo ranged from 14% to 26% with a mean ± s.d. of 17.27 ± 2.86%; intramuscular fat ranged from 2.45% to 6.60% with a mean ± s.d. of 5.11 ± 1.21%.

Table 2 shows that Rs and Xc, at any frequency, were not significantly correlated with LW. But resistance measurements decreased as LW increased. This trend is justified by the fact that subcutaneous fat interferes with the transmission of electrical signals. Rs and Xc, at any frequency, were negatively correlated with Lvol. This is justified by the fact that impedance is a geometric system and depends on the physical characteristics of the conductor, than on physical animal characteristics, and on frequency signal.

From the examination of regression, it is obvious that it is possible, using LW, Xc_{500} and Xc_{1000} as independent variables, to obtain a good prediction model for FFM, even if such a forecast is possible with one or two single parameters (Table 3). Table 3 shows also that the low residual values define the goodness of predicting FFM models in live animal.

Table 4 shows that the best predicting model was the last one, because when using LW, Xc_{500} and Xc_{1000} as independent variables, we obtained the best R^2 Adj, Durbin Watson statistic, AIC and significant level of independent variable.

Table 3 *Characteristic of model to predicted fat-free mass*

Model	Sum of squares	Mean square	*F*	Significance	Durbin Watson statistics	AIC
1						
Regression	45239.414	45239.414	303.220	0.000[a]		
Residual	2685.536	149.196			1.989	161.00
Total	47924.950					
2						
Regression	45913.593	22956.796	194.031	0.000[b]		
Residual	2011.357	118.315			1.941	157.43
Total	47924.950					
3						
Regression	46596.999	15532.333	187.143	0.000[c]		
Residual	1327.951	82.997			2.596	145.67
Total	47924.950					

[a] Predictors: (constant), PV.
[b] Predictors: (constant), PV, Xc_{500}.
[c] Predictors: (constant), PV, Xc_{500}, Xc_{1000}.

Table 4 *Regression models for predicting the fat-free mass*

Dependent variable	Independent variable	Model	R^2 Adj
FFM	LW	$FFM = 28.10 + 0.972LW + 12.21$	0.941
	LW, Xc_{500}	$FFM = -53.18 + 0.984LW + 0.119Xc_{500} + 10.87$	0.953
	LW, Xc_{500}, Xc_{1000}	$FFM = -30.59 + 0.993LW + 0.150Xc_{500} - 0.123Xc_{1000} + 9.11$	0.967

Table 5 *Statistics of unstandardized and standardized coefficients*

	Unstandardized coefficients		Standardized coefficients		
Model	*B*	s.e.	β	*t*	Significance
1					
LW	0.880	0.051	0.972	17.413	0.000
2					
LW	0.892	0.045	0.984	19.697	0.000
Xc_{500}	10.572	4.429	0.119	2.387	0.029
3					
LW			0.993	23.667	0.000
Xc_{500}			0.150	3.473	0.003
Xc_{1000}			−0.123	−2.870	0.011

The regression coefficients and standard errors for estimating FFM are presented in Table 5. Although LW accounted for 95% of the variation in FFM, the addition of LW, Xc_{500} and Xc_{1000} accounted for 97% of the variation in FFM. LW, Xc_{500} and Xc_{1000} were positively related to FFM. These results were similar to those reported by Swantek *et al*. (1999) to predict FFM in pigs. They are reported in Table 6 in the analysis of residuals for a better understanding of the model studied.

Figure 3 shows the height correlation between the actual and predicting FFM obtained with the 3rd statistical model.

Table 6 *Residual statistics of the best model considerate*

	Minimum	Maximum	Mean ± s.d.
Predicted value	320.8803	506.9624	427.9500 ± 49.52246
Residual	−24.92468	19.09573	0.00000 ± 8.36015
Std. predicted value	−2.162	1.595	0.000 ± 1.000
Std. residual	−2.736	2.096	0.000 ± 0.918

These results indicate that the use of bioelectrical impedance has excellent potential as a rapid, non-destructive procedure and is capable of being applied to the

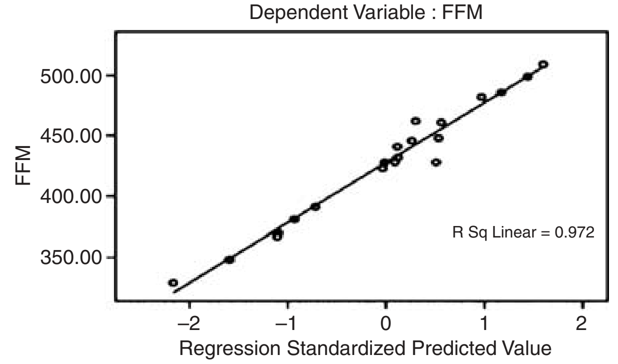

Figure 3 The relationship between fat-free mass (FFM) observed and FFM predicted for the 3rd statistical model. The height correlation between the actual and predicting FFM obtained with the 3rd statistical model is shown. These results indicate that the use of bioelectrical impedance has excellent potential as a rapid, non-destructive procedure and is capable of being applied to the live animal with minimal perturbation on subsequent performance in the buffalo species too.

live animal with minimal perturbation on subsequent performance in the buffalo species too.

Conclusions

The goodness of BIA forecasting depends on homogeneity of parameters, dimension/volume of the studied species and significant sampling. Slanger *et al.* (1994) provided, in lambs, excellent correlations ($R^2 = 0.97$) between FFM and LW, Rs and Xc. BIA may be more precise when larger volumes are measured (Thomson *et al.*, 1997). The BIA measurements are lower in cattle, higher in pigs and highest in sheep (Marchello *et al.*, 1999). Thomson *et al.* (1997) showed good BIA results from animals in wide ranges of body condition and weight. Our studies indicate that BIA can be used for evaluating FFM in buffalo calf and can be used to indicate the best moment to slaughter animals.

Acknowledgment

We are grateful to Novi Gianfranco, Vito Vitale and Agostino Navarro for technical assistance.

References

ASPA (Scientific Association of Animal Production) 1991. Metodologie relative alla macellazione degli animali di interesse zootecnico ed alla valutazione e dissezione della loro carcassa (Slaughter and carcass evaluation and dissection procedures in livestock). ISMEA, Rome, Italy.

Berg EP, Neary MK, Forrest JC, Thomas DL and Kauffman RG 1996. Assessment of lamb carcass composition from live animal measurement of bioelectrical impedance or ultrasonic tissue depths. Journal of Animal Science 74, 2672–2678.

Domermuth W, Veum TL, Alexander MA, Hedrick HB, Clark J and Eklund D 1976. Prediction of lean composition of live market weight swing by indirect methods. Journal of Animal Science 43, 966–976.

Ferrara B, Infascelli F, Vale WG, Barnabe VH and Aguilar de Mattos JC 1994. Buffalo meat production: consumption, quality, carcass, sub-products. In Proceedings of IV World Buffalo Congress, vol 1, pp. 122–136. FAO/FINEP, São Paulo.

Ferrell CL and Cornelius SG 1984. Estimation of body composition of pigs. Journal of Animal Science 58, 903–912.

Forrest JC, Kuei CH, Orcutt MW, Schinckel AP, Stouffer JR and Judge MD 1989. A review of potential new methods of on-line pork carcass evaluation. Journal of Animal Science 67, 2164–2170.

Forro M, Cieslar S, Ecker GL, Walzak A, Hahn J and Lindinger MI 2000. Total body water and ECFV measured using bioelectrical impedance analysis and indicator dilution in horses. Journal of Applied Physiology 89, 663–671.

Fredeen HT, Martin AH and Sather AP 1979. Evaluation of an electronic technique for measuring lean content of the live pig. Journal of Animal Science 48, 536–540.

Gigli S, Failla S and Iacurto M 2001. Valutazione delle carcasse, caratteristiche chimico-fisiche e nutrizionali della carne bufalina. Atti del I Convegno Nazionale sull'Allevamento del Bufalo, 3–5 Ottobre 2001, Eboli, Italy.

Jenkins TG, Leymaster KA and Turlington LM 1988. Estimation of fat-free soft tissue in lamb carcasses by use of carcass and resistive impedance measurements. Journal of Animal Science 66, 2174–2179.

Keim NL, Mayclin PL, Taylor SJ and Brown DL 1988. Total body electrical conductivity method for estimating body composition: validation by direct carcass analysis of pigs. American Journal of Clinical Nutrition 47, 180–185.

Kempster AJ, Chadwick JP and Jones DW 1985. An evaluation of the Hennessey grading probe and the SFK Fat-O-Meater for use in pig carcass classifications and grading. Animal Production 40, 323–329.

Kushner RF and Schoeller DA 1986. Estimation of total body water by bioelectrical impedance analysis. American Journal of Clinical Nutrition 44, 417–424.

Lanari D 1973. Utilizzazione dei tagli campione nella stima della composizione delle carcasse bovine. Rivista di Zootecnia Veterinaria 1, 241–256.

Lukaski HC, Johnson PE, Bolonchuk WW and Lykkenn GI 1985. Assessment of fat-free mass using bioelectrical impedance measurements of the human body. American Journal of Clinical Nutrition 41, 810–817.

Marchello MJ and Slanger WD 1992. Use of bioelectrical impedance to predict leanness of Boston butts. Journal of Animal Science 70, 3443–3450.

Marchello MJ, McLennan JE, Dhuyvetter DV and Slanger WD 1999. Determination of saleable product in finished cattle and beef carcasses utilizing bioelectrical impedance technology. Journal of Animal Science 77, 2965–2970.

Miyatani M, Kanehisa H, Masuo Y, Ito M and Fukunaga T 2001. Validity of estimating limb muscle volume by bioelectrical impedance. Journal of Applied Physiology 91, 386–394.

Pietrobelli A, Morini P, Battistini N, Chiumello G, Nunez C and Heymsfield SB 1998. Appendicular skeletal muscle mass: prediction from multiple frequency segmental bioimpedance analysis. European Journal of Clinical Nutrition 52, 507–511.

Salinari S, Bertuzzi A, Mingrone G, Capristo E, Scarfone A, Greco AV and Heymsfield SB 2003. Bio-impedance analysis: a useful technique for assessing appendicular lean soft tissue mass and distribution. Journal of Applied Physiology 94, 1552–1556.

Sather AP, Jones M and Robertson WM 1989. The effect of genotype on prediction lean yield in heavy pig carcasses using the Hennessey grading probe, the destrom PG-100 and Fat-O-Meater electronic grading probes. Canadian Journal of Animal Science 69, 93.

Schoeller DA 2000. Bioelectrical impedance analysis: what does it measure? Annals of the New York Academy of Sciences 904, 159–162.

Slanger WD, Marchello MJ, Busboom JR, Meyer HH, Mitchell LA, Hendrix WF, Mills RR and Warnock WD 1994. Predicting total weight of retail-ready lamb cuts from bioelectrical impedance measurements taken at the processing plant. Journal of Animal Science 72, 1467–1474.

Statistical Packages for the Social Science (SPSS) 2003. 12.0 Package software.

Stahn A, Terblanche E and Strobel G 2007. Modelling upper and lower limb muscle volume by bioelectrical impedance analysis. Journal of Applied Physiology 103, 1428–1435.

Swantek PM, Crenshaw JD and Berg EP 1991. Validation of bioelectrical impedance to measure fat-free mass of swine. Journal of Animal Science 69(suppl. 1), 318.

Swantek PM, Crenshaw JD, Marchello MJ and Lukaski HC 1992. Bioelectrical impedance: a nondestructive method to determine fat-free mass of live market swine and pork carcasses. Journal of Animal Science 70, 169–177.

Swantek PM, Marchello MJ, Tilton JE and Crenshaw JD 1999. Prediction of fat-free mass of pigs from 50 to 130 kilograms live weight. Journal of Animal Science 77, 893–897.

Tagliabue A, Andreoli A, Comelli M, Bertoli S, Testolin G, Oriani G and De Lorenzo A 2001. Prediction of lean body mass from multifrequency segmental impedance: influence of adiposity. Acta Diabetology 38, 93–97.

Thomson BC, Thomas BJ, Ward CL and Sillence MN 1997. Evaluation of multifrequency bioelectrical impedance data for predicting lean tissue mass in beef cattle. Australian Journal of Experimental Agriculture 37, 743–749.

Velazco J, Morrill JL and Grunewald KK 1999. Utilization of bioelectrical impedance to predict carcass composition of Holstein steers at 3, 6, 9 and 12 months of age. Journal of Animal Science 77, 131–136.