# Body composition estimated by bioelectrical impedance analyses is diminished by prenatal stress in neonatal lambs and by heat stress in feedlot wethers

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# **INTRODUCTION**

Body composition correlates to carcass value in livestock, which makes the ability to accurately estimate body composition in the live animal beneficial (Berg and Marchello, 1994). Bioelectrical impedance analysis (BIA) is a clinical tool used to assess body composition in humans (Lukaski et al., 1985), but its use in livestock has been minimal. Lean and fat content contribute to profitability for livestock producers, and poor body composition can be caused by stress that occurs either during in utero development (De Blasio et al., 2007) or during postnatal growth (Boyd et al., 2015). Maternal hyperthermia-induced placental insufficiency (Brown et al., 2015) and sustained maternal inflammation (Cadaret et al., 2018) are two established causes of intrauterine growth restriction (IUGR). IUGR-born animals are characterized by asymmetrical growth restriction that alters lifelong body composition due to impaired muscle growth capacity (Yates et al., 2018). In addition, acute heat stress during periods of peak postnatal growth can alter body composition in livestock (Boyd et al., 2015). We postulate that BIA can detect these changes in the live animal. Thus, the objective of this study was to determine

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whether BIA measurements can predict changes to body composition in live neonatal lambs exposed to intrauterine stress and in heat-stressed feedlot lambs.

## MATERIALS AND METHODS

#### Animals and Experimental Design

These studies were approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln, which is accredited by AAALAC International.

**Experiment 1.** Maternal inflammation-induced IUGR (MI-IUGR) lambs were produced from Polypay ewes administered 0.1 µg/kg bacterial lipopolysaccharide (iv; Sigma-Aldrich; n = 8) every third d from 100 to 115 d of gestation (dGA) as described by Cadaret et al. (2018). Placental insufficiency-induced IUGR (PI-IUGR; n = 7) lambs were produced from ewes exposed to 40 °C and 35% relative humidity from dGA 40 to 90 as described by Brown et al. (2015). A second PI-IUGR group was supplemented with maternal O<sub>2</sub> (100%, 10 L/min) through an endotracheal catheter for 8 h/d from dGA 131 until parturition (PI-IUGR + O<sub>2</sub>; n = 9). Controls were maintained at thermoneutral conditions and injected with saline (n = 16).

**Experiment 2.** Rambouillet crossbred wethers  $(43.1 \pm 0.6 \text{ kg})$  were stratified by body weight (BW) and randomly assigned to be fed high-concentrate diets under thermoneutral (pair-fed controls, n = 14) or heat stress (40 °C; n = 12) conditions for

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30 d. Ractopamine HCl was supplemented in a  $2 \times 2$  factorial but had no effect on BIA results and was removed from the model.

## **Bioelectrical Impedance Analysis**

BIA was performed at d 3 and 25 for neonatal lambs and at d 0 and 14 (live animal) and on the hot carcass at necropsy (d 30) for feedlot wethers. We used a four-terminal Quantum V (RJL Systems, Detroit, MI) to measure reactance (Xc), resistance (Rs), and phase angle (PA). BIA used two sets of spaced electrode terminals to transmit an electrical current across the tissues. Electrodes were connected to aluminum 20G needles (Covidien) placed subcutaneous in live animals and intramuscular in hot carcasses. The two sets of electrode terminals (one red, one black) were placed 2.5 cm apart. In live animals, one set was placed ~25 cm from the point of the scapula. The second set of was placed  $\sim 5$  cm from the tail head. Electrodes were placed in the same approximate location in carcasses. Needles were placed

dorsally ~1 cm off the midline. Measurements were recorded in 5-s intervals for 30 s, resulting in six total measurements that were averaged and used in estimation equations for live animals and hot carcasses in Table 1 (Berg and Marchello, 1994; Slanger et al., 1994; Moro et al., 2019).

## Statistical Analysis

Data were analyzed for fat-free mass (FFM), fat-free soft tissue (FFST), nutrient components, and mass of collective muscle groups by analysis of variance using the mixed procedure of SAS with repeated measures. Data were analyzed for mass proper and as a fraction of BW. Animal was considered the experimental unit for both experiments. Data are presented as means  $\pm$  standard error.

## RESULTS

*Experiment 1.* Composition estimates for neonatal lambs are presented in Table 2. At 3 and 25 d, BW of control and PI-IUGR + O, lambs were

**Table 1.** Equations for carcass traits estimated from Bioelectrical impedance analysis (BIA) in heat-stressed wethers and intrauterine growth-restricted (IUGR) neonatal lambs

Estimate	Equation	Reference
	Live animal BIA	
FFM1	= (0.585*BW) - (0.28*Rs) + (0.578*Xc) + 16.35	Berg and Marchello, 1994
FFM2	= (0.578*BW) - (0.293*Rs) + (0.039*L) + 18.771	Berg and Marchello, 1994
FFM3	= (0.596*BW) - (0.286*Rs) + 19.711	Berg and Marchello, 1994
FFM4	= (0.643*BW) + (0.624*Xc) - 2.701	Berg and Marchello, 1994
FFST1	= (0.555*BW) - (0.247*Rs) + (0.390*Xc) + 16.260	Berg and Marchello, 1994
FFST2	= (0.542*BW) - (0.259*Rs) + (0.044*L) + 17.470	Berg and Marchello, 1994
FFST3	= (0.562*BW) - (0.251*Rs) + 18.529	Berg and Marchello, 1994
SUM	= 1.7 + 0.338*BW - 0.0531*Rs + 0.0494*L	Slanger et al. 1994
LSLRS	= 1.7 + 0.237*BW - 0.0396*Rs + 0.0308*L	Slanger et al. 1994
LSL	= 0.95 + 0.147*BW - 0.0329*Rs + 0.0222*L	Slanger et al. 1994
Moisture	= 0.66 + 0.94 * XcD + 0.09 * V	Moro et al. 2019
Protein	= -0.70 + 0.05*RsD $+ 0.03$ *V $+ 0.07$ *PA	Moro et al. 2019
Fat	= -2.11 + 0.10*RsD $+ 0.04$ *V	Moro et al. 2019
Lean	= -1.90 + 0.11*V + 0.18*RsD + 0.31*PA	Moro et al. 2019
	Hot carcass BIA	
FFM1	= (0.454*BW) - (0.134*Rs) + (0.217*Xc) - (0.244*T) + 26.609	Berg and Marchello, 1994
FFM2	= (0.396*BW) - (0.106*Rs) + 20.216	Berg and Marchello, 1994
FFM3	= (0.437*BW) - (0.130*Rs) + (0.229*Xc) + 16.950	Berg and Marchello, 1994
FFST1	= (0.433*BW) + (0.124*L) - (0.114*Rs) + (0.175*Xc) - (0.211*T) + 17.811	Berg and Marchello, 1994
FFST2	= (0.393*BW) - (0.089*Rs) + 17.773	Berg and Marchello, 1994
FFST3	= (0.419*BW) - (0.111*Rs) + (0.188*Xc) + (0.111*L) + 10.051	Berg and Marchello, 1994
SUM	= -4.5 + 0.598*BW - 0.0297*Rs + 0.096*Xc + 0.114*L + 0.103*T	Slanger et al. 1994
LSLRS	$= -3.6 + 0.440^{*}BW - 0.0214^{*}Rs + 0.0880^{*}Xc + 0.0527^{*}L + 0.0939^{*}T$	Slanger et al. 1994
LSL	$= -2.4 + 0.256^{*}BW - 0.0142^{*}Rs + 0.0521^{*}Xc + 0.0327^{*}L + 0.0748^{*}T$	Slanger et al. 1994

SUM = sum of leg, sirloin, rack, shoulder, neck, riblets, shank, and lean trim (kg); LSLRS = sum of leg, sirloin, loin, rack, and shoulder; LSL = sum of leg, sirloin, and loin (kg); Rs = resistance ( $\Omega$ ); Xc = reactance ( $\Omega$ ); L = length between electrodes (cm); XcD = resistive density (kg<sup>2</sup>/ (cm<sup>2</sup>  $\Omega$ )); V = biometrical volume (cm<sup>2</sup>/ $\Omega$ ); RsD = reactive density (kg<sup>2</sup>/(cm<sup>2</sup>  $\Omega$ )); PA = phase angle (°); T = temperature (°C).

Variable	Control	PI-IUGR	PI-IUGR + O <sub>2</sub>	MI-IUGR	P value
3 d of age					
BW, kg	$5.0 \pm 0.2^{a}$	$2.6\pm0.2^{\mathrm{b}}$	$4.6 \pm 0.1^{a}$	$3.9 \pm 0.4^{\circ}$	0.02
BW/BL, kg/cm	$0.018 \pm 0.001^{a}$	$0.011 \pm 0.001^{\rm b}$	$0.017 \pm 0.001^{a}$	$0.017 \pm 0.002^{a}$	0.009
FFM, kg	$6.7 \pm 0.3$	$7.1 \pm 3.2$	$8.1 \pm 1.7$	$6.9 \pm 2.1$	NS
FFM/BW, kg/kg	$1.4 \pm 0.3$	$1.8 \pm 0.3$	$1.7 \pm 0.3$	$1.2 \pm 0.3$	NS
Moisture, kg	$6.1 \pm 0.3^{a}$	$3.2 \pm 0.9^{b}$	$6.3 \pm 0.5^{a}$	$4.7 \pm 0.5^{\circ}$	0.008
Protein, kg	$1.52 \pm 0.09$	$1.48 \pm 0.65$	$1.87 \pm 0.41$	$1.53 \pm 0.45$	NS
Fat, kg	—	—	_	_	_
Moisture/BW, kg/kg	$1.301 \pm 0.091$	$1.362 \pm 0.503$	$1.343 \pm 0.105$	$1.226 \pm 0.068$	NS
Protein/BW, kg/kg	$0.321 \pm 0.021$	$0.583 \pm 0.206$	$0.398 \pm 0.083$	$0.491 \pm 0.238$	NS
Fat/BW, kg/kg	—	—	_	_	_
25 d of age					
BW, kg	$11.8 \pm 0.5^{a}$	$9.1\pm0.8^{\mathrm{b}}$	$11.4 \pm 0.4^{a}$	$9.2 \pm 0.6^{b}$	0.002
BW/BL, kg/cm	$0.024 \pm 0.001^{a}$	$0.024 \pm 0.002^{ab}$	$0.023\pm0.002^{ab}$	$0.021 \pm 0.001^{\rm b}$	0.02
FFM, kg	$8.7 \pm 0.6^{a}$	$6.4 \pm 0.7^{\rm b}$	$8.6 \pm 0.8^{\mathrm{ac}}$	$7.4 \pm 0.4^{\mathrm{bc}}$	0.10
FFM/BW, kg/kg	$0.82 \pm 0.04^{a}$	$0.70 \pm 0.04^{\rm b}$	$0.82 \pm 0.04^{a}$	$0.81 \pm 0.04^{a}$	0.03
Moisture, kg	$7.4 \pm 0.3^{a}$	$5.9\pm0.5^{\mathrm{b}}$	$7.9\pm0.7^{\mathrm{a}}$	$6.7 \pm 0.3^{b}$	0.04
Protein, kg	$2.04 \pm 0.16^{a}$	$1.48 \pm 0.19^{b}$	$2.09 \pm 0.23^{a}$	$1.76 \pm 0.13^{b}$	0.10
Fat, kg	$0.97 \pm 0.1^{a}$	$0.31 \pm 0.2^{\rm b}$	$1.23 \pm 0.3^{a}$	$0.65 \pm 0.2^{b}$	0.03
Moisture/BW, kg/kg	$0.653 \pm 0.042$	$0.662 \pm 0.045$	$0.702 \pm 0.059$	$0.739 \pm 0.038$	NS
Protein/BW, kg/kg	$0.187\pm0.026$	$0.164 \pm 0.013$	$0.185\pm0.019$	$0.193 \pm 0.011$	NS
Fat/BW, kg/kg	$0.086 \pm 0.012^{a}$	$0.031 \pm 0.021^{\text{b}}$	$0.108 \pm 0.022^{a}$	$0.069 \pm 0.016^{a}$	0.08

**Table 2.** Carcass characteristics estimated from Bioelectrical impedance analysis (BIA) in intrauterine growth-restricted (IUGR) lambs at 3 and 25 d of age

<sup>a,b</sup>means with different superscripts differ (P < 0.05)

Values are expressed as means  $\pm$  SE. FFM = fat-free mass; BW = body weight; BL = body length; PI-IUGR = placental insufficiency-intrauterine growth-restricted; MI-IUGR = maternal inflammation-intrauterine growth-restricted; NS = not significant.

greater (P < 0.05) than MI-IUGR and PI-IUGR lambs. MI-IUGR lambs had greater (P < 0.05) BW than PI-IUGR lambs at 3 but not 25 d. BW/ body length (BL) at 3 d did not differ among controls, PI-IUGR + O<sub>2</sub>, and MI-IUGR, but all were greater (P < 0.05) than PI-IUGR lambs. BW/BL at 25 d was lower (P < 0.05) in MI-IUGR lambs than controls, PI-IUGR, and PI-IUGR + O, lambs. Moisture content at 3 and 25 d was greater (P < 0.05) for control and PI-IUGR + O<sub>2</sub> lambs than PI-IUGR and MI-IUGR lambs. MI-IUGR lambs had greater (P < 0.05) moisture content than PI-IUGR lambs at 3 but not 25 d. Estimated protein content did not differ at 3 d but protein and fat content were greater (P < 0.05) in controls and PI-IUGR +  $O_2$  lambs than PI-IUGR and MI-IUGR at 25 d. Moisture/BW and protein/BW did not differ among groups at either d. Fat content and fat/BW could not be estimated at 3 d. Fat content/BW at 25 d was similar among controls, PI-IUGR + O<sub>2</sub>, and MI-IUGR, all of which were greater (P < 0.05) than PI-IUGR lambs. FFM/ BW was not different among groups at 3 d but was greater (P < 0.05) in controls, PI-IUGR + O<sub>2</sub>, and MI-IUGR lambs than PI-IUGR lambs at

25 d. It should be noted that predicted values for d-3 carcass and nutrient composition were highly variable, and some values were estimated to be more than 100% of BW. Conversely, values at d 25 were reasonable and realistic.

Experiment 2. Body composition and nutrient composition estimates for heat-stressed wethers at d 30 (hot carcass) are presented in Table 3. There were no differences in any estimated variables between groups at d 0 or 14 (data not shown). Average daily gain between d 0 and 14 and between d 0 and 30 tended to be less (P < 0.10) in heat-stressed weathers than controls. No equations for FFM or FFST detected differences between groups at d 0 or 14. At necropsy, reduced (P < 0.05) FFM and FFST in heat-stressed wethers were predicted by only one equation each, but were predicted by the mean of all respective equations. Moreover, reduced (P < 0.05) FFM/BW and FFST/BW in heat-stressed wethers were detected by all equations. Estimated sum of the leg, sirloin, rack, shoulder, neck, riblets, shank, and lean trim mass (SUM), the sum of leg, sirloin, loin, rack, and shoulder mass (LSRLS), and the sum of leg, sirloin, and loin mass (LSL) were not different between

Table 3. Carcass characteristics estimated fromBioelectrical impedance analysis (BIA) in heat-stressed wethers at necropsy

Variable	Control	Heat stress	P value
BW, kg	$48.1 \pm 0.8$	$48.3 \pm 1.1$	NS
ADG, kg	$0.11 \pm 0.01$	$0.08\pm0.01$	0.08
FFM1, kg	$25.9\pm0.5$	$24.9\pm0.8$	NS
FFM2, kg	$20.7\pm0.5$	$19.4 \pm 0.8$	NS
FFM3, kg	$21.9\pm0.5$	$20.9\pm0.7$	NS
FFM4, kg	$23.1\pm0.5$	$20.1\pm0.9$	0.01
FFM, kg	$22.9\pm0.4$	$21.4\pm0.8$	0.1
FFM1/BW	$1.063 \pm 0.009$	$1.026\pm0.017$	0.03
FFM2/BW	$0.850\pm0.010$	$0.798 \pm 0.015$	0.004
FFM3/BW	$0.900\pm0.009$	$0.861\pm0.013$	0.02
FFM4/BW	$0.948 \pm 0.009$	$0.829 \pm 0.030$	< 0.001
FFM/BW	$0.940\pm0.008$	$0.878 \pm 0.018$	0.003
FFST1, kg	$23.1 \pm 0.4$	$22.1\pm0.7$	NS
FFST2, kg	$21.7\pm0.3$	$19.2 \pm 1.1$	0.05
FFST3, kg	$19.8 \pm 0.4$	$18.7 \pm 0.7$	NS
FFST, kg	$21.5\pm0.4$	$20.1\pm0.7$	0.08
FFST1/BW	$0.949 \pm 0.008$	$0.912\pm0.015$	0.04
FFST2/BW	$0.900\pm0.012$	$0.789 \pm 0.045$	0.04
FFST3/BW	$0.811 \pm 0.008$	$0.774\pm0.012$	0.01
FFST/BW	$0.883 \pm 0.008$	$0.825\pm0.017$	0.004
SUM	$16.3 \pm 0.2$	$16.2 \pm 0.4$	NS
LSLRS	$11.2 \pm 0.1$	$11.2 \pm 0.3$	NS
LSL	$6.6 \pm 0.1$	$6.6 \pm 0.2$	NS
SUM/BW	$0.667\pm0.002$	$0.668 \pm 0.002$	NS
LSLRS/BW	$0.454\pm0.001$	$0.460\pm0.003$	NS
LSL/BW	$0.270\pm0.001$	$0.272\pm0.001$	NS

Values are expressed as means  $\pm$  SE. Bolded variables are the average values for the prediction equations. SUM = sum of leg, sirloin, rack, shoulder, neck, riblets, shank, and lean trim (kg); LSLRS = sum of leg, sirloin, loin, rack, and shoulder; LSL = sum of leg, sirloin, and loin (kg); NS = not significant; ADG = Average daily gain.

groups. Estimated nutrient composition (moisture, protein, fat, lean mass) is presented in Table 4 and did not differ between groups at either d.

#### DISCUSSION

Our findings show that the impact of prenatal stress on body composition in offspring was detectable at 25 d of age but not at 3 d. Reduced lean tissue mass is a hallmark of IUGR and is often coupled with increased fat deposition during early postnatal growth (Yates et al., 2018). The reduced BW and estimated fat mass in IUGR lambs at 25 d of age indicates that they had not undergone postnatal catch-up growth. It is unclear why body composition estimates at d 3 were inaccurate, but we speculate that it is due to the small size and low proportion of soft tissue relative to older lambs. In addition, it appears from these findings that body composition estimates are more accurately represented as fractions of BW due to size variability. We

**Table 4.** Nutrient composition estimated fromBioelectrical impedance analysis (BIA) in heat-stressed wethers at d 0 and 14.

Variables	Control	Heat stress	P value	
d 0				
Moisture, kg	$7.4 \pm 0.3$	$7.9 \pm 0.5$	NS	
Protein, kg	$3.1 \pm 0.1$	$3.0 \pm 0.1$	NS	
Fat, kg	$3.9 \pm 0.2$	$3.7 \pm 0.2$	NS	
Lean, kg	$16.0 \pm 0.4$	$11.9 \pm 0.4$	NS	
Moisture/BW	$0.159\pm0.005$	$0.177 \pm 0.1$	NS	
Protein/BW	$0.067\pm0.001$	$0.068 \pm 0.001$	NS	
Fat/BW	$0.086\pm0.002$	$0.083 \pm 0.001$	NS	
Lean/BW	$0.260\pm0.004$	$0.267\pm0.005$	NS	
d 14				
Moisture, kg	$7.2 \pm 0.5$	$7.4 \pm 0.7$	NS	
Protein, kg	$3.3 \pm 0.1$	$3.3 \pm 0.1$	NS	
Fat, kg	$4.4 \pm 0.2$	$4.1 \pm 0.2$	NS	
Lean, kg	$12.8 \pm 0.4$	$12.8 \pm 0.4$	NS	
Moisture/BW	$0.151 \pm 0.009$	$0.157\pm0.014$	NS	
Protein/BW	$0.069\pm0.001$	$0.069\pm0.001$	NS	
Fat/BW	$0.091\pm0.002$	$0.088\pm0.001$	NS	
Lean/BW	$0.267 \pm 0.005$	$0.272\pm0.006$	NS	

Values are expressed as means  $\pm$  SE. NS = not significant.

also found that BIA estimates accurately detected the impact of heat stress on body composition in feedlot wethers. Heat stress reduced weight gain, as expected (Morrison, 1983), and our BIA-estimated lean mass values indicate that this is due to reduced lean muscle growth, although equations estimating specific muscle weights did not detect differences. It is worth noting that the moderate nature of heat stress effects in this study was likely due to pair feeding of controls, and thus differences represent direct effects of the stress itself. Nevertheless, we conclude that BIA estimates for FFM and FFST reasonably reflected stress-induced changes in body composition.

# **IMPLICATIONS**

BIA appears to be an accurate method for estimating carcass characteristics in live animals. Its simplicity and consistency make it useable in livestock to improve selection efficiency and maximize profit. Estimating body composition in live animals will increase product uniformity and minimize merit- and yield-based discounts at harvest. Further studies will better optimize estimation equations for nutrient composition and muscle weights to increase precision and accuracy.

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