



Bioelectrical impedance analysis (BIA) in animal production: Review



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Abstract:

Bioelectrical impedance analysis (BIA) is a method based on the different levels of opposition to the flow of an ionic current through the different body tissues. Results are expressed by primary measures of resistance (Rs) and reactance (Xs). From such measures, equations are applied to determine the phase angle (PA) and impedance (Z). Bioimpedance analysis has been indicated as a reliable and precise method to determine the body composition and nutritional status in humans. BIA has recently been adapted to be applied on animal production. Therefore, the aim of this review is to provide an analysis on the potential use of bioelectrical impedance on zootechnical production. Through BIA, correlations among bioelectrical measures and tissue composition of swine, bovine, ovine, bubaline and fish carcasses can be established. In this regard, a growing number of demands were led by more precise and cost-effective methods to evaluate the body composition in the zootechnical sector, in which the analysis of

bioelectrical impedance proved to be a promising and minimally invasive technology to replace traditional methods.

Key words: Body composition, Impedance, Technology, Zootechnical production.

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Introduction

The pursuit of excellence in quality within the zootechnical chain in Brazil and in the world has been constant. To maintain the status quo, the emphasis should be placed on the use of technologies to guarantee the biosecurity of food on animal production adaptable to consumer requirements.

Different methods to evaluate the quality and composition of the most diverse meat and milk products are available in the literature. However, many of these methods, such as carcass dissection and physical and chemical composition require skilled and qualified labor, state-of-the-art equipment and considerable financial funds.

Hence, research and exploration of technologies and methodologies are fundamental to help evaluate efficiently the quality of zootechnical products. In view of that, bioelectrical impedance (BIA) is characterized for being a technology of quick measurement, minimally invasive, relatively cost-effective and less subjective. BIA shows great potential to predict tissue chemical composition of farm animal carcasses⁽¹⁾ as well as to evaluate the quality of raw cow milk⁽²⁾.

BIA analysis is based on the principle that body tissues have different impedances, it means an opposition to the flow of an ionic current. Such impedance is determined by primary measures of resistance (R_s) and capacitive reactance (X_c). The flux of the electric current opposes to the vector R_s , when it goes through intra and extra cellular environments⁽³⁾. Similarly, X_c also shows opposition to the current flux caused by the capacitance produced by the cell membranes⁽⁴⁾.

Hence, the search for alternative methods becomes necessary to determine and uphold the quality of zootechnical products if we are to consider the current juncture. In this regard, this article review aims to relate and unveil the principle of BIA and its use in animal production.

Electric bioimpedance

Body tissues have different levels of conductivity and opposition to the flow of an ionic current due to their chemical-physical composition⁽⁴⁾. From this principle, the analysis of electrical bioimpedance is carried out as a method of evaluating body composition, nutritional condition, total amount of body water, physiological properties, and an indicator of several clinical and pathological situations, such as prognostic of cancer, malnutrition and cardiac insufficiency⁽⁵⁾.

This method is relatively cost-effective, minimally invasive and of easy handling and applicability. It also offers quick measurement since it takes approximately 5 sec to gather the data⁽⁶⁾. Besides, differently from traditional methods, BIA does not require the sacrifice of animals to evaluate tissue composition and energetic level unless dissection is performed when applicable⁽⁷⁾. The principles of BIA have been applied and adapted for animal production to determine the tissue composition of swine⁽⁸⁾, bovines⁽⁹⁾, ovine^(10,11), bubalus⁽¹²⁾ carcasses as well as to evaluate the composition of raw cow milk⁽²⁾.

To use BIA, an ionic current should pass along the animal body. The current is usually alternate of low amplitude (500 to 800 μ A) and high frequency, which cannot be felt by the body. It usually takes four electrodes (a tetrapolar system) to apply the current: one pair of electrodes produces the current excitation while the other pair measures the difference in the potential reached⁽¹³⁾. In biological systems, humans or animals, the displacement of the current occurs through the dissolved ions in the body fluids, especially ions of sodium and potassium.

The literature shows that when the current is applied to the bioimpedometer, the different animal tissues present different opposition to the flow of the ionic current, which is called impedance (Z). The value of the impedance is frequency dependent⁽⁴⁾ and is the resultant composition of two vectors named resistance (R_s) and reactance (X_c).

When the current is applied, it disperses through the ions in the body fluids and is inversely proportional to its motility and directly proportional to the area that it traverses. From the primary values of R_s and X_c obtained by the bioimpedometer, the mathematical model proposed by Lukaski *et al*⁽¹⁴⁾ is used, showing dependence on the length of the conductor, on the frequency used and on the area of transversal section, in other words, the body volume: $Z = (R_s^2 + X_c^2)^{0.5}$.

The magnitude of the impedance is calculated by the square root of the sum of the squares of resistance and reactance combined to the circuit⁽¹⁴⁾; all measures are expressed in ohms (Ω).

The resistance occurs because of the opposition to the electric current when flowing across intra and extracellular environments⁽³⁾. The opposite can be obtained by the

conductance (C). According to Eickemberg *et al*⁽⁴⁾, this is inversely related to resistance ($C=1/R_s$), that is, the larger the amount of liquid, the weaker the current flow opposition. Therefore, the resistance shows opposite behavior to the amount of water in the body and the level of hydration in these environments.

Intracellular fluids are composed by nucleus and cytoplasm mostly formed by salts, proteins and water, considered good electrical conductors. Likewise, conductive materials, such as K and PO-4 ions⁽¹⁵⁾, also form the extracellular fluids.

The same applies to lean tissues formed by muscles and viscera, which are good conductors of ionic current due to their large amount of water and electrolytes. Adipose and bone tissues, on the other hand, conduct ionic current with less intensity, when compared to the muscles and viscera, being highly resistant⁽¹⁶⁾.

If the tissues were homogeneous, the opposition would be only resistive. However, the tissues are heterogeneous and show another type of opposition, the reactance, which originates from tissues that show composition and characteristics of electric capacitors (condensers) in their structure produced by the cell membranes. Cell capacitance is known as an energy storage property (to concentrate electrons) in tissue interfaces and cell membranes for a short period, having the characteristic of delaying the electric current⁽¹⁷⁾.

Cytoplasmic membranes involve two layers of protein material with hydrophilic properties, proving to be good electric conductors, and a lipid-insulating layer (the dielectric layer). Those membranes act as if they were a capacitor (X_c)⁽⁴⁾. This assessment is an indicator of body lean mass quantity, which can be related to the structure and function of cell membranes since each tissue component responds differently to the flow of an alternate applied current^(18,19).

The BIA method considers that a singular cylindrical conductor with homogenous length and transversal area represents the animal body. Nevertheless, such statement is objectionable once the composition and transversal section areas are considered heterogeneous⁽²⁰⁾.

Hence, it is necessary to relate BIA with proven methods in the literature to determine chemical and physical compositions so that it is possible to validate the method and its respective prediction equations, identify and narrow down errors and make technology more reliable and precise⁽²¹⁾.

Since BIA analysis can also provide information on body composition, factors such as body and environment temperature, and length of body segment must be taken into consideration. According to Hartmann *et al*⁽²²⁾ temperature and length affect significantly the measurements of resistance and reactance, which is explained by the temperature increase that boosts the vibration of the atoms.

Considering the nature of the biological fluids, the temperature exhibits a negative coefficient for resistance, that is, when the temperature decreases the resistance increases. Consequently, the conductance decreases proportionally⁽⁶⁾. Other variables can also interfere on the serum concentration of electrolytes, electrode placement spots, types of electrodes used, training of users on the bioimpedometer application, use of non-conductive materials, calibration of the device as well as breed, age and weight^(3,10). In this regard, impedance values are associated to the total amount of water and the composition of body tissues in animals⁽⁶⁾. Besides, the impedance can also be used as an indirect method to assess the bacteriological quality in raw milk.

Phase Angle

Whenever an electric current is applied to biological tissues, a detour is formed as soon as the current traverses the cell membranes. Part of the membranes has the ability to store energy, which means that there should be an observable delay in the electric current flow due to capacitance, generating a decrease in the current tension⁽²³⁾. This characteristic originates a phase change since there is no synchrony in the capacitor between the variations of current and tension, generating a geometric transformation of the angle of the capacitance under the designation of phase angle (PA)⁽⁴⁾.

According to Cintra *et al*⁽³⁾, the phase angle determined by BIA is a method to measure the relation existing between the resistance and the reactance in series or parallel circuits. Eickemberg *et al*⁽⁴⁾ explained that there might be variation from 0° to 90°, in which 0° involves a resistive circuit without any cell membrane or degradation, and 90° corresponds to a capacitive circuit in which all cellular membranes have extra cellular fluid.

PA depends on the capacitance and is negatively associated to resistance⁽²⁴⁾. It is related to quality, size and cellular integrity, given that its variation indicates alterations in the body composition, in the functioning of the membrane or in human health conditions⁽⁴⁾. Therefore, PA can be quantified geometrically by the formula $PA = (Xc/R)$, calculated by the relation between the arc tangent of Xc and Rs, where the result obtained is expressed in radians, being multiplied by $(180^\circ/\pi \cong 57,296)$ to convert in degrees⁽²⁵⁾. The equation, according to Baumgartner *et al*⁽²⁴⁾, is the following: $AF = [\tan^{-1}(Xc/R) \times 180^\circ/\pi]$.

Eickemberg *et al*⁽⁴⁾ set as a value of normality for healthy individuals a variation between 4 and 15 degrees. According to Selberg and Selberg⁽²⁶⁾, when the values of PA are higher, the reactance values show the same tendency. These, in turn, are related to good health conditions and intact cell membranes.

Contrarily, when PA rates are low, there is a relation with reactance, associating them to cell death, reduction of cell membrane selective permeability and even to the existence or worsening of some disease⁽²⁷⁾. When the assessment was performed in fish, Cox and

Heintz⁽²⁸⁾ described that the angle exceeding 15° indicated that the animals were in good health conditions whereas the angle inferior to 15° indicated that health conditions were compromised.

In this context, PA has been widely used as an indicator of the nutritional condition in human beings as well as in animal carcasses, of quality assessment and raw cow milk adulteration^(1,2,9).

BIA for body composition and carcasses evaluation of farm animals

The evaluation of the body composition is frequently used to improve management and zootechnical control on farm animal sector, enabling the responsible technician and raiser to select animals with higher performances, adapting them for the consuming market⁽¹⁶⁾. The most commonly used methods to predict the composition and the carcass of living or slaughtered animals are often time-consuming, expensive and invasive⁽¹⁰⁾. Consequently, researching about new methodologies and precise technologies is fundamental to meet such estimation. In this regard, BIA is a method minimally invasive, relatively inexpensive and one that has been used in the zootechnical sector to estimate the body composition of farm animals.

In humans⁽⁴⁾ BIA is used to assess the body mass index (BMI) since it considers the association between weight and the patient's squared height ($BMI = \text{weight}/\text{height}^2$). Lukaski *et al*⁽²⁹⁾, based on the principles of Ohm's law, related the cross-sectional area with the body volume, describing that the volume of a conductive mass can be the quotient between squared height and resistance ($V = \text{height}^2 / \text{LOL}$).

Aiming to reproduce the equations of volume used in humans for animals, Jenkins *et al*⁽³⁰⁾ replaced the height by the length of the carcass. Others⁽³¹⁾, replaced the height by the length of the conductor, represented by the distance between the electrodes that detect the current. Zollinger *et al*⁽⁹⁾ associated in their studies the body compositions of animals and the resistive (RDs) and reactive (XDc) densities and described the relation between the weight, the resistive and capacitive volume, expressed in Kg^2/cm^2 ohms.

By assessing the potential use of BIA to determine the composition of the soft portion of carcasses of hot and cold lambs, Moro *et al*⁽³²⁾ verified that BIA measurements obtained from cold carcasses anticipate its components with higher precision than measurements obtained from hot carcasses. Such fact is explained by the decrease in conductance in response to loss of fluids during the cooling, and changes in the distribution of electrolytes between intracellular and extracellular environments in tissues.

Acupuncture or hypodermic needles can be used as electrodes to perform the measures. They should be inserted in the animal under constant penetration depth, enabling the transmission without interruption of signal throughout the tissues⁽¹¹⁾. Besides, there should be extra care when animals are laid on non-conductive surfaces.

To make the relation of the variables of BIA with the body composition of animals possible, one should determine the levels of moisture, protein, mineral matter and lipids in the laboratory, among others. The position of the electrodes supposed to obtain the primary BIA measures performed in the back of the animals showed more accurate results than the ventral readings due to the differentiated tissues that each compartment shows⁽²¹⁾.

Significant changes are neither found in the variables of BIA nor in the membranes or extracellular compartments right after the slaughter, considering that the temperature shows little variation. Nevertheless, when the carcass starts to cool, biochemical changes in the cell membranes occur due to the *rigor mortis* effect as the carcass loses its ionic gradients with the raise of the temperature and time lapse of maturation⁽³³⁾.

According to Bertotti⁽³³⁾, this process is consistent with the enzymatic mechanisms since the phospholipid layers of the membranes suffer oxidation and give the membrane a porous aspect. Such mechanism described by Damez *et al*⁽³⁴⁾ causes an increase in permeability, facilitating the flow and mixture of the intra and extracellular fluids. Swentek *et al*⁽³⁵⁾ reported that the values for Rs and Xc in cold swine carcasses were approximately from 6 to 11 times higher than the ones in living swine, in addition to observing that Rs decreased proportionally to the temperature.

Altmann *et al*⁽¹⁰⁾ obtained very weak correlation between the reactance and the composition of the carcass, what can also be found in studies with lamb⁽³¹⁾. Others⁽⁸⁾, found a positive correlation among the resistance, live weight and amount of fat in swine. Consequently, there was an increase in the resistance due to the decrease of total amount of water in the body, resulting the raise of weight and fat in swine.

Gibbs *et al*⁽³⁶⁾ found changes on the fat-free mass and on the fat-free soft tissue caused by stress when they used BIA to determine body alterations in newborn lambs exposed to intrauterine heat stress. Likewise, it was observed that BIA showed potential to estimate marbling in bovines in Japan⁽³⁷⁾.

Berg and Marchello⁽³¹⁾ used the following variables in their models: weight of cold and warm carcasses, length of the carcass and temperature associated with Rs and Xc. Some

researchers⁽⁹⁾ reported that they obtained weak relationship amongst most of the components of the carcasses. Moro *et al*⁽¹⁾ added phase angle, bioelectric volume, resistive and reactive density to their equations to associate with the components of the carcass since the resistive density can be related to these components and the reactive density might indicate the association with the concentration of lean mass.

In addition, Silva *et al*⁽³⁸⁾, when using BIA analysis as a technique for the prediction of the carcass and muscle of light goats, observed that R_s was the only independent variable that determined subcutaneous fat with prescription. However, by including the carcass length combined with R_s in the model, they obtained an even higher level of determination, 0.943 ($P < 0.01$). To predict the amount of muscle, the insertion in the cold carcass weight equation combined with R_s , reported an accuracy of 0.998.

In a recent work⁽³⁹⁾, the authors showed the potential the parameters evaluated by BIA have to measure meat characteristics: the combination of R_s and X_c to predict intramuscular fat demonstrated adjustment of 79.3 % while for the physical-chemical characteristics the best adjustments were in the length of the sarcomere with 64.4 % and shearing force of 60.5 %.

The accuracy of prediction models improved with the inclusion of more information. Some models are shown in Table 1 along with their respective variables, which best adjusted to increase the capability to determine equations.

Table 1: Prediction equations from *in vivo* evaluations, of bioimpedance, in the carcasses of bovine, bubalus, lamb and swine

Authors	Dependent Variable	Equations	R ²
Bovine			
Velazco <i>et al.</i> , 1999	Fat-free Mass	$Y = 0,130 BW - 0,039 L + 0,0002 L^2 - 0,007 Z - 9,320 Vol_2 + 9,507 Vol_3 + 35,555 BMI - 34,249$	0,98
Bubalus			
Sarubbi <i>et al.</i> , 2008	Fat-free Mass	$Y = 28,10 + 0,972LW + 12,21$	0,94
Lamb <i>in vivo</i>			
Berg and Marchelho, 1994	Fat-free Mass	$Y = 0,555 LWt - 0,247 Rs + 0,390 Xc + 16,260$	0,77
	Fat-free Adipose tissue	$Y = 0,555 LWt - 0,247 RS + 0,390 XC + 16,260$	0,78
Moro <i>et al.</i> , 2019	Protein (kg)	$Y = - 0,70 + 0,05RsD + 0,03V + 0,07PA$	0,91
	Fat (kg)	$Y = - 2,11 + 0,10RsD + 0,04V$	0,87
	Lean mass (kg)	$Y = - 1,90 + 0,11V + 0,18RsD + 0,31PA$	0,89
Avril <i>et al.</i> , 2013	Fat-free Mass (kg)	$Y = 18,8 + 0,023 LW - 10,5 L^2/R$	0,85
	Fat Mass (kg)	$Y = 1,43 + 0,001 LW - 0,81 C$	0,65
Lamb Carcass			
Berg and Marchelho, 1994	Fat-free Mass	$Y = 0,439 HCWt + 0,167 L - 0,134 RS + 0,191 XC - 0,258 T + 19,914$	0,77
	Fat-free Adipose tissue	$Y = 0,433 HCWt + 0,124 L - 0,114 RS + 0,175 XC - 0,211 T + 17,811$	0,79
	Fat-free Mass	$Y = 0,583 CWt + 0,150 L - 0,027 RS + 0,013 XC - 0,287 T + 1,836$	0,77
	Fat-free Adipose tissue	$Y = 0,555 CWt + 0,096 L - 0,022 RS + 0,008 XC - 0,278 T + 3,868$	0,77
Swine			
Swantek <i>et al.</i> , 1992	Fat-free Mass	$Y = 0,486 LWt - 0,881 RS + 0,480 L + 0,880 XC + 7,950$	0,81
	Fat-free Mass (Cold)	$y = 0,267 CWt - 0,158 RS + 0,519 L + 0,103 Xc + 20,04$	0,83

Independent variables: BMI= BW^2/L^2 ; BMI= Body mass index; BW= Body Weight; C= Conductance; CWt= Cold carcass Weight; HCWt= Hot carcass Weight; L= Length; LW= Live weight; PA= Phase Angle; Rs= Resistance; RsD= Resistive density; T= Temperature; V= Bioelectric volume; Vol2= $L^2/(R^2 + Xc^2)^{0.5}$; Vol3= Geometric volume; Xc= Reactance; Z= Impedance.

It is possible to evaluate BIA in the whole extension of the carcasses. However, some restrictions present during the measure of bioimpedance brought forth the need to assess the analysis of BIA by segments, in other words, by small sections in the carcasses.

To provide precise information and meet the demands of the consumer market, Moro *et al*⁽³²⁾ reported recently that the analysis of bioimpedance proved to be a promising technology when compared to traditional methods, for it provides an easy and quick way to determine the composition of fat, protein and composition of water in commercial cuts.

However, to produce more accurate information and meet the demands of the consumer market, such as the composition of fat, protein and water composition in commercial cuts, Moro *et al*⁽³²⁾ described that bioimpedance analysis proved to be a promising technology to replace traditional methods.

BIA assessment in fish

Electric bioimpedance can be a useful tool not only for scientific studies on fish, but also for the pisciculture industry, considering that the body composition of a great quantity of living fish could be quickly evaluated⁽⁴⁰⁾. Besides, its use could result in 20 to 41 times reduction in the total cost of the assessment procedures when compared to the traditional methods of chemical analyses.

Wuenschel *et al*⁽⁴¹⁾ reported that by means of this technology, since it is not lethal, it is possible to monitor the changes in the body composition of fish as they grow and make changes in the diet, according to BIA evaluation of the responses to biological interactions. Another important finding⁽⁴²⁾ is that BIA can be useful to analyze the biotic and abiotic variations that can affect the body's constituents of animals, which in turn cannot be sacrificed, as endangered species.

According to some authors⁽⁴³⁾, the body geometry of fish favors the use of impedance as estimation of body composition, where an only measure can precisely represent the whole body ($R^2= 0.96$).

Aiming to use BIA analyses to determine the body composition of fish, Zaniboni-Filho *et al*⁽⁴⁴⁾ treated the fish with a diet composed by different rates of lipids (8.90% vs 18.68 %) to produce individuals with different body compositions. The results showed stronger correlations for the dorsal analyses of moisture and resistance in series (0.87); protein and resistance in series (0.87); ash and reactance in parallel (0.82). Weak correlations were observed among BIA data by ventral reading with the lipidic contents (0.44). These authors emphasized that BIA proved to be a proper method to determine the body composition of fish in accordance to the authors previously mentioned.

Arantes⁽⁷⁾ tested the use of Bioimpedance technique in Piava fish (*Leporinus obtusidens*) and found high level of the coefficient of determination obtained in the lateral region. The coefficients were respectively 0.92, 0.85 and 0.87 among BIA method and the parameters of body composition obtained by the chemical method, such as moisture, protein and ethereal extract. These results showed that BIA proved to be valid to estimate body composition and qualify the levels of energy of Piava fish.

The effectiveness of BIA to evaluate fish composition is beneficial for fishing management and ecological investigations. It enables the evaluation of the energetic flow among and within populations, besides assessing the answers regarding environmental changes.

Traditional measurement methods, although highly accurate, are time-consuming, expensive and lethal. On the other hand, BIA method represents a quick and non-lethal technique, which, explains 80 % of the variability in the percentage of lipids in fish, demonstrating high accuracy in estimating and monitoring the body conditions of fish that live in rivers in inland Alaska territory⁽⁴⁵⁾. In addition, the technique can be beneficial for determining bioenergetic changes related to variations in the environment.

Fitzhugh *et al*⁽⁴⁶⁾ used BIA to establish a relation with the energy available for the reproduction of sea fish. The authors observed that the phase angle remained less than 15° after the peak spawning season. Hartmann *et al*⁽⁴⁰⁾ reported that BIA showed potential to measure the reproductive status, maturity and gonad development, for under these conditions fish present alterations in their body fat percentage.

According to some authors⁽²⁸⁾, phase angles measuring more than 15° indicated that the fish were healthy, while values of less than 15° showed fish with compromised health due to changes in the aquaculture environment. Differently from the models used for other animals, the phase angle showed high productivity among fish.

Changes on the phase angle are also found when the fish face a long period of fasting. The fast forces the organism to use the nutrients stored to meet their energetic needs⁽⁴⁷⁾. The use of stored nutrients causes changes in the intra and extracellular fluids, loss of body protein, progressive cellular dehydration and decrease of phase angles. Furthermore, Zavadlav *et al*⁽⁴⁸⁾ suggested estimating the storage length and sensory properties of the squid samples with FA measurements, since they showed close correlation.

On the other hand, it was observed⁽⁴⁹⁾ weak and non-significant correlations among PA, Rs and Xc and the components of body composition, reporting that the reason for the weak correlation is that the methodology of BIA was not able to detect alterations in body composition of fish. This fact corroborates the results of other authors⁽⁵⁰⁾, in which the morphological measures estimated by BIA in fish that face threat of extinction in inhabiting deserted regions were redundant, demanding caution in the field evaluation, as it presents great sensitivity.

According to Cox *et al*⁽²¹⁾, fish can be kept in ice for up to 9 h after being slaughtered with no alteration in Rs or Xc. Delays in freezing affect first Xc, then Rs and PA. This order is due to Xc representing the integrity of the cell membrane, which after 12 hours starts to swell in the face of *rigor mortis*. Thus, the cell membrane bursts releasing intracellular liquid in extra cellular spaces.

To meet the demand of the consumer market, Fan *et al*⁽⁵¹⁾ found that it is possible to define the quality, freshness and muscle softening of rainbow trout by means of impedance and during storage on ice. The same authors described that the procedure enabled the detection of the *rigor mortis index* via impedance, condition in which a strong and negative correlation between rigor mortis and (K) potassium ($r = -0.938$, $P < 0.01$) was evidenced and a strong positive correlation was found with $r = 0.981$ ($P < 0.01$) regarding hardness. Some similarity was described by Yuan *et al*⁽⁵²⁾, who demonstrated that the bioimpedance analysis showed a good correlation with the value of K after 24 h of storage in ice, suggesting that BIA effectively reflects the change in the biochemical compounds related to the freshness of fish meat.

To narrow down errors during the research, Hartmann *et al*⁽⁴⁰⁾ recommend that the minimum sample should be of 60 fish and a minimum layer of fat of 29 % among fish to obtain correlations ≥ 0.80 . Therefore, the results might show higher reliability to estimate the body composition of fish.

However, the assessment of potential sources of errors and variations during BIA measurements is an important step in the protocol development to ensure the application of data obtained by this method. Champion *et al*⁽⁴²⁾ evaluated some challenges that the technique presents. For the technique to be effective, the authors suggest some criteria to be followed: the anatomical location of the electrode insertion; the fish size and variation among species; the environment/body temperatures; the time length between capture and death of the fish; the degradation of post-mortem biological tissue; the standardization of the catching method to reduce physiological stress.

According to Cox *et al*⁽²¹⁾, a change in the electrodes on the dorsal to the ventral side of a fish changes not only the distances between the electrodes, but also the types of tissues that are examined. Hafs and Hartmann⁽⁵³⁾ identified better correlations among BIA and tissue components when the electrodes were placed along the dorsal line.

The fact that the fish have large and thick scales and might impair the contact between the skin and the electrode, some scales might have to be extracted. In short, the use of BIA in fish has shown promising results to estimate the body composition. However, further studies are needed to develop protocols and prediction equations to enhance the accuracy and applicability of this technique to a level that allows its use on field and in the industry.

Assessment of BIA in bovine milk

Current analyses to verify the quality, composition or even the adulteration of milk are performed mostly by analytical methods in certified laboratories. Within the environment of a laboratory, however, it is hard to carry out tests and offer real-time prediction for the technicians. Therefore, it is important to develop new methodologies to monitor the composition and quality of milk to fulfill the expectations of the consumer market.

It is useful and promising to use the electrical properties of milk. Hence, Felice *et al*⁽⁵⁴⁾ suggested a method of quantification of bacteria in raw cow milk based on the variation of electric capacitance. Mabrook *et al*⁽⁵⁵⁾ observed that the electric conductance decreases as the percentage of fat in the milk raises. It was reported⁽⁵⁶⁾ that when the temperature of the milk was raised, the mobility and the number of ions in the solution also raised, causing a decrease in Z. This validates the results found by others⁽²⁾, who reported the same similar results.

The fat from the milk can be considered an obstacle for the flow of the electric current, since large globules covered by a thin and non-conductive membrane form fat. Milk with high percentage of fat showed high resistance and consequently low conductivity⁽⁵⁷⁾.

The strong correlation between the conductivity (C) and the milk somatic cell count (SCC) should also be noted. The raise in milk's C is directly proportional to the increase of the udder inflammation and SCC. This inflammation causes alteration in the tissues permeability, resulting in a raise on the flow of Na⁺ and Cl⁻ ions from the blood to the interior of the alveolar lumen of the mammary gland⁽⁵⁸⁾.

It was observed strong correlations among the variables Rs, Xs, C, Z and PA and the total solid-not-fat components in raw cow milk, also coinciding with the coefficient of determination of the prediction equations⁽²⁾. In the same study, when the temperature of milk was at 5 °C, the average of Z was 147 ohms and 1.21 % of fat. Veiga *et al*⁽⁵⁹⁾ found different average values for Z in whole milk (219.56 ohms), semi-skimmed (203.57 ohms) and skimmed (170.08 ohms) when they analyzed the electric bioimpedance of cow milk fat. Other authors⁽⁶⁰⁾ reported average values of 89 ohms for Z.

It was developed an impedance spectroscopy sensor to detect adulteration of milk based on the measures of PA⁽⁶¹⁾. In this study, the PA decreased when water from the tap was added. As the values for pH changed towards a basic solution, the PA and the conductivity also decreased. When urea and whey were added, the PA and the conductivity showed a raise. This is because the variables have the same nature. In other words, the nature of the change in PA is the same of the conductivity.

Durante *et al*⁽⁵⁶⁾ observed that the adulteration of the composition of raw cow milk and UHT milk (ultra-high temperature) differed chiefly in the variable resistance. Raw milk

showed little variation in face of adulteration by hydrogen peroxide. However, the adulteration was present in the samples of UHT milk. Such fact was possibly related to the fat rate and a lack of homogeneity of the raw milk. Sodium hydroxide samples showed tendency to decrease the resistance due to a larger presence of Sodium ions, demonstrating that the spectroscopy system showed consistent results concerning adulteration of cow milk.

Methodologies to identify the composition, adulteration and conductivity of milk⁽⁵⁶⁾ using spectroscopy of impedance⁽⁶²⁾ and conductivimeters are different from BIA. It is important to emphasize that the technique of spectroscopy of impedance can provide data on the structure and composition of milk properties by means of different electric frequencies. BIA, on the other hand, uses an alternate current of low amplitude (500 to 800 μ A) and high frequency (50 kHz).

Aspects such as breed, stage of lactation, genetics, and nutrition milking frequencies, age of the animal as well as the recipients and types of electrodes should be considered when bioimpedance of cow milk is analyzed.

In this sense, the use of spectroscopy or simply bioimpedance in single frequency are promising techniques, which might provide quick and cost-effective results concerning composition, quality and potential adulteration of cow milk.

Conclusions

The growing increase in the demands of the consumer market toward food safety, well-being and composition of products of animal origin highlights the need to investigate precise and cost-effective methods that assure quality. Bioimpedance analysis, thus, proved to be a promising, fast, relatively inexpensive and minimally invasive technology to characterize and predict the body composition of domestic animals and the quality of raw bovine milk, showing great capacity for replacing traditional methods. Nevertheless, in order to expand the use of BIA, it is important to highlight the need for controlling, as much as possible, the potential sources of errors and variations, regardless of the species used to define protocols and standardize the analysis. Moreover, further research is needed to assess the interaction of BIA with the variation of the most diverse environments and nutritional status. To ensure information and models that are more accurate, it is recommended to associate BIA data with modeling methods and statistical packages. Hopefully, in the near future, BIA can be used both in the field and industrial plants.

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Conflict of interest declaration

The authors declare they have no conflicts of interest with the work presented in this review article.

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