EVALUATION OF ULTRASOUND IMAGING TO PREDICT LOIN EYE AREA IN TAMBAQUI*

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ABSTRACT

Tambaqui is the major native Characiform fish species in Brazil. The loin-eye area (LEA) is a feature currently used in breeding programs for livestock and may be a phenotype susceptible to selection also in tambaquis. The purpose of this study was to evaluate the efficiency of two different LEA measurements. LEA data were taken using the vegetable paper (VP) and ultrasonography (US) techniques measured in three LEA regions (LEA1) at the end of the operculum region, (LEA2) at the insertion of the dorsal fin, and (LEA3) in the region of insertion of the anal fin from 29 individuals in a fish processing plant located in the State of Rondônia, Brazil. The data were analyzed in the R program using both the Student's t-test and the Pearson correlation. The mean values for the two methods were not statistically different at the 5% risk level, indicating that the US can efficiently estimate the LEA compared with the actual value given by VP. The correlation was moderate (0.61) for LEA2 and can be attributed to the loin cut into two halves lengthwise in tambaquis usually done during commercial fish processing. Ultrasound has proven to be an efficient method for accurately estimating the LEA in tambaqui for further genetic breeding programs.

Keywords: *Colossoma macropomum*; fish; prediction; aquaculture; processing; loin

AVALIAÇÃO DA UTILIZAÇÃO DE ULTRASSONOGRAFIA PARA PREDIÇÃO DA ÁREA DE OLHO DE LOMBO EM TAMBAQUIS

RESUMO

Tambaqui é a maior espécie de peixe da ordem Characiformes no Brasil. A área de olho de lombo (AOL) é uma característica usada atualmente em programas de melhoramento genético em animais de criação terrestre. O objetivo deste estudo foi avaliar a eficiência de dois tipos diferentes de medidas de AOL. As medidas de AOL foram tomadas com o uso de papel vegetal (PV) e ultrassonografia (US) em três regiões do animal: (AOL1) região final da região do opérculo, (AOL2) na inserção da nadadeira dorsal e (AOL3) na região da inserção da nadadeira caudal em 29 indivíduos advindos de uma planta processadora localizada no estado de Rondônia, Brasil. Os dados foram analisados no programa R com o uso do test t de Student e a correlação de Pearson. Os valores médios para ambas metodologias não foram estatisticamente diferentes ao nível de 5%, indicando que US pode eficientemente estimar a AOL quando comparado com o valor real gerado por PV. A correlação foi moderada (0,61) para AOL2 e isto pode ser atribuído ao corte em duas metades do lombo geralmente realizado em tambaquis nas plantas de processamento. A técnica de ultrassom provou ser um método eficiente para estimar acuradamente a AOL em tambaqui em futuros programas de melhoramento genético.

Palavras chave: ***Colossoma macropomum***; peixe; predição; aquicultura; processamento; lombo

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INTRODUCTION

Fish farming has increased significantly over the last decades in Brazil (OSTRENSKY *et al.*, 2008). Among the native species, tambaqui (*Colossoma macropomum*) is the most important species, with steady growth since 1994 and an overall farming production in 2013 of 88,719.00 t (IBGE, 2014). Tambaqui is native to the Amazon basin and farmed mostly in the northern and northeastern regions of Brazil (ARAUJO-LIMA and GOULDING, 1997).

The importance of this species for aquaculture in Brazil and other South American countries has demanded the development of better farming management systems as well as fingelings of genetic quality (GOMES *et al.*, 2003). Currently, four out of five tambaquis commercialized in Brazil come from captivity (JACOMETO *et al.*, 2010). Production efficiency depends not only on nutritional and reproductive studies, but also on genetic improvement programs aiming to enhance the quality of fingerlings produced, which directly reflects the productivity indices of fish farming (RESENDE, 2009). Genetic improvement programs focus on traits that more directly relate to economic performance, such as weight gain and feed conversion (GJEDREM, 2000). However, fillet yield and external coloration can be also target traits for selection in breeding programs (THOSEDEN *et al.*, 2013).

An important trait used in livestock breeding programs is the loin-eye area (LEA) (PRADO *et al.*, 2004; TEIXEIRA *et al.*, 2011). In pigs, the LEA shows high heritability values and selection for a larger LEA results in desirable modifications in a percentage of lean muscle cuts and backfat thickness at the tenth rib. Thus, these characteristics make LEA an important trait to be included in selection breeding programs of swine production (BOURDON, 1997).

LEA in tambaquis can become a target trait for genetic improvement programs to develop strains having larger loins. Therefore, the development of methodologies capable of accurately measuring the LEA in wild and captive populations of tambaquis for estimating the genetic parameters related to this trait is necessary. Thus, the current study aimed to evaluate the efficiency of LEA measurements obtained by B-mode ultrasound and generate an easy-to-apply methodology for use in breeding programs.

MATERIAL AND METHODS

In tambaqui, as well as in the so-called round fishes, the loin is the part of the body on either side of the epaxial musculature (Figure 1A). This area is split by the vertebral neural arch and vertebral spinous process extending horizontally backward and marginally downward on the dorsal area (CARR *et al.*, 2014).

Tambaquis were sampled in the fish processing enterprise "Pescado Zaltana", located in Rondônia, Brazil. A total of 29 fish with average weight of 2.5 kg (± 300 g) were included. The fish used in this study were processed and then cut vertically downward into two parts. Therefore, the tambaqui loins used to measure LEA by drawing methodology were bisected. Three LEA regions were selected to evalute LEA: (I) at the end of the operculum region (LEA1), (II) at the insertion of the dorsal fin(LEA2), and (III) in the region of the insertion of the anal fin (LEA3) (Figure 1B). The weight of the entire loin from each fish was recorded.

Two methods were used to determine the LEA. First, before processing LEA, each tambaqui was measured using a portable MyLab™One VET digital ultrasound machine (Esaote S.p.a., Florence, Italy) with a rectal linear probe of 6.0/10 MHz transducer frequency. The probe was placed over each of the three points to generate cross-sectional images of the LEA (Figure 2). After processing each of two parts of the bisected tambaqui´s loin, they were delimited with a line tangent to the edges of the cross-section of the three LEA areas as described. The LEA drawn on vegetable paper (VP) was scanned and then processed using the ENVI EX 4.8 software. This software allows for assignment of different colors to each of the three LEA and to the background. In addition, it generates the number of pixels referring to the area of the three marked regions (LEA) in different colors and the total number of pixels of the scanned area. The LEA was obtained by dividing the pixels of each of the three regions and the total scanned area in percentage. The LEA in percentages of pixels was converted into area (cm^2) by correlating it with the total area of the VP.

Statistical correlations between ultrasound and VP LEA measurements were carried out by parametric paired Student's t-tests and Pearson's correlation. The variables of the LEA (VP) and the LEA (US) were tested for normal distribution and homogeneity of variances. Statistical analyses were performed using the software 'R' version 2.13.2 for Windows, package fBasics.

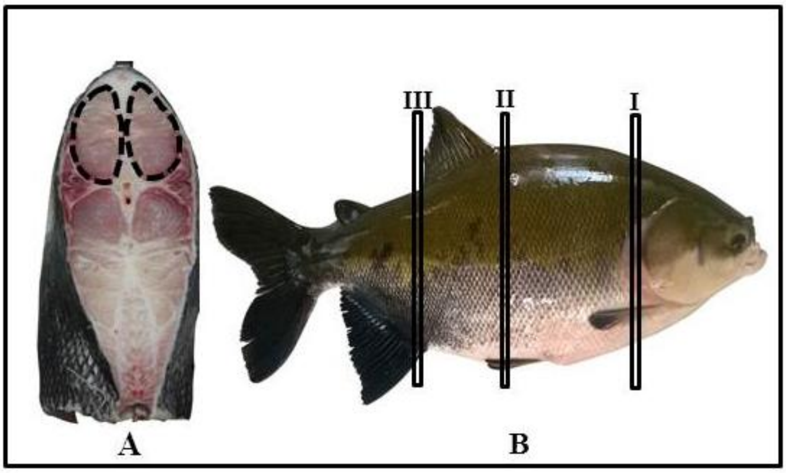

Figure 1. (A) Loin area in tambaqui *Colossoma macropomum* traced in black. (B) Positions of LEA data measurements in tambaquis: LEA1 (I), LEA2 (II) and LEA3 (III).

Figure 2. (A) Ultrasound cross-sectional images of the tambaqui *Colossoma macropomum* loin taken in LEA 2 position traced in black.

RESULTS

Descriptive statistics of the VP and US LEA measurements are shown in Table 1. Normality and homogeneity were met for all measurements (LEA1, LEA2 and LEA3) for each of the LEA methodologies (VP and US). The paired Student's t-test comparisons were not statistically significant (P>0.05), which ensures that the null hypothesis is accepted, i.e., there were no differences in measurement among LEA1, LEA2, and LEA3 achieved by VP and US. Table 2 shows the Pearson correlations within each LEA between VP and US, ranging from 0.231 to 0.609. The correlation value in the LEA2 region was the highest between VP and US (0.609; P<0.01), as well as for the loin weight with LEA2-US (0.447; P<0.05).

Table 1. Descriptive statistic of Loin eye area (LEA) data obtained using vegetable paper (VP) and ultrasonography (US).

	VP			US			LW*
	LEA1	**LEA2**	**LEA3**	**LEA1**	**LEA2**	**LEA3**	
N	29	29	29	29	29	29	29
Minimum	4.650	9.720	2.390	4.950	10.270	2.450	20.000
Maximum	11.990	12.930	5.250	12.310	13.790	4.710	30.000
Mean	7.300	11.707	4.019	10.375	12.093	3.744	26.552
Median	7.260	11.760	4.050	10.480	12.030	3.810	25.000
Standard error mean	0.279	0.157	0.118	0.254	0.165	0.097	0.560
Standard deviation	1.506	0.845	0.638	1.369	0.789	0.523	3.019

**LW = loin weight*

Table 2. Correlations among the measures of LEA collected by ultrasound (LEA1_US, LEA2_US and LEA3_US) and vegetable paper (LEA1_VP, LEA2_VP and LEA3_VP) compared to the morphometric variable loin weight (LW).

	LEA1_VP	**LEA2_VP**	**LEA3_VP**	**LEA1_US**	**LEA2_US**	**LEA3_US**	**LW***
LEA1_VP	1	-	-	0.375*	-	-	0.124
LEA2_VP		1	-	-	0.609**	-	0.435*
LEA3_VP			1	-	-	0.231	0.204
LEA1_US				1	-	-	0.137
LEA2_US					1	-	0.447*
LEA3_US						1	0.085
LW							1

**P-value <0.05; **P-value <0.01; *LW = loin weight.*

DISCUSSION

Precise establishment of goals is essential for planning and executing a successful genetic breeding program. Thus identifying and determining which traits are the best to be measured in a selection program is an important decision. The estimation of the genetic parameters, such as heritability (h^2) of the selected traits and the genetic correlations between them, is mandatory. Once these parameters are established, it is possible to decide which selection method shall be applied and which features shall be incorporated into the improvement program.

Ultrasound has been extensively used in domestic species, especially for measuring livestock carcasses. LIMA NETO *et al.* (2009) estimated the genetic parameters of carcass traits including LEA in the Guzerat breed cattle using ultrasound. Also, different levels of energy in diets were associated to LEA measured by ultrasound in lambs (CARTAXO *et al.*, 2011). Studies to assess the efficiency of ultrasound in estimating carcass traits have been performed in livestock (MERCADANTE *et al.*, 2010; SILVA *et al.*, 2012). PRADO (2004) compared different methods to estimate LEA and back fat in different genetic groups of beef cattle. The results indicated that the use of ultrasound for the evaluation of LEA showed a confidence level of 80% compared with other carcass measurements.

Corroborating the efficiency of ultrasound, TAROUCO *et al.* (2005) assessed animals of the Braford cattle breed and found a correlation between ultrasound and direct carcass measurements of 0.963 for LEA indicating a high relationship between the methodologies. Previous studies using the ultrasound imaging methodology in fish have focused on reproduction for sex determination (CREPALDI and ROTTA, 2007; MARTIN-ROBICHAUD and ROMMENS, 2001). However, carcass evaluation using ultrasonography has also been performed. CREPALDI *et al.* (2008) found high correlations between US images and yield traits, such as carcass, head, shank fillet and belly fillet in surubins (*Pseudoplatystoma* spp.). HAFFRAY *et al*. (2013) estimated the genetic parameters for carcass traits head and fillet yields in the rainbow trout (*Oncorhynchus mykiss*) predicted *in vivo* by ultrasound measurements. The authors observed that the images obtained quickly and noninvasively by real-time ultrasound have a high genetic correlation with traits of economic interest.

Genetic improvement programs for tambaqui in Brazil have been conducted mainly for weight gain. However, programs including other traits of economic interest, e.g., increasing the LEA may be of considerable value for the development of genetic varieties of tambaqui having greater carcass yield. The loin is a preferred cut of tambaqui and increasing its area can add value to the final tambaqui processing yield. Therefore, inclusion of the LEA in a selection program requires precise measurements of this region without slaughter of the animals. These actions are important to determine the heritability and repeatability genetic parameters necessary to determine the LEA as a selection target.

Pearson´s statistical correlation of LEA 1 and 3 between measures of LEA obtained using US and PV were low. These low correlation values may be related to the experimental conditions as well as the region from which the measures were taken. PV measures were taken after daily tambaqui processing in the plant. Tambaquis are usually bisected, and therefore the loin in divided into two halves lengthwise. This procedure could explain the low correlation in the LEA 1 and 3, which may be more sensitive to the bisection during processing. However, correlation between US and VP of the LEA 2, which is located at the thicker region of loin, was moderate even after the bisection. In addition, the sample size (N = 29) may have impacted the low-to-moderate correlations observed in this study and future evaluations using larger sample sizes may provide higher correlations.

The paired t-test between the VP and US measurements was not significant, and the means of LEA obtained by VP and US did not differ statistically for the three LEA regions assessed herein. This outcome further supports our finding that, considering the measurement obtained by VP as the actual measurement, the ultrasound technique was conclusive in estimating LEA in tambaquis. According to SUVANICH *et al.* (1998), ultrasound measures enable the analysis of body content with the advantages of being noninvasive, accurate, easy to use and informative. However, TAROUCO *et al.* (2005) showed that some factors may contribute to the precision of measurements taken by ultrasound and directly on the carcass. The appraiser ability is important to the accuracy and repeatability of the LEA ultrasound imaging. Also, the lack of a specific probe for LEA in fish can decrease the precision of the measurements. In the current study a linear large animal transrectal probe was used, which was the most adapted for the size of the tambaquis assessed. Maybe adaptors similar to the used for beef cattle loin area measurement should be developed in order to obtain better images.

CONCLUSION

The present study showed that ultrasound imaging can be a tool of great potential for collecting phenotypic data of LEA in tambaqui and other round fish. Sources of variation, such as contemporary groups, age of animals must be taken into account in using ultrasound images for LEA and other carcass traits measurements. The use of ultrasound imaging of LEA of broodstock, and also wild populations of tambaqui is the first step to better understanding of this trait to produce a database to estimate the genetic parameters necessary to include and establish LEA in further genetic improvement programs.

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