

Temperature, pH and sarcomere length of *Caiman yacare* in different body weight categories at slaughter

Temperatura, pH e comprimento de sarcômero de Caiman yacare em diferentes categorias de peso corporal ao abate

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ABSTRACT

The objective of this work was to characterize the internal temperature of the carcass, pH and sarcomere length in four categories of body weight at a slaughter. The categories were: category 1, animals up to 1.5 kg, category 2, animals from 1.501 to 2.5 kg, category 3, animals from 2.501 to 3.5 kg and category 4, animals over 3.501 kg. The time intervals evaluated were 0.5, 2, 4, 6, 8, 10, 12, 14, 16, 18, 24 and 36 hours after bleeding, therefore, a factorial scheme (4 x 12) was used, in which four categories of pre-slaughter weight and the behavior of the carcasses during the post-slaughter time were evaluated. There was no interaction effect between the weight categories and the time for the variation of the carcass internal temperature, pH and sarcomere length, but when only the time of rigor was evaluated, there were significant differences for the analyzed parameters. The internal temperature of the initial average carcass (0.5 hours) was 16.02°C, reaching 3.80°C at 36 hours post-slaughter. In addition, the lowest average pH found was at 24 hours, with a value of 5.39. Regarding the sarcomere length, the smallest mean length observed was 1.41 µm at 16 hours post-slaughter and the final mean length (36 hours) was 1.89 µm. It was concluded that the analyzed margin of the categories of body weight at slaughter did not interfere in the variation of temperature, pH and sarcomere length during the post-mortem of the Pantanal caiman.

Keywords: alligator meat, Pantanal alligator, cooling temperature, meat pH, sarcomere length

RESUMO

O objetivo do trabalho foi caracterizar a temperatura interna da carcaça, o pH e o comprimento de sarcômero em quatro categorias de peso corporal ao abate. As categorias foram: categoria 1, animais com até 1,5 kg, categoria 2, animais de 1,501 a 2,5 kg, categoria 3, animais de 2,501 a 3,5 kg e categoria 4, animais acima de 3,501 kg. Os intervalos de tempo avaliados foram 0,5, 2, 4, 6, 8, 10, 12, 14, 16, 18, 24 e 36 horas, após a sangria, portanto, foi utilizado um esquema fatorial (4 x 12) que avaliou quatro categorias de peso e o comportamento das carcaças ao decorrer do tempo. Não houve efeito de interação entre as categorias de peso e o tempo para a variação da temperatura interna da carcaça, pH e comprimento de sarcômero, mas quando avaliado apenas o tempo de rigor, houve diferenças significativas para os parâmetros analisados. A temperatura interna da carcaça média inicial (0,5 horas) foi de 16,02°C, chegando a 3,80°C nas 36 horas pós-abate. Além disso, o menor pH médio encontrado foi as 24 horas, com valor de 5,39. Em relação ao comprimento de sarcômero o menor comprimento médio observado foi de 1,41 µm nas 16 horas pós-abate e o comprimento médio final (36 horas) foi de 1,89 µm. Conclui-se que a margem analisada das categorias de peso corporal ao abate, não interferiram na variação de temperatura, pH e comprimento de sarcômero durante o *post mortem* do jacaré-do-Pantanal.

Palavras-chave: carne de jacaré, comprimento de sarcômero, jacaré-do-Pantanal, pH da carne, temperatura de resfriamento

INTRODUCTION

The Pantanal alligator (*Caiman yacare*) is one of the most abundant species of crocodilians in Brazil, occurring in high densities in the Pantanal field. The sustained use of its genetic stocks has been recognized as an important strategy to promote the conservation of natural ecosystems in the Pantanal (Farias *et al.*, 2013). According to current legislation (IBAMA, 2015), the natural management of Pantanal alligator populations is permitted by collecting eggs in the wild and subsequently rearing the animals in confinement.

According to Nickum *et al.* (2017), the great interest in crocodilians has always been related to leather exploration. However, for decades their meat has been sold in restaurants and specialized markets, obtaining a position of great relevance in the structures of the

production chains of these animal species.

Some studies have been developed involving changes in the pre-rigor state, which associate time, temperature and pH of alligator carcasses, indicating that the final characteristics of the meat are directly influenced by conditions during the pre-slaughter, slaughter and post-slaughter periods (Taboga *et al.*, 2003; Rodrigues *et al.*, 2007; Vicente Neto *et al.*, 2007; Vieira *et al.*, 2012). However, in the Brazilian conditions the characterization of the *rigor mortis* process of alligator carcasses is not yet well defined.

Subsequently, the objective of the work was to characterize the development of the rigor mortis process of Pantanal alligators slaughtered in four different live weight categories, through assessments of the internal temperature of the carcass, the variation in pH and the

change in length of sarcomere during 36 hours post-slaughter.

MATERIALS AND METHODS

We used 32 Pantanal alligators from the company Caimasul, regulated with registration number 5849143 at the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA), located in the municipality of Corumbá-MS. The animals were divided into four pre-slaughter weight categories, containing 8 animals each, as follows: category 1, animals weighing up to 1.5 kg; category 2, animals weighing 1.501 to 2.5 kg; category 3, animals weighing 2.501 kg to 3.5 kg; and category 4, animals weighing more than 3.501 kg.

The animals were slaughtered, under the Ministry of Agriculture registration number SIF/DIPOA 0001/1515, which compresses federal inspection. They were subjected to a period of fasting, lasting 24 hours before slaughter. The animals then had their jaws tied and remained in tanks with chlorinated water, 0.5 ppm, and then taken to the slaughter room, where they were stunned with a Zilka pneumatic gun. After stunning, the animals were de-molded, bled, skinned and eviscerated. After the slaughter and inspection procedures were completed, the carcasses were stored under cooling in a cold storage room. The internal temperature of the carcasses was measured using a digital thermometer, introducing its metal rod to a depth of 3.0 cm at three points on the muscular masses of the back, loin and tail, at time intervals of 0.5, 2, 4, 6, 8, 10, 12, 14, 16, 18, 24 and 36 hours after bleeding. The temperature of the cold storage chamber at the moment the

carcasses entered in each time interval mentioned was also observed.

To determine the pH, a portable pH meter (Hanna Hi 991 663) with a piercing electrode was used. Three measurements were made in each animal unit in the same locations, measuring the internal temperature of the carcasses, as well as at the same time intervals.

Using tweezers and a scalpel, samples measuring 15 mm in length, 10 mm in width and 5 mm in thickness were taken from the right side of the *Ilio-ischio-caudalis* muscle, at time intervals of 0.5, 2, 4, 8, 10, 12, 16, 24 and 36 hours after bleeding, to measure sarcomere length. Subsequently, the samples were kept in 50 ml falcon tubes, containing a 10% formaldehyde solution for 24 hours, after which the solution was replaced with 70% ethyl alcohol, where the samples were stored until the moment of histological processing (Rodrigues *et al.*, 2004).

According to Behmer (1976), histological processing involved removing samples from 70% ethyl alcohol, embedding them in paraffin, and making sections from the blocks with a microtome. Afterward, the slides were stained with phosphotungstic hematoxylin.

Images were captured using a microscope coupled to a camera, with a final magnification of 1000×, using QCapture Software Pro 7 (QImaging, Surrey, British Columbia, Canada). The sarcomere length of the readings was determined using the IP WIN32 ImagePro Plus software (Acromag, Wixom, MI, USA), counting 10 sarcomeres from 20 random fibers from 20 fields of images collected for each animal.

The experimental design was completely randomized. To evaluate the internal temperature, pH and sarcomere length, a factorial scheme (4 x 12) was used, in which four different pre-slaughter weight categories and the behavior of the carcasses during the slaughter were evaluated. An ANOVA was performed and the means were subjected to the Tukey test ($p \leq 0.05$). In all statistical analyzes the SAS Inst. Inc. Cary NC USA (2000) program was used. No statistical analysis was performed for the cold room temperature.

RESULTS AND DISCUSSION

According to the results found for the variation in internal carcass temperature, pH and sarcomere length, there was no interaction effect ($p > 0.05$) between the live weight categories at slaughter and the post-bleeding period evaluated. Furthermore, there were no significant differences among the four categories of live weight at slaughter, and the analyzed parameters differed significantly only during the post-slaughter period (Table 1).

Table 1. Cold chamber temperature (TC), internal carcass temperature (TI), pH and sarcomere length (CS) of different weight categories at slaughter, as a function of post-bleeding time of Pantanal alligator.

Category	Time (hours)	TC (°C)	TI (°C)	pH	CS (μm)
Weight 1 (<1,5)	0,5	2,5	16,20±10,09	8,27±1,52	1,61±0,02
	2	4,3	9,56±3,45b	6,54±0,21	1,67±0,08
	4	5,2	10,18±4,07	7,29±0,54	1,59±0,01
	6	4,8	8,78±2,67	7,68±0,93	-
	8	4,8	9,58±3,47	6,75±0,01	1,64±0,05
	10	3,9	5,70±0,41	6,41±0,34	-
	12	3,8	4,88±1,23	7,05±0,30	1,59±0,01
	14	3,6	4,16±1,95	6,99±0,24	-
	16	3,6	4,14±1,97	6,86±0,11	1,37±0,22
	18	3,6	4,67±1,44	6,76±0,02	-
	24	2,9	3,61±2,50	5,22±1,53	1,68±0,09
	36	3,0	3,70±2,41	6,54±0,21	1,88±0,29
Weight 2 (1,501 a 2,5 kg)	0,5	3,7	15,23±9,12	7,25±0,50	1,66±0,07
	2	4,4	8,61±2,50	7,28±0,53	1,63±0,04
	4	4,7	7,50±1,39	7,16±0,41	1,52±0,07
	6	4,8	5,58±0,53	6,90±0,15	-
	8	4,7	4,31±1,80	6,80±0,05	1,59±0,01
	10	4,6	3,81±2,30	6,61±0,14	-
	12	4,8	4,41±1,70	6,96±0,21	1,58±0,02
	14	4,6	3,31±2,80	6,72±0,03	-
	16	4,6	3,85±2,26	6,47±0,28	1,46±0,13
	18	3,4	3,53±2,58	6,31±0,44	-
	24	4,2	4,16±1,95	6,10±0,65	1,59±0,01
	36	2,9	3,82±2,29	5,95±0,80	1,87±0,28
Weight 3	0,5	4,4	15,49±9,38	6,64±0,11	1,61±0,02

(2,501 a 3,5 kg)	2	4,5	7,56±1,45	7,43±0,68	1,56±0,07
	4	4,7	7,76±1,65	6,87±0,12	1,48±0,11
	6	4,8	5,53±0,58	7,45±0,70	-
	8	4,0	4,58±1,53	6,60±0,15	1,51±0,08
	10	3,1	3,72±2,39	6,86±0,11	-
	12	2,9	3,50±2,61	6,80±0,05	1,58±0,02
	14	2,9	3,52±2,59	6,67±0,08	-
	16	4,5	3,98±2,13	6,76±0,02	1,46±0,13
	18	2,9	3,73±2,38	6,71±0,04	-
	24	4,8	4,25±1,86	5,02±1,73	1,59±0,01
	36	3,0	3,88±2,23	6,68±0,07	1,87±0,28
Weight 4 (>3,501 kg)	0,5	4,5	17,17±11,06	6,78±0,03	1,61±0,02
	2	4,3	8,83±2,72	7,10±0,35	1,51±0,08
	4	4,2	6,32±0,21	7,41±0,66	1,61±0,02
	6	4,3	6,13±0,02	7,50±0,75	-
	8	3,8	4,71±1,40	6,45±0,30	1,38±0,21
	10	3,2	3,70±2,41	7,15±0,40	-
	12	5,8	4,77±1,34	6,74±0,01	1,47±0,12
	14	3,2	3,86±2,25	6,75±0,02	-
	16	4,9	4,08±2,03	6,66±0,09	1,42±0,17
	18	5,0	4,51±1,60	6,59±0,16	-
	24	4,2	4,38±1,73	5,20±1,55	1,58±0,01
	36	2,5	3,82±2,29	6,50±0,25	1,90±0,31
Main Effects					
Weight (kg)	1 (<1,5)	3,92	7,09±0,98	6,87±0,12	1,63±0,04
	2 (1,501 a 2,5)	4,28	5,68±0,43	6,71±0,04	1,61±0,02
	3 (2,501 a 3,5)	3,87	5,63±0,48	6,71±0,04	1,58±0,01
	4 (>3,501)	4,16	6,03±0,08	6,74±0,01	1,56±0,03
Time (hours)	0,5	3,77	16,02±9,91 ^a	7,23±0,48 ^{ab}	1,62±0,03 ^b
	2	4,37	8,64±2,53 ^b	7,09±0,34 ^{bc}	1,60±0,01 ^{bc}
	4	4,70	7,94±1,83 ^{bc}	7,18±0,43 ^{ab}	1,55±0,04 ^c
	6	4,67	6,50±0,39 ^c	7,38±0,63 ^a	-
	8	4,32	5,79±0,32 ^{cd}	6,65±0,10 ^e	1,53±0,05 ^c
	10	3,70	4,23±1,88 ^d	6,76±0,01 ^{de}	-
	12	4,32	4,39±1,72 ^d	6,89±0,14 ^{cd}	1,52±0,07 ^c
	14	3,57	3,72±2,39 ^{de}	6,78±0,03 ^{de}	-
	16	4,40	4,01±2,10 ^d	6,69±0,06 ^{de}	1,41±0,18 ^d
	18	3,87	4,11±2,00 ^d	6,59±0,16 ^{ef}	-
	24	4,07	4,10±2,01 ^d	5,39±0,72 ^g	1,64±0,05 ^b
	36	2,90	3,80±2,31 ^{de}	6,42±0,33 ^f	1,89±0,30 ^a
Probability					
Weight (P)		-	0,0793	0,0851	0,0806
Time (T)		-	<0,0001	<0,0001	<0,0001
Interaction (PxT)		-	0,0892	0,0932	0,0955

V.C. ² (%)	-	12,10	5,30	5,10
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¹Means \pm standard deviation followed by Tukey test at 5% probability; ² C.V. Coefficient of Variation

The behavior of internal carcass temperature and the pH during the cooling period were analyzed separately for each slaughter weight category, and shown in Figure 1.

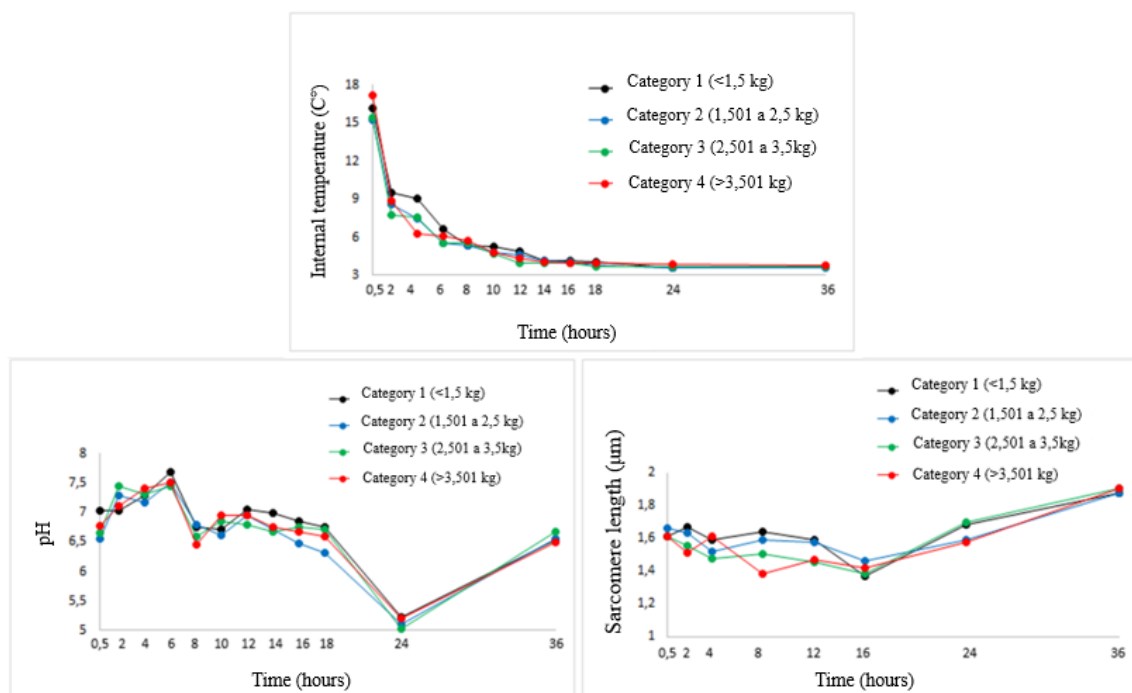


Figure 1. Comparison of the variation in internal temperature, pH and sarcomere length, among different categories of live weight at slaughter, as a function of post-bleeding time, during the post-slaughter period of the Pantanal alligator.

This means that both temperature and pH are used as parameters to monitor meat quality. According to the results obtained for the internal temperature of the carcass during the cooling period (*rigor mortis*) the average initial temperature of the carcass in the first 0.5 hour was 16.02°C inside the cooling chamber. The average temperature of 4.23°C in the internal carcass was reached approximately 10 hours after bleeding and the lowest temperature was reached after 14 hours inside the cooling chamber (Table 1). Statistical analysis showed that there were no significant differences ($p>0.05$) for the internal temperatures of

the carcasses at intervals of 10, 12, 16, 18 and 24 hours. These results indicate that the internal temperature of the carcass behaved in accordance with the expectations described by Marsh *et al.* (1987) for the same post-slaughter period, for the evaluation of beef. Vieira *et al.* (2012) evaluating the *rigor mortis* process of Pantanal alligators, observed that as time passed in the post-mortem of the animals, the internal temperature of the carcass decreased, starting at 2°C in 0.5 hour post-slaughter and going to 4.2°C in 36 hours post-slaughter. However, in the present study the behavior of the internal temperature

was not similar, as there was an oscillation between the internal temperature of the carcass, which decreased and increased after 10 hours of slaughter. This is due to the opening and closing of the cold chamber during the post-slaughter period, since in the evaluated processing unit, due to the large amount of animals being slaughtered in the company, the slaughter was carried out in two days. In addition to the animals slaughtered for the present study, there were also more animals slaughtered for the processing and sale of meat and skin. Therefore, this fact justifies the greater opening of the chamber and consequently, the oscillation in the decrease in the internal temperature of the carcass.

Even with fluctuations in the internal temperatures of the carcass and cold chamber (Figure 1), the values obtained corroborate Sousa *et al.* (2021). According to these authors, immediately after slaughtering the Pantanal alligator, the carcasses have an internal temperature ranging from 17°C to 19°C. Furthermore, they must be stored in cooling chambers with temperatures of 0°C to 5°C, for at least 24 hours, and reach an internal temperature, in the center of the muscle mass, of a maximum of 7°C before deboning. In other animal species such as the turkey, for example, Costa *et al.* (2006) observed a linear decline of 40.83°C until reaching 2.40°C at 18.5 hours post-slaughter in animals with a live weight of 16.1 kg at the time of slaughter, on average. In wild boar meat, Machiori & Felício (2003), obtained temperatures in the *Longissimus dorsi* muscle of 30.2°C (1 h), 12.4°C (6 h) and 5.4°C (24 h). Fernandez *et al.* (2009) achieved temperatures of 39.07°C (0.5 h), 12.0°C

(7 h) and 0.28°C (24 h), in animals with live weight ranging from 45 to 53 kg. The difference in the time of decrease in the internal temperature of the carcass, between the Pantanal alligator evaluated in the present study and the meat of the aforementioned animal species, occurred mainly due to the weights of the carcasses, since in the other species the slaughtered animals had larger live weights, consequently increasing the time for the carcass temperature to decrease within the cooling chamber.

Regarding pH, in the present work, an initial average value was found to be 7.23 in 0.5 hours; 5.39 after 24 hours; and 6.42 at 36 hours, which indicate quadratic behavior, oscillating between decrease and increase of the pH over the analyzed time interval. This behavior differs from that found by Vieira *et al.* (2012) when they evaluated the rigor mortis process of the *Ilio-ischio-caudalis* muscle of the Pantanal alligator, which presented an initial pH value of 6.7 after 15 hours, 5.7 after 36 hours and final pH of 5.6, with only decline and stagnation of pH, a behavior that also corroborates with the findings by Toboga *et al.* (2003) who also evaluated the alligator tail muscle during 36 hours post-bleeding.

Hoffman *et al.* (2000) verified the variation in post-slaughter pH in carcasses of Nile crocodiles slaughtered by shot and obtained values of 6.88 in the first hour, 7.28 in 12 hours and 6.28 in 48 hours post-slaughter. Considering that the procedure mentioned for slaughter is characterized as non-humane, the stress suffered by the animal pre-slaughter caused rapid consumption of glycogen and the pH remained higher than 6.2 even after 24 hours post-slaughter. However, in the present work, even using a method as the stunning (Zilka

pistol), the pH remained below 6.2 only for 24 hours, reaching 6.42 in 36 hours (final pH), which shows that the evaluated carcasses can generate meat with unsuitable characteristics, requiring a reevaluation of the pre-slaughter procedures addressed by the processing unit. This is due the speed of pH decrease, as well as the final pH obtained, which are influenced by several aspects such as temperature, humidity, light, genetic inheritance of the animal and management before slaughter (Alves *et al.*, 2016; Neto & Rosenthal., 2011).

There are reports in the literature that the pre-slaughter stunning method was also used, but even so, the final pH obtained was above 6.2. This was the case of Cossu *et al.* (2007) who evaluated the meat of alligators of the species *Caiman latirostris* and *Caiman yacare* slaughtered with a cut in the neck and stunned with a thread inserted into the spinal cord. The authors observed an average pH of 6.88 in 4 hours, 6.64 in 12

hours and 6.49 in the 24 hours post-slaughter.

The *rigor mortis* process in farm animals demonstrates different sarcomere lengths during the entire biochemical process. These changes that occur in length are due to the contraction and relaxation that occur in the *post-mortem* period (Oliveira *et al.*, 2004). In this study, the initial average length (0.5 hour) of sarcomere removed from the *Ilio-ischio-caudalis* muscle of the Pantanal alligator was 1.62 μm , and decreased to 1.53 μm at 8 hours, 1.41 μm at 16 hours and increased again to 1.64 μm in 24 hours and 1.89 μm in 36 hours, post-slaughter (Figure 2). Therefore, the maximum contraction that occurred in the sarcomere was at 16 hours *post-mortem*. Vieira *et al.* (2012) observed the maximum sarcomere contraction in the 15 hours post-slaughter, with an average length of 1.5 μm , for the same muscle taken from a Pantanal alligator. The same authors also point out the final sarcomere length (36 hours) of 1.9 μm .

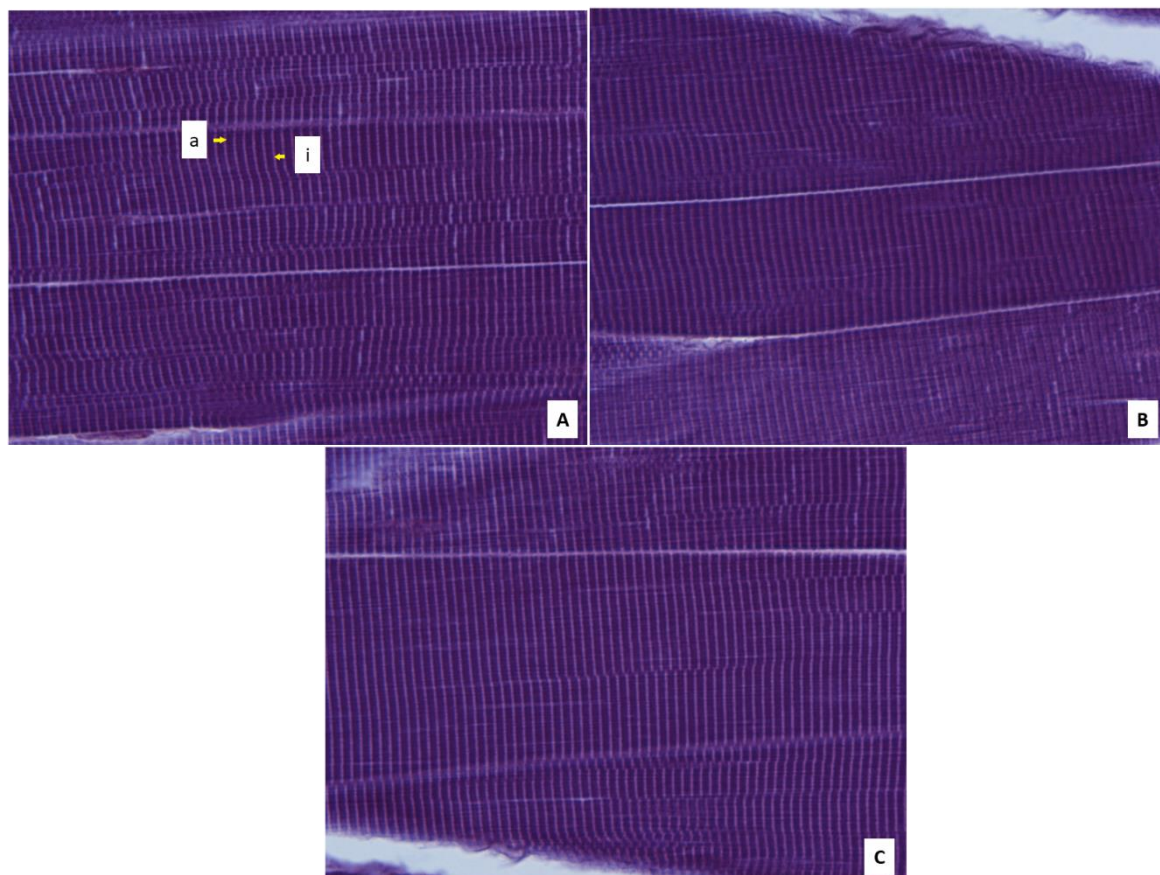


Figure 2. Photomicrographs of the *Ilio-ischio-caudalis* muscle of a Pantanal alligator, showing bands a (dark) and i (light) of an animal in the live weight category at slaughter 4 (above 3.5 kg), at the time intervals 0.5 hours (A), 16 hours (B) and 36 hours (C).

In general, there is a lack of studies that address the variation in sarcomere length in alligators during the development of *rigor mortis*. However, when comparing this species with common animal production species, such as sheep, the maximum contraction of the sarcomere was after 15 hours of slaughter with a length of 1.46 μm (Oliveira *et al.*, 2004), which confirms the result found in the present study. However, in animals with less consumed meat, such as wild boars, the *Triceps brachii* muscle showed maximum shortening 7 hours post-bleeding, and maximum relaxation 24 hours post-bleeding, with sarcomere lengths of 1.61 μm and 1.97 μm , respectively (Fernandez *et al.*, 2009).

CONCLUSION

It is concluded that the different weight categories at slaughter do not affect the variation in temperature, pH and sarcomere length during the post-mortem process of the Pantanal alligator. However, during the development of the *rigor mortis* process there were fluctuations in the temperature and pH of the carcasses, being lowest temperature of 3.80°C reached in the 36 hours post-slaughter and the lowest pH value (5.39) in the 24 hours post-slaughter. In addition, the maximum muscle contraction occurred 16 hours post-slaughter, with a sarcomere length of 1.41 μm .

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