Hematological markers and biochemical profiles in terms of gender and age of captive collared peccaries (*Tayassu tajacu*) in eastern Amazon

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ABSTRACT. Complete blood counts and blood biochemical analyses are laboratory tests that allow the monitoring of physiological condition, nutrition, and health in free-living or captive wild animals. When interpreting these tests, it is essential to compare the results with reference ranges that are suitable for the species. Few studies have been conducted on the hematological and biochemical characteristics of *Tayassu tajacu*, particularly for animals raised in the Amazon biome. The objectives of this study were to evaluate the influence of age and gender on the hematological

and biochemical profiles of captive *T. tajacu*, and to establish reference intervals for these parameters. Complete blood counts and biochemical analyses were performed using manual methods and semi-automatic equipment, respectively. There were significant differences in relation to age in hematocrit and hemoglobin levels, and mean cell volumes, in captive *T. tajacu*. No basophils were observed, and the neutrophil:lymphocyte ratio was less than 1. Levels of total protein, urea, phosphorus, and alkaline phosphatase were significantly affected by age ($P < 0.05$). Gender did not affect any of the results. The hematological and biochemical parameters for this species were determined, and may be used as reference ranges for captive *T. tajacu*.

Key words: Reference interval; Complete blood count; Leukogram; Tayassuidae

INTRODUCTION

Collared peccaries (*Tayassu tajacu*) are ungulate mammals belonging to the order Artiodactyla, suborder Nonruminantia, superfamily Suoidea, and family Tayassuidae (Nowak, 1991). Therefore, the collared peccary does not belong to the same family as wild boar and domestic pigs (Suidae). Despite some similarities, the collared peccary differs from wild boar and domestic pigs because this species has a stomach that is divided into four compartments and produces volatile fatty acids (García and Leal, 2003), their hind limbs have three digits, and they lack a gallbladder and a scent gland located near the tail (Sowls, 1997). The geographical distribution of this species ranges from South America to Central America and the southern United States (García and Leal, 2003). In the Amazon region, collared peccaries are a major source of animal protein for indigenous and colonist populations (Redford and Robson, 1987; Schettini et al., 2005).

Collared peccaries can adapt well to captivity; therefore, they could be commercially exploited (Batista et al., 2008). They are a subsistence source of protein and are marketed for breeding, meat, and leather (Nogueira-Filho and Lavorenti, 1997). Captive breeding, as well as its commercial applications, may contribute to the conservation of the species because it is vulnerable to extinction due to poaching (Silva et al., 2011). A survey conducted by Homma (1992) revealed that 5413 tons of collared peccary skin was marketed between 1953 and 1970, which is an alarming number.

Researching the species can result in more efficient conservation and zootechnical exploitation (Garcia et al., 2009); therefore, the behavior, nutrition, reproductive health, and management of these animals should be investigated. Some studies have been conducted on husbandry and reproduction in this species (Costa and Paula, 2005; Mayor et al., 2007; Silva et al., 2011), but few studies have evaluated the health of free-living animals or animals raised in captivity, particularly in regard to blood biochemistry and hematology (Almeida et al., 2011). Among the laboratory tests used to assess animal health, complete blood counts and biochemical analyses are notable for their ease of implementation and interpretation. Understanding the reference ranges for different species is important for the correct interpretation of these tests. Reference intervals are obtained from a healthy population using appropriate statistical tests, and represent an estimate within which 95% of clinically healthy individuals must be found (George et al., 2010).

The present study aimed to establish reference intervals for the hematology and blood

biochemistry of captive *T. tajacu* in eastern Amazonia, and to evaluate the effects of age and gender on the parameters obtained.

MATERIAL AND METHODS

The study was approved by the research ethics committee of Universidade Federal do Pará, and the number of the certificate of application for ethical review was 092-12. The project was registered (No. 30710) with the System of Authorization and Information on Biodiversity of the Chico Mendes Institute for Biodiversity. The study was conducted on two farms that are authorized by the Brazilian Institute of Environment and Renewable Resources (IBAMA) in Pará, eastern Amazonia, Brazil. The scientific breeding of collared peccaries was conducted at Embrapa Amazônia Oriental (IBAMA No. 1501.5219/2011) in Belém (1°28'S, 48°27'W) and the commercial breeding facility (IBAMA No. 526 063) was located in Santa Isabel (1°17'58"S, 48°9'40"W).

On both farms, the animals were kept in facilities measuring 36 m^2 under natural conditions of temperature, humidity, and light-dark cycle. Each installation contained a drinker and a feeder, providing the animals with free access to feed (Albuquerque et al., 2009). The climate was equatorial, with an average air temperature of between 24° and 27°C and an average annual rainfall greater than 2000 mm.

Animals

Seventy-three clinically healthy collared peccaries were used, with 36 from Embrapa and 37 from Santa Isabel. To certify their health, their behavior, mucosal appearance, degree of hydration, and presence of wounds, abscesses, or lesions in the skin and hooves were checked. The control of endoparasites was conducted according to breeding management principles using a broad-spectrum oral anthelmintic, mebendazole for swine (Mebendazole Poultry and Swine, Vetnil Veterinary Products, São Paulo, Brazil), at a dosage of 80 mg of dewormer for every 50 kg of animal. The animals were identified with numbered ear tags, and were raised in collective pens with individuals of the same family group. Their food was based on a balanced ration for the growth and fattening of pigs (Makaru Ltda., Ananindeua, PA, Brazil); it contained 2500 kcal/kg of energy with 14% protein. In addition, the animals were given fruit, fodder, and water *ad libitum*.

The animals were divided into three age groups (G), according to the rating established by Venturieri and Le Pendu (2006): G1 infant (animals less than 1 year old, N = 21, 13 males and 8 females); G2 juveniles (1- to 2-year-old animals, N = 16, 8 males and 8 females); and G3 adults (animals older than 2 years, N = 36, 17 males and 19 females). The experiments in this study conformed to the current laws of Brazil.

Sample collection

For the collection of blood, the animals were physically restrained with a hand net. Between 8:00 am and 10:00 am, 3-mL samples of blood were collected for hematological analyses by saphenous or cephalic venipuncture using disposable syringes and needles into collection tubes with EDTA. For biochemical studies, 3 mL blood was collected into tubes without anticoagulant, left at room temperature for clot formation, and centrifuged at 2000 *g* for 10 min. The resulting serum was frozen at -20°C until analysis. No hemolytic, icteric, or lipemic samples were processed.

Hematological and biochemical analyses

Total red cell blood counts (RBCs) and total white cell blood counts (WBCs) were performed using the manual method. Whole blood was diluted in saline (0.9%; 1:200) and Turk liquid (1:20) for the determination of RBCs and WBCs, respectively, and counts were performed in a Neubauer hemocytometer (Bioanalytic GmbH, Germany). The results were multiplied by the dilution factor and expressed as the number of cells/µL blood. The platelet estimate was determined by blood smears.

The number of hematocrits was determined using the microhematocrit technique. Capillary tubes were filled with blood and centrifuged at 12,000 *g* for 5 min, and hematocrit determination was performed on a microhematocrit card reader. The cyanmethemoglobin technique was used to determine the hemoglobin concentration. The hematocrit and hemoglobin results were expressed as a percentage and g/dL, respectively. The mean cell volume (MCV) and mean cell hemoglobin concentration (MCHC) were calculated based on the results obtained for the RBCs, hemoglobin, and hematocrits.

Differential leukocyte counts were performed on blood smears that were stained with Quick Panoptic (Newprov Produtos Para Laboratório Ltda., Pinhais, PR, Brazil). One hundred leukocytes were counted and differentiated into neutrophils, band neutrophils, eosinophils, basophils, monocytes, and lymphocytes. The results are expressed in absolute values (10^3/µL).

Biochemical measurements were performed using commercial Cepa kits (MBIOLOG Diagnósticos Ltda., Contagem, MG, Brazil) in a BIOPLUS biochemical analyzer (BIOPLUS Produtos para Laboratório Ltda., Barueri, SP, Brazil). Biochemical testing was performed to determine the levels of urea, creatinine, calcium, and phosphorus, as well as the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), and alkaline phosphatase (ALP) enzymes. The determination of total proteins was performed by refractometry (ITEST, Medição e Automação Ltda., São Paulo, SP, Brazil).

Statistical analysis

To establish reference values, we followed the recommendations of the Clinical and Laboratory Standards Institute (CLSI), as described by Ferreira and Andriolo (2008). The results for each hematological and biochemical variable were tested for normality (Gaussian) using the Kolmogorov and Smirnov test. Data that did not have a Gaussian distribution were tested again for normality after a square root transformation. For data with a Gaussian distribution, the reference values were determined to be the mean ± 2SD. For data without a Gaussian distribution even after transformation, the 2.5th and 97.5th percentiles were used as reference intervals.

To evaluate the effect of gender and age on the biochemical and hematological variables, we used the Tukey test for normally distributed data and the Kruskal-Wallis test for data that were not normally distributed. The program BioEstat 5.0 (Ayres et al., 2007) was used to calculate the statistics. The results are reported as means ± SDs, and the differences were considered significant when $P < 0.05$.

RESULTS

The hematological and blood biochemistry results are shown in Table 1. All of the hematological and biochemical parameters followed Gaussian distributions, except for the MCHC and band neutrophils. Eosinophil and monocyte counts and the neutrophil:lymphocyte (N:L) ratio were normally distributed after a square root transformation.

Table 1. Reference intervals for hematological and biochemical parameters of *Tayassu tajacu* (N = 73).

Parameter	Unit	Data distribution	Mean ± SD	Reference range
RBC	$x10^6$ /µL	Gaussian	10.3 ± 1.17	7.3-13.1
Hematocrit	%	Gaussian	50.3 ± 4.52	40-60
Hemoglobin	g/dL	Gaussian	14.7 ± 1.77	11.5-20
MCV	Fl	Gaussian	48.9 ± 4.25	37.3-58.9
MCHC	%	Non-Gaussian	29.1 ± 1.81	26.7-34.6
Platelets	$x10^3$ /µL	Gaussian	280.6 ± 106.1	81-583
WBC	$x10^3$ /µL	Gaussian	14.6 ± 5.9	5.9-35
Neutrophils	$x10^3$ /µL	Gaussian	5.9 ± 2.5	1.9-12.2
Bands	$x10^3$ /µL	Non-Gaussian	0.2 ± 0.2	0-1.4
Eosinophils	$x10^3$ /µL	Gaussian after transformation	0.9 ± 0.7	0.1-3.7
Lymphocytes	$x10^3$ /µL	Gaussian	7.3 ± 3.8	2.1-21.0
Monocytes	$x10^3$ /µL	Gaussian after transformation	0.2 ± 2.2	0.09-1.1
N:L	no unit	Gaussian after transformation	0.9 ± 0.62	0.3-3.8
ALT	U/L	Gaussian	31.8 ± 12.4	6.0-67.0
AST	U/L	Gaussian	27.1 ± 9.5	9.0-46.0
GGT	U/L	Gaussian	7.9 ± 5.9	0-27.0
ALP (G1, N = 21)*	U/L	Gaussian	274.7 ± 155.1	91-541
ALP (G2, N = 16)*	U/L	Gaussian	181.12 ± 96.1	77-370
ALP (G3, N = 36)*	U/L	Gaussian	77.46 ± 32.3	30-135
Urea	mg/dL	Gaussian	25.8 ± 6.1	15-46
Creatinine	mg/dL	Gaussian	2.0 ± 0.4	1.4-3.5
Total Protein	mg/dL	Gaussian	10.5 ± 0.9	6.2-14.3
Calcium	mg/dL	Gaussian	9.9 ± 0.7	8.0-11.5
Phosphorus	mg/dL	Gaussian	8.0 ± 2.0	4.5-17.4

RBC, total red cell blood count; WBC, total white cell blood count; N:L, neutrophil:lymphocyte ratio; MCV, mean cell volume; MCHC, mean cell hemoglobin concentration; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transferase; ALP, alkaline phosphatase. *Reference intervals established for different age groups.

Table 2 shows the hematological parameters by age. The only parameters of the complete blood count that were influenced by age were hematocrits, hemoglobin, and MCV, which were significantly lower ($P < 0.05$) in infants (G1) than in adults (G3). Regarding the differential leukocyte counts, basophils were not observed and the N:L ratio was less than 1 for all groups.

Table 2. Hematological parameters of *Tayassu tajacu* according to age (means ± SD).

Parameter	G1 (N = 21)	G2 (N = 16)	G3 (N = 36)
RBC ($x10^6$/µL)	10.2 ± 0.9	10.4 ± 1.5	10.3 ± 1.1
Hematocrit (%)	48.0^B ± 2.8	50.4^{AB} ± 4.5	51.5^A ± 4.6
Hemoglobin (g/dL)	13.8^B ± 1.1	14.5^{AB} ± 1.7	15.2^A ± 1.9
MCV(fl)	47.0^B ± 3.5	49.0^{AB} ± 5.2	50.0^A ± 3.8
MCHC (%)	5.3 ± 0.1	5.3 ± 0.1	5.4 ± 0.1
Platelets ($x10^3$/µL)	305.2 ± 111.1	255.6 ± 107.1	277.3 ± 102.5
WBC ($x10^3$/µL)	16.2 ± 5.2	14.1 ± 5.6	13.9 ± 6.4
N. targeted ($x10^3$/µL)	6.2 ± 2.4	5.6 ± 2.5	5.8 ± 2.5
Bands ($x10^3$/µL)	0.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
Eosinophils ($x10^3$/µL)	0.9 ± 0.3	0.8 ± 0.2	0.9 ± 0.3
Lymphocytes ($x10^3$/µL)	8.5 ± 3.3	7.1 ± 4.0	6.6 ± 4.0
Monocytes ($x10^3$/µL)	0.8 ± 0.1	0.9 ± 0.1	0.86 ± 0.1
N:L	0.8 ± 0.1	0.9 ± 0.3	0.99 ± 0.2

RBC, total red cell blood count; WBC, total white cell blood count; N:L, neutrophil:lymphocyte ratio; MCV, mean cell volume; MCHC, mean cell hemoglobin concentration. Superscript capital letters indicate significant differences between columns ($P < 0.05$). G1, infants; G2, juveniles; and G3, adults.

A comparison of the biochemical parameters between age groups (Table 3) showed that the measured values for total protein, urea, phosphorus, and ALP were influenced by the age

of the animals. ALP enzyme activity was significantly higher in the G1 and G2 age groups than in G3. Juveniles (G2) and adults (G3) had higher concentrations ($P < 0.05$) of total protein than infants (G1), whereas infants had a higher concentration of phosphorus than adults ($P < 0.05$). G1 had lower values for urea than G2 ($P < 0.05$) or G3 ($P < 0.05$). Gender did not affect any of the hematological or blood biochemistry results.

Table 3. Biochemical parameters of *Tayassu tajacu* according to age (means ± SD).

Parameter	G1 (N = 21)	G2 (N = 16)	G3 (N = 36)
ALT (U/L)	33.5 ± 13.6	32.2 ± 8.9	31.3 ± 13.6
AST (U/L)	31.4 ± 7.6	23.8 ± 7.1	27.6 ± 10.4
GGT (U/L)	7.0 ± 5.9	7.3 ± 4.8	7.8 ± 6.2
ALP (U/L)	274.7^{A} ± 155.1	181.12^{A} ± 96.1	77.46^{B} ± 32.3
Urea (mg/dL)	24.06^{B} ± 6.1	31.50^{A} ± 8.1	25.85^{AB} ± 6.7
Creatinine (mg/dL)	2.0 ± 0.6	1.9 ± 0.5	2.0 ± 0.4
Calcium (mg/dL)	10.3 ± 1.0	10.0 ± 1.0	9.7 ± 0.7
Phosphorus (mg/dL)	10.6^{A} ± 3.0	8.9^{AB} ± 1.7	7.5^{B} ± 1.9
Total Protein (mg/dL)	9.1^{B} ± 1.3	11.36^{A} ± 1.8	10.5^{A} ± 1.0

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transferase; ALP, alkaline phosphatase. Superscript capital letters indicate significant differences between columns ($P < 0.05$). G1, infants; G2, juveniles; and G3, adults.

DISCUSSION

Complete blood counts and blood biochemical analyses are laboratory tests that allow the monitoring of physiological condition, nutrition, health (Lochmiller et al., 1986; Furtado and Kashivakura, 2006), and stress (Batista et al., 2008, 2009) in wild animals that are free-living or captive. When interpreting these tests, it is essential to compare the results with reference ranges that are suitable for the species (George et al., 2010). Few studies have been conducted on the hematological and biochemical characteristics of *T. tajacu* (Santos, 1999; Schettini et al., 2005; Almeida et al., 2011), particularly on animals raised in the Amazon biome (Schettini et al., 2005; Pereira, 2009).

In this study, the hematological and blood biochemistry characteristics of *T. tajacu* were determined using the statistical procedures recommended by CLSI, including the use of parametric and nonparametric tests, as appropriate for the data distribution (Ferreira and Andriolo, 2008). Although the majority of biochemical and hematological values are not parametric (Votja et al., 2011), we found that 17 of the 22 parameters analyzed were normally distributed, and only two (MCHC and band neutrophils) were not normally distributed, even after a square root transformation. The size of the reference population used in this study (73) was higher than that recommended for determining a normal distribution (30) (Ferreira and Andriolo, 2008), and higher than sample sizes in other studies of *T. tajacu* (Santos, 1999; Schettini et al., 2005; Almeida et al., 2011). The use of an appropriate statistical methodology is a prerequisite for establishing reliable reference ranges for diagnostic screening tests, which are aimed at evaluating health and disease in a given population (Votja et al., 2011).

In the present study, gender did not influence any of the parameters evaluated. However, other studies performed on the same species have reported a gender effect: males had significantly higher values than females for leukocytes (Pereira, 2009) and total protein (Almeida et al., 2011).

The results of the hematological analyses are similar to those described by other authors

(Pereira, 2009; Almeida, 2011). The N:L ratio was less than 1, which was also found by Almeida et al. (2011) and Pereira (2009), demonstrating that this species has a lymphocytic profile. In the present study, age affected hematocrit and hemoglobin levels and the MCV ($P < 0.05$). Infants had the lowest values for these parameters, probably because of the reduced availability of iron in their diet, as some animals in this age group were still breastfeeding and milk is a poor source of iron (Kegley et al., 2002). However, despite the difference between the age groups, these hematological parameters were within the reference intervals established by other authors (Santos, 1999; Pereira, 2009; Almeida et al., 2011).

AST, ALT, and GGT activity was similar to that observed by other authors (Lochmiller et al., 1986; Schettini et al., 2005), and was not influenced by the age or gender of the animals. However, ALP was significantly affected by age, with high values in G1 and G2. The high ALP activity in infant and juvenile animals was probably due to high osteoblastic activity during growth (Fernandez and Kidney, 2007). The effect of age on ALP has also been reported in non-human primates (Takeshita et al., 2011), dogs (Rosset et al., 2012), cats (Levy et al., 2006), and pigs (Arantes et al., 2007). Therefore, reference ranges for this enzyme should be established for different age groups. In addition to the effect of age, Lochmiller et al. (1986) reported that the quality of food influences ALP results, with enzyme activity being higher in malnourished animals.

The concentrations of total protein and urea were low in infant animals and adults. The levels of these metabolites can be used to evaluate the nutritional status of an animal (Lochmiller et al., 1986), and the difference may have been due to differences in food intake between the groups. Despite the difference observed between the age groups, the values of urea and total protein were similar to those observed by other authors (Lochmiller et al., 1986; Schettini et al., 2005), and probably have no clinical significance. The high phosphorus levels observed in infants may be related to the greater need for this mineral during early growth phases (Andriguetto et al., 1981). Phosphorus plays a vital role in animal metabolism, and promotes the rapid, efficient, and proper development of bones and teeth (Arouca et al., 2009). Nogueira-Filho et al. (2006) stated that growing peccaries need a diet with more phosphorus and calcium than maintenance-level requirements.

The hematological and biochemical parameters of captive *T. tajacu* were determined and could be used as a reference. The gender of the animals had no effect on the parameters analyzed, but age did. However, only changes in ALP activity were sufficiently significant to establish different reference ranges for different age groups.

Conflicts of interest

The authors declare no conflict of interest.

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