

## Biochemical composition of the hoof capsule of buffaloes and its influence on hoof quality

[Composição bioquímica do estojo córneo de bubalinos e sua influência na qualidade do casco]

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### ABSTRACT

The purpose of this study was to establish the biochemical parameters of the abaxial wall, dorsal wall and sole of the hoof of the medial thoracic, lateral, and medial pelvic digits of buffalos. The hoof samples were subjected to destructive biochemical analyses to identify the dry material (DM), mineral matter (MM), organic matter (OM), crude protein (CP) and ether extract (EE) contents. Sulfur (S), calcium (Ca), potassium (K), phosphorus (P), zinc (Zn) and copper (Cu) levels were determined based on nondestructive biochemical analyses. The parameters of dry material, mineral matter, organic matter, crude protein and ether extract of hoof capsule of the digits of buffalos can be determined by means of both destructive and nondestructive biochemical analysis. In addition, this study revealed that the highest concentrations of DM, CP and minerals such as, K, Zn and Cu are concentrated in the digits that bear the greatest body mass weight, suggesting that there is a positive correlation between the aforementioned parameters and the strength and growth of the hoof capsule in the digits. As for the element S, this study demonstrated that its highest concentration is located in the lateral digits of the pelvic members.

Keywords: buffaloes, keratinized epidermis, minerals, crude protein

### RESUMO

*O presente estudo objetivou estabelecer os parâmetros bioquímicos da muralha abaxial, muralha dorsal e sola do casco dos dígitos torácico medial e pélvico lateral dos bubalinos. Foram realizadas análises bioquímicas destrutivas das amostras dos cascos para se obterem os teores de matéria seca (MS), matéria mineral (MM), matéria orgânica (MO), proteína bruta (PB) e extrato etéreo (EE). As análises bioquímicas não destrutivas foram empregadas para se avaliarem os níveis de enxofre (S), cálcio (Ca), potássio (K), fósforo (P), zinco (Zn) e cobre (Cu). Concluiu-se que os parâmetros matéria seca, matéria mineral, matéria orgânica, proteína bruta e extrato etéreo do estojo córneo dos dígitos de bubalinos podem ser definidos por análises bioquímicas destrutivas e não destrutivas. Os maiores valores dos elementos minerais estão concentrados nos dígitos que suportam o maior peso do animal, sugerindo que existe uma relação positiva entre esses parâmetros. Além disso, este estudo revelou que as maiores concentrações de MS, PB e minerais como, K, Zn e Cu estão nos dígitos que carregam o maior peso de massa corporal, o que indica que há uma correlação positiva entre os parâmetros acima referidos, resistência e crescimento do estojo córneo dos dígitos. Em relação ao elemento de S, este estudo demonstrou que a sua maior concentração situa-se nos dígitos laterais dos membros pélvicos.*

Palavras-chave: búfalo, epiderme queratinizada, minerais, proteína bruta

### INTRODUCTION

Basic studies aimed at gaining a better understanding of the morphology, cellular and biochemical composition of the ruminant hoof, especially that of buffaloes, are scanty and

therefore deserving of the attention of researchers. This is an interesting subject, given that so little is known about the cellular and biochemical events involved in the formation and composition of the hoof capsule of buffaloes, which, in theory, render this species more resistant to foot diseases (Campos, 2012; Silva et

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*al.*, 2015). Thus, by clarifying some of the structural aspects of the hoof capsule of the digits of this species, this knowledge can presumably be employed to improve the hoof quality and reduce the vulnerability to foot diseases of some bovine species. This subject is controversial, but new scientific research involving buffaloes may represent advances in the study of the pathophysiology of foot diseases in other animal species.

In cattle, the quality and strength of the hoof capsule can be influenced by metabolic, hormonal, genetic, environmental and nutritional factors (Bajanowski *et al.*, 2001; Ferreira *et al.*, 2005; Muelling, 2009; Scholey *et al.*, 2012). Among the nutritional factors, fatty acids, minerals, vitamins and amino acids participate in the formation of the hoof capsule of the digits. A sulfur-containing amino acid that is particularly important is cysteine, which is essential for the formation of a good quality hoof capsule (Tomlinson, *et al.*, 2004; Ferreira, *et al.*, 2005; Bertenchini, 2013).

This study aimed to quantify parameters of the biochemical composition of the abaxial wall, dorsal wall and prebulbar region of the sole of the hoof capsule of the medial and lateral digits of the pelvic and thoracic limbs of buffaloes.

## MATERIAL AND METHODS

The research was conducted in the Department of Large Animal Surgery and Laboratories of Physics and Food Analysis at the Federal University of Goiás at Jataí, GO, Brazil, from June 2014 to August 2015. The research project was approved by the Ethics Committee on Animal Use, Office of the Vice Dean for Research and Innovation of UFG (CEUA-PRPI-UFG), under Protocol No. 20/2014.

The analyses involved 56 hoof capsules taken from 14 female adult, pigmented, Jafarabadi buffaloes, 24 to 60 months old, pasture raised, without concentrated dietary supplementation, totaling 112 lateral and medial digits distributed equally between thoracic and pelvic limbs.

Duplicate samples, with approximate dimensions of 10mm x 10mm, were collected from the keratinized tissue of the hoof, dorsal wall, abaxial wall and prebulbar sole of the medial

third of each digit. The samples were cleaned to remove all traces of the laminar corium and dirt. The clinical specimens were then stored in plastic bags and frozen at -15°C.

The biochemical composition of the hoof was evaluated by the destructive technique, which required grinding the samples, and by the non-destructive technique, which did not require fragmentation of the material. The destructive technique (Silva and Queiroz, 2002) was applied in the analysis of dry material (DM), mineral matter (MM), crude protein (CP) and ester extract (EE). The technique consisted of grinding the samples in a Marconi MAO 48 micro-mill and sifting the ground material through an intermediate mesh sieve to obtain fragments of approximately 0.5mm and a standard minimum weight of two grams.

Before analyzing the dry material (DM), the samples were weighed on a precision balance, placed in a ceramic crucible of known weight, and oven-dried at 105°C for two hours. To stabilize the samples, they were then placed in a glass desiccator with silica and left there for 40 min, after which they were weighed again. The value of DM was determined based on the difference between the original weight of the samples and the second weight.

The mineral matter (MM) was analyzed after analyzing the dry material. To this end, the same samples were heated in a JUNG muffle furnace at 600°C for four hours. The furnace was then turned off and the samples were left in it while the temperature gradually decayed to 200°C. The samples were then oven-dried at 105°C for one hour, followed by 40 min of storage in the glass desiccator containing silica. Finally, the material was weighed again and the value of MM was determined based on the difference in weight between the DM and the weight of the samples after this procedure.

To analyze the crude protein (CP), the samples were preweighed and placed in a test tube containing a 10:1 ratio of sodium sulfate + copper sulfate, which acted as a catalyst. Five ml of pure sulfuric acid was then added to each sample, and the samples were placed in a block digester, where they were left for 1.5 hours. The digester temperature was gradually increased from 70°C to 400°C. The samples were held at

this temperature until their color changed from black to green. The mixture was then allowed to rest for 20 min, after which 20 ml of distilled water were added to each sample. The material was then distilled in a nitrogen distiller for three min until it was reduced to 100 ml of distillate. It was then titrated with HCl until the color of the distillate changed from yellow to pale pink. The amount of HCl used in titration was converted to (CP) relative to (DM).

For the analysis of the ester extract (EE), the samples were first weighed and then wrapped in a 15 cm envelope of qualitative-grade filter paper cartridge and inserted into a previously weighed volumetric flask. The flask was then coupled to an extractor and a condenser (Soxhlet system) and washed intermittently with 200 mL of petroleum ether solvent, at heating level three, for four hours. The cartridge was then removed and the solvent was refluxed, and still under heating, up to 0.1 cm of solvent was recovered from the volumetric flask. The flask was placed in an oven heated to 105°C for one hour, and then in the glass desiccator with silica for 40 min. After this step, the flask was weighed again, and the value of (EE) was determined based on the difference between the flask's initial and final weight.

The minerals were subjected to a non-destructive analysis to quantify the contents of sulfur (S), calcium (Ca), potassium (K), phosphorus (P), zinc (Zn) and copper (Cu) by energy dispersive X-ray fluorescence spectroscopy (EDXRF), using a Shimadzu EDX-720 spectrometer equipped with a rhodium (Rh) tube (Bajanowski, 2001). This device detects mineral elements in the range of sodium (Na) to uranium (U) and provides a fluorescence spectrum showing the energy peaks released by each element, which are identified by a specific program and then quantified according to the chosen parameters. The data files provided by the spectrometer were saved in text files (TXT) and transformed into data files (DAT). The angle ( $2\theta$ ) vs. count (u.a.) charts were prepared using graphics software.

The findings were analyzed using SigmaPlot 12.0<sup>®</sup> software. The means were compared

statistically, using the *t*-test for two means and ANOVA for three means, at a 5% level of significance (Sampaio, 2010).

## RESULTS

The results of the biochemical analysis of dry materials (DM), mineral matter (MM), organic matter (OM), crude protein (CP) and ester extract (EE) of the hoof capsule of the digits of buffalos obtained by the destructive method are listed in Table 1. Mean values of 85.55% DM, 0.73% MM, 99.23% OM, 91.67% CP and 0.87% EE were found for the digits of the thoracic and pelvic limbs.

A comparison of the means of the parameters evaluated for the thoracic and pelvic limb digits showed values of 87.2% (DM) and 93.2% (CP) for the medial digits and 84.5% (DM) and 90.1 (CP) for the lateral digits. The thoracic medial digits showed higher values for DM and CP than the lateral digits ( $P < 0.05$ ).

The mean values of DM, MM, OM, CP and EE of the lateral and medial digits of the pelvic limbs, of the lateral digits of the thoracic and pelvic limbs, and of the medial digits of the thoracic and pelvic limbs, were compared and revealed no significant difference ( $P > 0.05$ ).

Table 2 shows the results of the biochemical analysis and quantification of the minerals sulfur, calcium, potassium, phosphorus, zinc and copper found in the hoof capsule of buffaloes. The mean values found in the digits of the thoracic and pelvic limbs were: 83.53% S, 8.13% Ca, 3.47% K, 3.74% P, 0.81% Zn and 0.31% Cu.

A comparative analysis of the mean percentages of minerals found in the lateral and medial digits of thoracic limbs and the lateral and medial digits of the pelvic limbs revealed no statistically significant differences. However, phosphorus showed a statistically significant difference in the comparison between the two mean percentages, with the lateral digits of the pelvic limbs showing higher percentages of phosphorus than the medial digits ( $P < 0.05$ ).

Table 1. Biochemical analysis and quantification of the dry material, mineral matter, organic matter, crude protein and ester extract found in the hoof capsule of buffaloes, using a destructive technique

DIG/ ARH	Biochemical Composition – Destructive technique				
	DM	MM (%DM)	OM (%DM)	CP (%DM)	EE (%DM)
TL	85.9±1.77a	0.66±0.10a	99.3±0.20a	91.6±2.45a	0.87±0.03a
PL	85.2±0.99a	0.54±0.04a	99.5±0.10a	91.7±2.57a	0.90±0.02a
TL-LD	84.5±1.07a	0.71±0.20a	99.3±0.20a	90.1±2.41a	0.77±0.02a
TL-MD	87.2±1.02b	0.60±0.10a	99.4±0.10a	93.2±1.42b	0.92±0.08a
PL-LD	85.1±0.32a	0.49±0.05a	99.5±0.05a	92.2±2.80a	0.89±0.01a
PL-MD	85.3±1.52a	1.12±0.09a	98.9±0.95a	91.3±2.83a	0.92±0.02a
TL-LD	84.5±1.07a	0.71±0.20a	99.3±0.20a	90.1±2.41a	0.77±0.22a
PL-LD	85.1±0.32a	0.49±0.05a	99.5±0.05a	92.2±2.80a	0.89±0.011a
TL-MD	87.2±1.02a	0.60±0.10a	99.4±0.10a	93.2±1.42a	0.91±0.08a
PL-MD	85.3±1.52a	1.12±0.95a	98.9±0.95a	91.3±2.83a	0.91±0.02a
DW	85.9±1.78a	0.77±0.20a	99.2±0.12a	91.0±1.55a	0.89±0.03a
ABW	85.0±1.86a	0.54±0.01a	99.5±0.14a	91.7±1.33a	0.96±0.04a
SOL	85.8±0.29a	0.53±0.02a	99.5±0.20a	92.3±2.45a	0.87±0.02a
MEAN	85.55	0.73	99.23	91.67	0.87

DM–dry material, MM – mineral matter; OM– organic matter; CP– crude protein; EE – ester extract; DIG – digits; ARH – Anatomical region of the hoof; TL – thoracic limb; PL – pelvic limb; TL-LD – thoracic limb lateral digit; PL-LD – pelvic limb lateral digit; TL-MD – thoracic limb medial digit; PL-MD – pelvic limb medial digit; DW – dorsal wall; ABW– abaxial wall; SOL – sole. Means followed by different letters in the same column and the same variable are significantly different ( $p<0.05$ ). Means followed by the same letters in the same column and the same variable do not differ ( $P>0.05$ ).

Table 2. Biochemical analysis and quantification of the minerals sulfur, calcium, potassium, phosphorus, zinc and copper found in the hoof capsule of buffaloes, using a non-destructive technique.

DIG/ RC/LA	Biochemical Composition – Non-destructive technique					
	S	Ca	K	P	Zn	Cu
TL	82.8±3.51a	8.70±2.41a	3.53±1.51a	3.85±0.56a	0.79±0.13a	0.31±0.04a
PL	84.2±2.38a	7.57±1.08a	3.41±1.83a	3.64±0.39a	0.82±0.07a	0.31±0.03a
TL-LD	81.0±1.74a	10.3±1.51a	3.44±1.66a	4.24±0.41a	0.73±0.16a	0.30±0.03a
TL-MD	84.7±4.22a	7.10±2.15a	3.62±1.69a	3.46±0.39a	0.85±0.08a	0.30±0.05a
PL-LD	84.5±0.73a	7.21±0.86a	3.18±1.12a	3.96±0.05b	0.83±0.09a	0.33±0.04a
PL-MD	84.3±3.71a	7.92±1.33a	3.63±2.64a	3.32±0.29a	0.82±0.05a	0.29±0.02a
TL-LD	81.0±1.74a	10.3±1.51b	3.44±1.66a	4.24±0.41a	0.73±0.16a	0.31±0.03a
PL-LD	84.5±0.73b	7.21±0.86a	3.18±1.12a	3.96±0.05a	0.83±0.09a	0.33±0.04a
TL-MD	84.7±4.22a	7.10±2.15a	3.62±1.69a	3.46±0.39a	0.85±0.08a	0.30±0.05a
PL-MD	84.3±3.71a	7.92±1.33a	3.63±2.64a	3.32±0.29a	0.82±0.06a	0.29±0.02a
DW	86.2±2.66a	6.93±1.80a	2.07±0.49a	3.61±0.52a	0.91±0.31b	0.27±0.01a
ABW	82.2±2.17a	9.25±1.87a	3.40±1.35b	4.00±0.50a	0.84±0.42ab	0.34±0.02b
SOL	82.2±2.42a	8.22±1.61a	4.94±1.33b	3.63±0.42a	0.74±0.32a	0.32±0.04b
MEAN	83.53	8.13	3.47	3.74	0.81	0.31

S – sulfur; Ca – calcium; K – potassium; P – phosphorus; Zn – zinc; Cu – copper; DIG – digits; RC – Region of the hoof; LA – anatomical location; TL – thoracic limb; PL – pelvic limb; TL-LD – thoracic limb lateral digit; PL-LD – pelvic limb lateral digit; TL-MD – thoracic limb medial digit; PL-MD – pelvic limb medial digit; DW – dorsal wall; ABW – abaxial wall; SOL – sole. Means followed by different letters in the same column and the same variable are significantly different ( $P<0.05$ ). Means followed by the same letters in the same column and the same variable do not differ ( $P>0.05$ ).

A comparison of the lateral digits of the thoracic and pelvic limbs showed no significant differences between the mean percentages of K, P, Zn and Cu. However, the lateral digits of the thoracic and pelvic limbs showed a statistically significant difference between S and Ca. In addition, no statistically significant differences were found in the comparison of the mean percentages of S, Ca, K, P, Zn and Cu ( $P>0.05$ ) in the medial digits of the thoracic and pelvic limbs.

Comparing the mean percentages of minerals found in the regions of the dorsal wall, abaxial wall and sole, a statistically significant difference was observed ( $P<0.05$ ). The results indicated that the abaxial wall and sole had a higher content of K than the dorsal wall. Moreover, the dorsal wall was also found to have higher content of Zn than the sole. The data also indicated that the abaxial wall and sole had a higher content of Cu than the dorsal wall.

## DISCUSSION

For starters, an interesting finding was that the dorsal wall, abaxial wall and sole of the hoof capsule of the medial digits of the thoracic limbs contained larger amounts of crude protein (CP) and higher percentages of dry materials (DM). This finding may be attributed to the fact that a greater portion of the animal's body weight is supported by these digits than by the lateral digits, thus requiring greater strength of the hoof capsule. Other researchers have presented similar findings, reporting that the medial digits of the thoracic limbs bear a greater burden of the animal's body weight and that this could influence the strength of the hoof capsule (Schaller, 1999; König and Liebich (2004); Greenough, 2007; Campos, 2012).

It was impossible to determine to what extent the pigmentation of the hoof capsule of the analyzed digits influences the concentration of the biochemical elements investigated here, because all the buffalo hooves examined in this study were pigmented. The literature consulted in this study did not provide information about biochemical analyses of the hoof capsules of buffaloes or cattle, pigmented or depigmented. However, research on the biochemical composition of the hooves of Mangalarga Marchador horses, conducted by Slaater *et al.*

(1997), Nascimento (1999) and Faria *et al.* (2005), revealed that pigmentation did not have a significant effect on the biochemical concentrations in the samples.

Based solely on the individual influence of the mineral elements and pigmentation on the strength of the hoof capsule of the thoracic limb, it is impossible to establish a positive correlation between these two parameters. This finding is corroborated by the fact that lower levels of phosphorus were identified in the medial digits of these limbs, which bear a greater portion of the animal's weight, according to Schaller (1999) and Campos (2012). However, Faria *et al.* (2005) found a higher concentration of MM, particularly phosphorus, in the hooves of Pantaneiro horses than in those of Mangalarga Machador horses, and suggested that this could influence the quality and strength of the hoof, although they did not explain whether their analysis involved the fore or hind limbs. This indicates that there are different interpretations of the subject and explains the importance of this study.

It is therefore very likely that the effect of phosphorus on hoof strength can be influenced by other minerals that act simultaneously. Authors such as Grosenbaugh and Hood (1992) did not describe the conditions needed for the action of phosphorus to give the hoof maximum strength. However, they stated that this is an important element in the formation of the cementing substance in the hoof because it is rich in phospholipids, which binds its protein envelopes.

With respect to the statistical difference between the concentration of phosphorus in the lateral and medial digits of the pelvic limbs, unlike what was found in the thoracic limbs, a higher concentration of this element was found in the lateral digits, which bear more of the animal's weight. Nevertheless, a comparison of the concentrations of this element in the medial and lateral digits of the thoracic limbs and in the lateral digits of the pelvic limbs showed similar values. Although these findings are not explained by consistent scientific reasoning, it is believed that it may have to do with the way these limbs are inserted in the animal's body structure and their ability to absorb impact when they touch the ground (Van Der Tol *et al.*, 2003).

Because the thoracic limbs are not inserted in the animal's thorax through joints, the hoof causes less impact as it touches the ground. In the pelvic limbs, the hip joint dampens this impact less. Vermunt and Greenough (1995) stated that a higher occurrence of digital diseases, including white line disease, digital dermatitis, heel erosion, and sole ulcers were related with this type of insertion of the pelvic limb in the animal's body structure. Romani *et al.* (2004) and Tomasella *et al.* (2014) stated that the higher incidence of foot diseases is identified in the pelvic limbs. Thus, given the lower phosphorus content in the digits of the pelvic limbs, it can be inferred that this element, *per se*, probably exerts little influence on the hoof strength of these limbs.

Upon analyzing the levels of sulfur, the highest concentration of this element was found in lateral digits of the pelvic members that bear a greater body mass weight and withstand greater weight-bearing impacts. It is known that the pelvic members have synovial joints, also known as diarthrosis, *i.e.*, movable joints that connect bone to bone, increasing the impact on the digits of these members. Conversely, the thoracic members have fibrous joints, which decreases the impact on digits (Ferreira *et al.*, 2005; Campos, 2012). Sulfur is known to be an important structural component of sulfhydryl amino acids such as methionine, cystine and cysteine, which are essential for the formation of keratin, the main constituent of the hoof capsule. These amino acids are linked by disulfide bonds, forming a very tough three-dimensional network (Schrooyen *et al.*, 2001), which partly explains the higher levels of this element in the digits that bear a greater portion of the animal's weight.

The higher concentrations of zinc (Zn) in the regions of the dorsal and abaxial wall may be related to the more intense growth of these two regions than that of the sole. Given that zinc is an important element in the synthesis of keratin and that intense cell proliferation occurs in the coronary corium, it is likely that there is a higher growth rate in the wall of the hoof capsule of the digits than in other regions of the hoof. Barsuto *et al.* (2008) demonstrated that the chelated form of Zn in the diet of animals triggers the formation of the zinc methionine complex, favoring greater growth of the hoof capsule and organization of the morphology of the horn

tubules. Thus, one can not overlook the fact that the quality of buffalo hooves may benefit from higher levels of Zn in the walls of the digital hoof capsule.

It is known that copper is an important mineral to ensure the strength of the hoof capsule, and that its concentration may result in better hoof quality. No information about this element in cattle hooves was found in the literature (Mulling *et al.*, 1999; Faria *et al.*, 2005; Basurto *et al.*, 2008; Muelling, 2009); hence, it can be inferred that the concentrations of copper (Cu) found in the buffalo hooves of this study can be used as a biochemical parameter for comparisons with other ruminants. The notion that Cu is important to hoof quality is reinforced by studies conducted by Hoblet and Weiss (2001) and Lana (2007), who reported that this element is active in different physiological processes such as the synthesis of melanin and the formation of sulfhydryl groups, among them methionine, cysteine and cystine, which increase the hoof's strength.

Lastly, the findings about the biochemical composition of buffalo hooves add to the body of knowledge about the structure of these animals' hooves, which can be used for extrapolations to other species or for comparative analysis, including cattle and horses. Another positive aspect was the possibility of associating the dry material, mineral matter, organic matter, crude protein and ester extract with the strength of the digits that bear the greater portion of the animal's body weight and the impact when the hooves touch the ground. Thus, as buffaloes are considered a species resistant to foot diseases, the analyzed parameters and the knowledge gained in this study can guide other studies, particularly those involving cattle breeds that are more susceptible to diseases of the digits.

## CONCLUSIONS

The parameters of dry material, mineral matter, organic matter, crude protein and ester extract in the hoof capsule of the digits of buffaloes can be defined by destructive and nondestructive biochemical analyses. The highest contents of mineral elements are concentrated in the digits that bear the greater portion of the animal's weight, suggesting that there is a positive relationship between these parameters and the

strength and growth of the hoof capsule of the digits.

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