

URINALYSIS, URINARY PROTEOME AND METABOLOME OF ZOO-HOUSED GIRAFFES (*Giraffa camelopardalis*) THROUGH NONINVASIVE SAMPLING METHOD

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Abstract

The study of non-domestic animals withholds more difficulties compared to the domestic counterpart, thus using noninvasive techniques to collect biological samples might play an important role in assessing the health status of wild animals.¹ The present study established the reliability of urine sampling from the ground. A preliminary study was run with 10 urine samples of 10 cows (*Bos taurus*) housed in a dairy farming in Northern Italy. Urine samples, collected both in sterile cups and from the ground, were analyzed and compared. Results revealed no statistical differences in the variables investigated ($p > 0.05$, dipstick parameters and USG, protein quantification and UPC and protein electrophoresis), which proved the reliability of this noninvasive sampling method. This method was used for sampling 103 urine samples from 44 zoo-housed giraffes (*Giraffa camelopardalis*) of four Italian zoos. Urine samples were used to establish the urinalysis reference values in this species and to study the urinary proteome for the first time. The urinary reference values reported as median (lower and upper limit) were: urine specific gravity (USG), 1.030 (1.006 - 1.049); urine total proteins (uTP), 17.58 (4.54 – 35.31) mg/dL; urine creatinine (uCr), 154.62 (39.59 – 357.95) mg/dL; urine protein: creatinine ratio (UPC), 0.11 (0.07 – 0.16). In giraffes, most urinary proteins had a low molecular mass (MM) and were present in low quantities. Proteomics disclosed fifteen different proteins, which were involved in the defense against microbes and in the ability of giraffes to concentrate urine. Albumin, lysozyme C, and ubiquitin were the most represented urinary proteins in giraffes. In addition, to define the urinary metabolome profile, 35 urine samples from 35 zoo-housed giraffes of five Italian zoos were used. Metabolomics allowed to identify and quantify 39 molecules and the most represented metabolites were hippurate, creatinine and phenylacetyl glycine. This analysis provided information on physiological adaptations of giraffes. Besides, urinary metabolites were influenced by sex: urinary metabolome profile of female showed higher level of acetate, succinate, and lactate, conversely hippurate, phenylacetyl glycine, and thymine were more concentrated in male urines. Similarly, the age affected the concentration of three urinary metabolites, namely formate, alanine, and valerate.

Key words: *Bos taurus*; *Giraffa camelopardalis*; giraffes; proteomics; metabolomics; urine; zoo

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