Determination of Enzyme Activity, Proteinuria and Creatininuria in Bovine Urine

D. Proverbio,* A. Belloli,* G. Greppi,* G. Vacirca,* V. Grieco°
*University of Milan, Italy, Veterinary Pathology and Medicine Dept.**
(Director Professor G. Vacirca)
Pathological Anatomy and Poultry Pathology Dept.°
(Director Professor G. Mandelli)

Summary

The Authors examined the presence of NAG, gamma GT, LDH, alpha HBDH and activity in the urine of healthy cattle. They reported urinary reference values of these enzymes, of proteinuria and creatininuria. Tests were supported with post-mortem and histological examinations of kidneys.

It is known that urinary diseases, especially those of the kidneys, can cause some alteration of urinary enzyme activity.[1,3,5,6,8,9]

Only a little is known of enzyme activity in cow's urine and about their utility and clinical application. The purpose of this study is to prove the urinary activity of N-acetil-glucosaminidase (NAG), gamma Glutamil-Transferase (GGT), Latic Dehydrogenase (LDH), alpha-Hydroxy Butyrodehydrogenase (HBDH) and Alkaline Phosphatase (AP) in healthy cows' urine.

At the same time we have shown physiological reference values both of enzymes activity and of proteinuria and creatininuria. Analyses have been supported with post-mortem and histological examination of kidneys.

Methods

The object of our studies were 67 female Italian Friesian cows between 4 and 7 years old, destined to slaughter houses. Before slaughtering, we subjected all the subjects to clinical visits and haematological examinations with test tubes without anticoagulant.

The blood samples were centrifugated at 3000 rpm for 10 minutes and sera obtained were kept at +4°C. Within 5 hours after the blood samples were collected, we determined serum levels of creatinine, urea, total protein, LDH, alpha HBDH, gamma-GT and AP. (Table 1)

After slaughtering we took urine samples that were kept in sterilized containers at +4°C until the moment of testing.

On urine samples we determined complete physical and clinical examination, the values of creatinine and microprotein and the enzyme activity of NAG, LDH, alpha HBDH, gamma-GT and AP.

The right procedure to measure enzyme activity and urinary protein determined in single urine samples rather than in 24 hour urine collections needs correlation of values with urinary creatinine.

In fact, urinary creatinine excretion in the presence of a stable glomerular filtration rate is constant in 24 hour and depends only on urinary volume and body size; therefore it is possible to correlate the urinary protein and the enzyme values with creatininuria and cancel out the "volume factor".

In short, at the end of slaughtering we took both kidneys from each subject and post-mortem macroscopic and microscopic examinations were made which permitted us to identify 21 subjects with kidneys without macroscopic important reports (WMR) and with 3 different histological types of reports (colored with hematoxylineosin) that we had divided in 3 classes:

FIRST-CLASS:	5 couples of kidneys histological examination: both WMR
SECOND-CLASS:	5 couples of kidneys histological examination: only one kidney in each couple had lymphocyte cells spread into cortical interstices
THIRD-CLASS:	11 couples of kidneys histological examination: in both couples of kidneys lymphocyte cells had spread into cortical interstices.

The presence of lymphocytes and plasma cells in the kidney's cortical interstice of the second and third class was so low that it did not change the renal parenchima's cell architecture and it did not cause damage to tubular and glomerular structures that could change the urine's physical-chemical structure.

Table 1.

	METHODS
U R E A S.	Enzymatic colorimetric method modified Berthelot's reaction
CREATININE S. / U.	Colorimetric Kinetic method with alkaline picrate
TOTAL PROTEIN	Biuret method
MICROPROTEINURIA	Blue Coomassie method
ALKALINE PHOSPHATASE S. / U. (AP)	p-Nitrophenylphophate kinetic method
GAMMA-GLUTAMIL-TRANSFERAS S. / U. (Gamma - G T)	Colorimetric kinetic method
LACTIC DEHYDROGENASE S. / U. (LDH)	Optimized standard method conforming to DGKCH
alpha HYDROXY BUTYRODEHYDROGENASE S. / U. (alpha-HBDH)	Optimized standard method conforming to DGKCH
N-ACETIL-ß-GLUCOSAMINIDAS U. (NAG)	Colorimetric method

Results

Table 2 shows the average, the standard deviation, (calculate with abacus concepts STAT WIEW II program) and serum reference values calculated on the 21 examined subjects, that are normal for the cow.[7,11,13]

Table 3 shows average values of urinary enzyme activity and microproteinuria correlated with creatininuria on the 3 different classes of kidneys. The table does not show any distinction among the 3 classes.

Table 4 shows the average, the standard deviation and the reference values of urinary enzyme activity and microproteinuria correlated with creatininuria on all 21.

Discussion

The urinary activities considered by us and microproteinuria are present in healthy calves' urine.

We showed just as an indication the same reference values are based on the haematological examinations and above all on the macroscopic and histological post mortem examination of both kidneys. The histological examination on the same kidneys has shown the same abnormalities but has excluded that these were so considerable as to impair the test results. In fact there were no lesions that could determine tubular cell damage with enzyme release. Also a glomerular damage responsible for proteinuria and a tubular deficit limiting microprotein re-absorption were excluded.

Table 2. Average (X), standard deviation (Dst) and serum reference values (R.V.) calculated on the 21 examined subjects.

	$\bar{X}$	Dst	R.V.
CREATININ mg/dl	1.182	0.236	1.182 ± 0.236
U R E A mg/dl	23.118	9.903	23.118 ± 9.903
A P U / l	59.727	34.856	59.727 ± 34.856
γ G T U / l	15.045	4.695	15.045 ± 4.695
L D H U / l	1492.182	437.493	1492.182 ± 437.493
alpha-HBDH U / l	846.364	342.037	846.364 ± 342.037
PROT. TOT. g / dl	8.291	0.616	8.291 ± 0.616

Table 3. Average values of urinary enzimatic activities and Microproteinuria correlated with creatininuria on the 3 different classes.

	$\bar{X}$ 1^CLASSE	$\bar{X}$ 2^CLASSE	$\bar{X}$ 3^CLASSE
NAG/C	0.23	0.33	0.15
AP/C	0.53	0.06	0.35
γGT/C	0.59	0.89	0.53
LDH/C	2.53	1.67	1.20
α-HBDH/C	0.33	0.20	0.32
MICROP./C	0.25	0.23	0.50
C	5.65	11.82	13.39

CREATININURIA (C) : mmoli/L
ENZYMES : U/L
MICROPROTEINURIA : mg/dl

Table 4: Average (X), standard deviation (Dst) and the reference values (R.V.) of urinary enzimatic actyvities and microproteninuria correlated with creatininuria on all the 21 subjects.

	$\bar{X}$	Dst	R.V.
NAG/C	0.216	0.234	0.216±0.234
AP/C	0.325	0.437	0.325±0.437
γGT/C	0.627	0.515	0.627±0.515
LDH/C	1.755	1.864	1.755±1.864
α-HBDH/C	0.410	0.680	0.410±0.680
MICROP./C	0.221	0.198	0.221±0.198
C	11.607	7.616	11.607±7.616

CREATININURIA (C) : mmoli/L
ENZYMES : U/L
MICROPROTEINURIA : mg/dl

It is interesting to note that among 67 cows without any symptoms of kidney damage at clinical and haematological examination, only 21 had no macroscopic and histological kidney damages at post mortem examination. These data confirm that bovine kidney pathology often takes a subclinical course that makes the clinical suspicion difficult.

We would like to specify that kidney parenchymal damage may cause a change in urinary enzyme activity but not in serum activity that changes only when the lesion has already invaded most of the kidney's parenchyma.[7]

Following our results we hope to use urinary enzyme activity and microproteinuria measurements to quickly identify kidney damage and in particular a tubular lesion as in human medicine.[2,10,12,14]

In fact, it is known that in the absence of inflammatory causes or urogenital neoplasias, the origin of urinary enzymes is almost exclusively tubular and that the presence of urinary low molecular weight protein is due to glomerular lesion with tubular re-absorption deficit.

Finally, we want to point out the microprotein/creatinine ratio that in our case was about 0,4. Grauer (1992) considers this ratio as "diagnostic index" in the dog to determine subjects with subclinical kidney pathology.

KEY WORDS: cattle, enzymuria, reference values

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