EFFECT OF AN UNBALANCED CA/P DIET ON BLOOD PARAMETERS AND UROLITHIASIS IN GROWING CALVES

Matteo Gianesella[1], Elisabetta Giudice[2*], Vanessa Messina[3], Chiara Cannizzo[1], Emanuele Florian[4], Giuseppe Piccione[3], Massimo Morgante[1]
[1]*Dipartimento di Scienze cliniche Veterinarie, Università degli Studi di Padova-Italy*
[2]*Dipartimento di Sanità Pubblica Veterinaria, Università degli Studi di Messina- Italy*
Phone +39 090 3503739; e-mail: elisabetta.giudice@unime.it
[3]*Dipartimento di Scienze Sperimentali e Biotecnologie Applicate, Università degli Studi di Messina-Italy*
[4]*AZOVE, Società Agricola Cooperativa , Ospedaletto Euganeo- Padova- Italy*
[*]*Corresponding author*

Summary. The influence of minerals on the urolithiasis syndrome is characterized by high phosphorus (P), calcium (Ca), magnesium (Mg), urea and creatinine serum levels. The aim of this study was to evaluate the influence of an unbalanced Calcium /Phosphorus (Ca/P) diet on blood parameters in beef cattle with a high risk of urolithiasis. Sixty-eight beef calves were divided into 2 groups on the basis of their age (40-days old and one year old calves). The two groups, A and B, were monitored during 3 different periods, lasting each 40 days and, during the first two periods, the animals were fed according to the farming practices. Then they were fed with two different diets from 80th to 120th days. The first diet was characterized by a Ca/P ratio of 1:3 (moisture 13.4%) and the second one was constituted by an elevate moisture percentage (28.71%), and a Ca/P ratio of 1:1. During the three periods blood samples were collected to assess serum urea, creatinine, Ca, P, Mg and potassium (K). This study suggests that Ca/P ratio in the diet plays an important role in reducing the occurrence of mineral calculi in beef cattle, and that a good balanced diet could have an important preventive role for this pathology.

Keywords: Ca/P ratio, urolithiasis, cattle, blood parameters, diet minerals.

PAŠARO SU NESUBALANSUOTU CA IR P KIEKIU POVEIKIS VERŠELIŲ KRAUJO SUDĖČIAI IR ŠLAPIMO PŪSLĖS AKMENŲ SUSIDARYMUI

Matteo Gianesella[1], Elisabetta Giudice[2*], Vanessa Messina[3], Chiara Cannizzo[1], Emanuele Florian[4], Giuseppe Piccione[3], Massimo Morgante[1]
[1]*Klinikinės veterinarijos institutas, Padujos universitas, Italija*
[2]*Visuomenės sveikatos fakultetas, Mesinos universitetas, Italija*
el. paštas: elisabetta.giudice@unime.it; tel. +39 090 3503739
[3] *Biotechnologijų ir eksperimentinės veterinarijos institutas, Mesinos universitetas, Italija*
[4] *AZOVE, Žemės ūkio kooperatyvas, Paduja, Italija*
[*]*Atsakingas autorius*

Santrauka. Šlapimo pūslės akmenys dažniausiai susidaro padidėjus fosforo (P), kalcio (Ca), magnio (Mg), šlapalo ir kreatinino kiekiui gyvulio kraujo serume. Atliktas bandymas, kurio metu tirtas pašaro su nesubalansuotu Ca irP kiekiu poveikis veršelių kraujo sudėčiai ir šlapimo pūslės akmenų susidarymui. 68 vyriškos lyties veršeliai ir buliukai buvo suskirstyti į dvi grupes pagal amžių, kiekvienoje grupėje buvo po lygų vienodo amžiaus gyvulių skaičių (40 amžiaus dienų ir vienerių metų). Veršeliai buvo tiriami trimis periodais, po 40 dienų kiekvienas. Pirmuosius du periodus (80 dienų) veršeliai ir buliukai buvo šeriami pašaru, atitinkančiu fiziologines normas. Nuo 80 iki 120 dienos A grupės veršeliai buvo šeriami pašaru, kur Ca ir P santykis buvo 1:3 (pašaro drėgnumas 13,4 proc.), o B grupės – pašaru su Ca ir P santykiu 1:1 (pašaro drėgnumas 28,7 proc.). Šlapalui, kreatininui, Ca, P, Mg ir kaliui (K) nustatyti kiekvieno periodo metu iš veršelių buvo imamas kraujas. Ištirta, kad Ca ir P santykis atrajotojų pašare yra labai svarbus veiksnys, lemiantis šlapimo pūslės akmenų susidarymą. Norint išvengti šios patologijos, reikalinga sisteminga šių medžiagų kiekio gyvulio kraujo serume kontrolė.

Raktažodžiai: Ca ir P santykis, šlapimo pūslės akmenys, galvijai.

Introduction. One of the most important and insidious frequent pathology in cattle is urolithiasis. Uroliths are pathological mineral concretions localized in the kidney or in another tract of urinary system (Shainduran et al., 2007). The urolithiasis is often associated to a dehydration status, but an unbalance diet supplement of minerals was seen to be the principal cause of the calculi formation (Tirunè, 2006). Usually the calculi formation results from a combination of physiological, nutritional and management factors and the food is one of the most important factor (Canzi, 2001; Ozmen, 2004; Sommardahal et al., 1997). In fact, an excessive intake of Ca, Mg and P take to an unbalance of these elements both in blood and urine, so to precipitate and form crystals and amorphous deposits (Ozmen, 2004). Stress can increase the excretion of Ca that associated to alkaline urinary pH represent an important source for the crystallization of original nuclei of calculi. Formation of phosphate calculi is encouraged by high concentrate, low roughage, low Ca/P ratio, high Mg diets

and alkaline urine (Canzi, 2001). In ruminants, P is recycled through saliva and excreted via feces (Canzi, 2001). High grain and low roughage diets decrease the formation of saliva and increase the amount of P excreted in the urine. The influence of these diet supplements on the urolithiasis syndrome is characterised by high P, Ca, Mg, urea and creatinine levels in serum (Ahmed et al.,1989). Many authors investigated urolithiasis frequency in cattle and the relationship between dietary Ca/P ratio and the onset of pathology. In lambs the level of P should not exceed 0.57% and Mg 0.28% (Rogers, 1999). The Ca/P ratio should be of 2/1 in cattle, a Ca/P imbalance results in high urinary phosphate excretion which is an important factor in the genesis of calculi (Rosol and Capen, 1997).

Moreover, in cattle was observed that excessive intake of Mg, over 1.4%, or Mg/P ratio more than 1.6%, facilitates the onset of urolithiasis (Rosol and Capen, 1997). It was found that elevated Ca serum levels can have a protective role in the insurgence of urinary calculi in growing calves (Kallfelz et al., 1987). High risk situations are represented by Ca/P relationship of equality (1:1) and by imbalanced ratio as Ca/P 1:2 (Canzi, 2001).

The aim of the present study was to evaluate the influence of an unbalanced Ca/P diet on blood parameters in beef cattle with an high risk of urolithiasis.

Materials and methods. Sixty-eight beef calves, mixed breed, from a farm with high incidence of urolithiasis were equally divided into 2 groups (A and B) on the basis of their age .The group A was composed by 40-days old subjects. The group B was constituted by one-year old calves. All animals were monitored for three periods, each lasting 40 days. Blood samples and urine collection were performed at the end of each period. During the periods 1 and 2 the animals were fed as standard farming practices. In the period 3, the two groups were further divided into two subgroups and were fed with two different diets. The first diet (D), was characterized by a C/P ratio of 1:3 (moisture 13.4%), the second diet (W), was a wet food with an elevate moisture percentage (28.71%) and with a Ca/P ratio of 1:1. During the three different periods the water was available *ad libitum*. In table 1 and 2 are shown the results of analysis composition of the foods administered as D diet and W diet. During the three periods in the morning, at the same hour (9.00), clinical conditions were regularly recorded. Immediately after collection urine specific gravity and pH were measured by means of a refractometer (Clinical Refractometer Cosmo R-308®) and by means of a portable pHmeter (Piccolo, Hanna Instruments, Leighton Buzzard, Bedfordshire, UK) respectively. On blood samples urea, creatinine, Ca, P, Mg and K levels were assessed by means of an automated analyzer (*BM Hitachi 911,* ROCHE, Basel, Switzerland).

Two-way repeated measure analysis of variance (ANOVA), followed by the Bonferroni's test, was applied to evaluate the influence of period and diet. *P values* <0.05 were considered statistically significant. Bonferroni's test was applied for post-hoc comparison. Unpaired Student's t-test was applied to evaluate the influence of age. All the data were analyzed using Statistica 7 software (Statsoft Inc.).

Table 1. **Chemical composition of the diet characterized by a low water content, called "D diet", expressed in percentage, with the Ca/P ratio**

Parameters	D diet
Ethereal Extract	87.40%
Ash	9.80 %
Ca	1.43%
Mg	0.32%
P	0.62%
K	1.22%
Ca/P ratio	1:3

Table 2. **Chemical composition of the diet characterized by an high water content, called "W diet", expressed in percentage, with the Ca/P ratio**

Parameters	W diet
Ethereal Extract	71.20%
Ash	6.80%
Ca	0.59%
Mg	0.22%
P	0.38%
K	1.20%
Ca/P ratio	1:1

Results. Table 3 shows the mean values of urinary parameters measured during our study, expressed in their conventional units of measurement, together with the standard error and the statistical significances. In tables 4 and 5 are shown mean values of the studied serum parameters, expressed in their conventional units of measurement, with standard error and the statistical significances.

The application of two-way ANOVA showed a significant effect of period on some parameter studied, both in groups A and B as follow: group A-creatinine ($F_{(2,64)}$=162.4; P<0.0001); Ca ($F_{(2,64)}$=51.78; P<0.0001); P ($F_{(2,64)}$=21.42;P<0.0001) and Mg($F_{(2,64)}$=7.04; P=0.0017); group B-specific gravity ($F_{(2,64)}$=3,71; p=0.0298); pH ($F_{(2,64)}$=3,28; p=0,0440); urea ($F_{(2,64)}$=7.19; P=0.0015); creatinine ($F_{(2,64)}$=59.41; P<0.0001); Ca ($F_{(2,64)}$=129.6; P<0.0001); P ($F_{(2,64)}$=41.18; P<0.0001); Mg ($F_{(2,64)}$=9.78; P= 0.0002) and K ($F_{(2,64)}$=6.16; P=0.0036). No statistical significant effect of period on urea and K, was observed in group A. A statistically significant effect of diet composition was observed only on urea serum levels both in group A ($F_{(2,64)}$=7.08;P=0.0121) and group B ($F_{(2,64)}$=34.30;P<0.0001).

The application of Student's t test showed a significant effect of age on urea (P<0.0001;t=11.4; df=32), creatinine (P=0.04;t=2.05;df=32), Mg (P<0.0001;t=5.77;df=32) and K (P=0.0016;t=3.43;df=32) in animals fed with the D diet, and a significant effect of age on urea (P<0.0001;t=7.99;df=32), Mg (P<0.0001;t=4.65;df=32) and K (P<0.0001;t=4.99; df=32) in animals fed with W diet.

Table 3. **Average values of the urinary parameters monitored during our experiment, expressed in their conventional units of measurement, with the standard error and the relative significance, for both groups** (A and B)**, during different feeding conditions** (administration of D and W diets)

Time	Groups	Specific Gravity	pH
D Diet			
Period 1 (40 days)	A	1009.90±1.86	6.98±0.23
	B	1025.80±3.22	7.46±0.28*
Period 2 (80 days)	A	1020.60±3.15	7.33±0.15
	B	1028.60±2.21	7.21±0.08•
Period 3 (120 days)	A	1016.80±2.35	7.82±0.02
	B	1025.00±2.21	7.72±0.11
W Diet			
Period 1 (40 days)	A	1017.80±2.60	7.07±0.16
	B	1019.90±4.80*•	7.29±0.11
Period 2 (80 days)	A	1020.70±3.60	7.09±0.13
	B	1022.60±0.23	7.78±0.06
Period 3 (120 days)	A	1018.90±2.76	8.07±0.08
	B	1024.50±1.90	7.68±0.12

Significance: *Vs Period 2;•Vs Period 3

Table 4. **Average values of the parameters considered, expressed in their conventional units of measurement with the related standard error and statistical significance observed in different experimental conditions in 34 beef cattle, fed with a diet with low water content** (D diet)

Parameters	Group A		
	Period 1 (40 Days)	Period 2 (80 days)	Period 3 (120 days)
Urea (mmol/l)	3.53±0.23•	3.70±0.30•	4.40±0.18
Creatinine (mmol/l)	129.4±6.52 *•	77.06±3.76	71.38±2.39
Ca (mmol/l)	2.50±0.02*	2.78±0.03•	2.56±0.02
P (mmol/l)	2.45±0.05*	2.82±0.11	2.64±0.07
Mg (mmol/l)	0.95±0.02	0.96±0.01	1.01±0.01
K (mmol/l)	4.67±0.05*	4.93±0.05•	4.62±0.06
Group B			
Urea (mmol/l)	3.67±0.32•	3.24±0.19•	2.10±0.15
Creatinine (mmol/l)	106.2±7.05*•	81.0±4.6	83.9±4.9
Ca (mmol/l)	1.9±0.1*•	2.54±0.02	2.65±0.02
P (mmol/l)	1.9±0.1*•	2.93±0.05•	2.59±0.10
Mg (mmol/l)	0.77±0.22*•	0.95±0.01	0.91±0.02
K (mmol/l)	4.45±0.08	4.17±0.06	4.28±0.09

Significance: * vs Period 2 ($P<0.0001$); • vs Period 3 ($P<0.05$)

Discussion. Our results showed that both age and diet have an influence on serum electrolyte levels. Relatively to age, an adaptation process of urea metabolism after neonatal period was observed by other authors in calves, characterized by a decrease during the first weeks of life, reaching the lower range at about 11 weeks after birth not related to the kind of food (Biergele and Ilgaza, 2003). Although our research showed that a significant effect of time on urea serum level was only in the group B, so we can affirm that the modifications of urea serum level observed are dependent by the diet type.

The urea and creatinine serum levels seem to be dependent by the serum levels of other electrolytes. In particular, others researches demonstrate that calves fed with an high magnesium or magnesium and phosphorus ratio had blood urea and creatinine levels greatly elevated (Kallfelz et al., 1987). Other data, obtained from dogs, report a significant increase of creatinine blood levels only when urethral obstruction and bladder rupture occur (Braun et al., 2003). A significant effect of diet was observed on Mg during the period 3 in both groups. The only parameter that had a statistical significance for the diet factor was the urea, showing a statistical significance both for A and B group during the period 3. The urea serum levels showed an increase during the administration of D diet and a decrease during the administration of the W diet in group A and the opposite trend in the group B. We could explain the trend of urea

serum levels well as diet administered also because of the age fluctuation of this parameter. In fact several studies, demonstrated that the physiological range of different biochemical parameters are subjected to variations depending exclusively from the age and metabolism change in relation of a major utilization of protein substrate (Carcangiu et al., 2002). Another study demonstrated that the higher values of these elements were associated to lower mean of phosphorus, and in our research the phosphorus effectively had a diminution trend (George et al., 2007). Moreover, the subjects with high levels of urea are usually the same affected by urolithiasis (George et al., 2007). During our experiment we did not find subjects affected by urolithiasis.

Table 5. **Average values of the parameters considered, expressed in their conventional units of measurement with the related standard error and statistical significance observed in different experimental conditions in 34 beef cattle, fed with a diet with low water content** (W diet)

Parameters	Group A		
	Period 1 (40 days)	Period 2 (80 days)	Period 3 (120 days)
Urea (mmol/l)	3.63±0.25•	3.53±0.80	2.37±0.14
Creatinine (mmol/l)	140.1±11.3 * •	90.0±7.59	90.0±7.66
Ca (mmol/l)	2.46±0.07*	2.76±0.02•	2.57±0.01
P (mmol/l)	2.32±0.08*•	2.83±0.07	2.67±0.05
Mg (mmol/l)	0.89±0.01*	1.03±0.02•	0.97±0.01
K (mmol/l)	4.82±0.07	4.75±0.05	4.82±0.11
	Group B		
Urea (mmol/l)	3.47±0.23*•	4.06±0.3	4.59±0.28
Creatinine (mmol/l)	105.7±7.09*•	83.1±3.27	81.7±3.87
Ca (mmol/l)	2.08±0.04*•	2.51±0.02	2.54±0.02
P (mmol/l)	2.09±0.16*•	2.69±0.07	2.59±0.06
Mg (mmol/l)	0.86±0.02	0.88±0.02	0.87±0.01
K (mmol/l)	4.30±0.09•	4.44±0.08•	4.02±0.11

Significance: * vs Period 2 (P<0.0001); • vs Period 3 (P<0.0001)

Results obtained from other authors demonstrated that is very difficult observe uroliths if Mg levels in the food are lower than 0,23% and P levels are lower than 0,46% ethereal extract (Ulutas, 2005).

A significant effect of time, probably due to the age, was observed on creatinine, Ca, P, and Mg. Other studies demonstrated that in puppies, foals and lambs the serum electrolytes amount are higher than in the adult animals (Lefebvre et al., 2008). A constant significant decrease on creatinine and P levels was observed during the period 3. Ca showed a statistically significant decrease in the group A and a statistical significant increase in the group B during the period 3. Considering that the group A is constituted by neonates, the showed trend during the period 3 indicate a physiological adapting of neonates to the maintenance of homeostasis till the first months of life (Steinhardt and Thieslcher, 1999).

Conclusion. In conclusion considering that the Ca/P ratio in diets used in this experiment does not seem to have sufficient effect in inducing urolithiasis in the animals of this study, this suggests that Ca/P ratio play an important role in reducing the occurrence of mineral calculi in beef cattle, so an important preventive role is once again attributed to food in preventing this pathology. The age of the animals and the adaptive response of metabolism to an unbalanced diet are another important factors determining the onset of the pathology. Further investigations should be done to test a different diet with another percentage of minerals content.

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