

## HAPTOGLOBIN AND SERUM AMYLOID A IN SUBACUTE RUMINAL ACIDOSIS IN GOATS

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### ABSTRACT

Ruminal acidosis is a frequent disorder that occurs in goats as a consequence of feeding mistakes in animals not adapted to a diet of easily fermentable carbohydrates. The subacute form of the disease is difficult to diagnose because no apparent signs are shown and the acid-base parameters may remain within the normal range. The present study aimed at testing the hypothesis that haptoglobin (Hp) and serum amyloid A (SAA), the two major acute phase proteins in ruminants, may be useful as markers of subacute acidosis in goats.

A subacute acidosis was induced in six Murciano-Granadina goats through a diet of 60% mixed feed-40% alfalfa hay offered during 5 days to goats not adapted to eat mixed feed. Two goats were rumen-fistulated to investigate the effect of feeding on ruminal pH. Sampling of blood and urine of all animals was done before the induction of the acidosis, during 5 days after the onset of induction and for 18 days after the induction (recovery period).

Ruminal pH in the fistulated goats dropped to less than 5.5 during the induction period, and half of the goats had diarrhea on the third day after the induction of acidosis. Acid-base parameters showed that the acid-base compensatory mechanisms were efficient in maintaining the equilibrium. Serum Hp had a moderate increase during the induction period, while SAA did not change. These results suggest that Hp might be a potential marker for ruminal acidosis in goats.

**Key-words:** ruminal acidosis, goats, haptoglobin, serum amyloid A.

## HAPTOGLOBINA Y PROTEÍNA AMILÓIDE SÉRICA A EN ACIDOSIS RUMINAL SUBAGUDA EN CABRAS

### RESUMEN

La acidosis ruminal es un trastorno frecuente en cabras como consecuencia de errores en el manejo alimentario en animales no adaptados a dietas que contienen carbohidra-

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tos fácilmente fermentables. La forma subaguda de la enfermedad es de difícil diagnóstico toda vez que no muestra evidencia de signos clínicos claros y los parámetros ácido-básicos pueden permanecer en el rango normal. El presente estudio tuvo por objetivo probar la hipótesis de que la haptoglobina y la proteína amilóide sérica A, las dos proteínas de fase aguda más importantes en rumiantes, pueden ser útiles como marcadores de acidosis subaguda en cabras.

Se indujo acidosis ruminal a seis cabras de la raza Murciano-Granadina, no adaptadas al consumo de concentrado, mediante el suministro de una dieta con 60% de concentrado y 40% de heno de alfalfa durante 5 días. Dos cabras fueron sometidas a fistulación ruminal para comprobar el efecto del tratamiento sobre el pH del rumen. A todos los animales se les tomaron muestras de sangre y orina el día anterior a la inducción, durante el período de inducción y hasta 18 días después de la inducción (período de recuperación).

El pH ruminal cayó a menos de 5,5 durante el período de inducción de acidosis en las cabras fistuladas, mientras que la mitad de las cabras tuvieron diarrea al tercer día de la inducción de acidosis. Los parámetros gasométricos indicaron que los mecanismos compensatorios fueron eficientes para mantener el equilibrio ácido-básico. La haptoglobina sérica presentó un aumento moderado durante el período de inducción de acidosis, mientras que la amilóide sérica A no presentó cambios. Los resultados sugieren que la haptoglobina puede utilizarse como un potencial indicador de acidosis ruminal en cabras.

**Palabras clave:** acidosis ruminal, cabras, haptoglobina, proteína amilóide sérica A.

## INTRODUCTION

Ruminal acidosis is a metabolic disorder caused by feeding errors in ruminants that may be manifested in acute or subacute form. It represents a significant economic problem due to direct effects caused by alterations in the ruminal metabolism that could lead to death and indirect effects which could lead to rumenitis, liver abscesses and laminitis (1, 2). In bovine herds the incidence of this problem may achieve up to 40% (3) while in goat herds it may reach 18% (1).

Acute ruminal acidosis is caused by an excessive intake of easily fermentable carbohydrates both in adapted and non-adapted animals (1). Rapid fermentation of carbohydrates alters the ruminal function with strong metabolic consequences (4). Ruminal alterations include

the proliferation of acid-resistant bacteria (*Lactobacillus* and *Streptococcus bovis*) and an increase in the production of volatile fatty acids and lactate, which causes a sharp drop of the ruminal pH to less than 5.0 in most of the cases (5, 6). Subacute ruminal acidosis is also produced by diets with excess easily fermentable carbohydrates, but the diagnosis and the characterization are more difficult than for acute acidosis, because the plasma pH may remain in the physiological range (7) and the signs may be just a decrease in feed intake. Usually subacute acidosis is caused by feeding grains or mixed feeds in amounts higher than the bacterial fermentation may withstand to keep an adequate production of volatile fatty acids and lactate in the rumen (8).

Subacute ruminal acidosis may be defined as a drop in ruminal pH to val-

ues ranging from 6.25 to 5.5, although the duration and intensity of pH drop may vary substantially (8). On the other hand, Oetzel (4) defined subacute acidosis as periods of decrease of ruminal pH to levels between 5.5 and 5.0 (4).

In many cases ruminal pH monitoring would be useful to prevent outbreaks of acidosis that will cause serious economic losses. Monitoring herds for acidosis demands the use of adequately sensitive blood or ruminal indicators because signs are usually unnoticed, mainly in subacute cases (7). For many authors, the main difference between acute and subacute acidosis is higher concentration of serum lactate (up to 100 mmol/L) and ruminal pH values below 5.0 in the acute form (8, 9, 10), while lactate values in the subacute form could be normal or slightly increased ( $> 2$  mmol/L) (11). Further, in subacute acidosis, the blood acid-base parameters may not be altered (5, 6). Probably, most of the changes in subacute acidosis remain restricted to the ruminal environment, resulting from the adaptation process associated with the switch from a forage diet to a grain diet (12).

The acute phase proteins (APPs) have been used as useful markers of inflammation/infection processes in ruminants (13, 14, 15, 16, 17). In these animals, haptoglobin (Hp) and serum amyloid A (SAA) are the major APPs, and have a great potential to be used as diagnostic indicators in metabolic problems (18). In cases of subacute acidosis induced with acidotic diets in steers, Gozho et al. (19) found an increase in the plasma concentration of both Hp and SAA for a diet consisting of 60% grain compared with a hay diet (19). They suggested that Hp and SAA are useful to detect subacute

acidosis in cattle. The main objective of the present study was to determine blood levels of Hp and SAA in cases of subacute acidosis induced by feeding a diet high in mixed feed to goats. Additionally, the ruminal pH in subacute acidosis was investigated in two fistulated animals.

## MATERIALS AND METHODS

### Animals and diet

Six healthy Murciano-Granadina goats were used in this experiment. Mean age and body weight were 5.8 years (range 3 to 9 years) and 49.6 kg (range 37 to 58 kg), respectively. The goats were housed in individual boxes with straw bedding and were fed alfalfa hay *ad libitum*, with free access to water. Two of the goats were subjected to a ruminal fistulation one month before the experiment in order to obtain ruminal contents for pH determinations. Only two animals were rumen-fistulated in accordance to the Ethics Committee of the University of Murcia (Spain). Measurements in rumen-fistulated animals were made to confirm the acidosis in the rumen caused by the feeding treatment, not to monitor the ruminal pH values throughout the experiment.

After a period of four weeks for adaptation to alfalfa hay, the *ad libitum* quantity consumed per day (mean= 0.9 kg) was lowered to 90% of the daily voluntary intake for one week in order to stimulate appetite. Subacute acidosis was then induced by offering a diet of 60% mixed feed (made of barley and wheat in equal proportions) and 40% alfalfa hay, for 5 continuous days. During the induction period (days 1 to 5), half of the forage was given in the morning (at 8:30 am) and the other half in the afternoon (at 3:00 pm), while the mixed feed was all given at noon. After the induction

period, the diet returned to alfalfa hay *ad libitum* for the remaining of the experiment (days 6 to 18, recovery time). The six animals were used as a control group (sampling before the induction of acidosis) and as a experimental group (sampling after the start of the induction and the recovery period), being the experimental unit each animal.

### Parameters measured

Ruminal fluid was collected directly from the ruminal contents through the cannula inserted in the two fistulated animals by means of a probe attached to a 50 mL-syringe. Sampling was conducted during the induction period at three times per day (8:30 am, 3:00 pm and 6:00 pm). These measurements were made to confirm the ruminal acidosis caused by the mixed feed by direct sampling of the rumen, since samples obtained using an esophageal probe are usually contaminated by saliva, which may distort the pH measurement.

Urine samples were obtained after the blood collection either by voluntary urination or by urination induced after covering the nose and the mouth of the goat for few seconds. The samples were maintained in refrigeration until their transport to the laboratory. Urinary and ruminal pH were determined using a pHmeter (Basic 20, Crison Instruments, Alella, Spain).

Blood samples were always collected at 8:00 in the morning from the day before the induction of the acidosis (day 0) and during the whole period of induction (days 1, 2, 3, 4 and 5). Thereafter, samples were collected on days 8, 10, 12 and 18 after the onset of the induction (recovery period).

Blood was collected from the jugular vein both in vacuum tubes with heparin and in plain tubes (Becton, Dickinson and Co., Plymouth, UK). Samples with heparin were used for determining fibrinogen by the method of precipitation by heat (20) and for acid-base and electrolyte parameter measurement, as well as lactate concentration determination using an automated analyzer (ABL System, Radiometer, Copenhagen, Denmark). Samples for acid-base parameters determination were processed within one hour of blood collection.

Blood in plain tubes was centrifuged (3,000 rpm for 10 min) and the serum kept at -20°C until the analysis of APPs. Hp was determined by a colorimetric method (Tridelta Development Ltd., Ireland) in an automatic analyzer (Cobas Mira Plus, ABX Diagnostica, France) and SAA was determined by a solid-phase sandwich ELISA assay (Tridelta Development Ltd., Ireland) using an automated microplate reader (Power Wave XS, Bio-Tek Instruments, USA).

### Statistical analysis

Arithmetic means and standard deviations were calculated using routine descriptive statistical procedures. To assess differences between the control period (day zero) and induction of acidosis (days 1 to 5) and recovery (days 8 to 18) periods, one-way ANOVA for repeated measurements with Tukey separation of the means in case of significance was performed.

Statistical procedures were calculated by using the SPSS statistical program (SPSS Inc., Chicago, USA). Statements of significance were based on  $P < 0.05$ .

## RESULTS

Ruminal pH values dropped to a mean of 5.5 (range from 5.38 to 6.13) in the two fistulated goats used to monitor the changes in the ruminal pH caused by the diet, after 24 hours of consuming mixed feed and during all the induction period (days 1 to 5). Diarrhea was observed from the third day of treatment in half of the goats. The acid-base and electrolytic parameters in blood were normal, except for mild, non-significant increases in anion gap and lactate values in the first two days

of treatment (Table 1). Also, a numerical but not significant decrease in the urinary pH was observed from a mean of 7.8 on the first day to a mean of 7.5 on the other 4 days of induction (data not shown).

Hp, SAA and fibrinogen concentrations are shown in Table 2. There was a 3.4 to 3.8-fold increase in Hp during the fourth and fifth days after the induction of acidosis compared with days 0 and 1 ( $P=0.08$ ). SAA and fibrinogen concentrations did not change in response to the induced acidosis.

**TABLE 1.** Mean values of blood acid-base and electrolyte parameters before and after the induction of subacute acidosis in goats.

Days pre and post induction*	pH	pCO <sub>2</sub> (mmHg)	pO <sub>2</sub> (mmHg)	HCO <sub>3</sub> (mM)	BE (mM)	Na <sup>+</sup> (mM)	K <sup>+</sup> (mM)	Ca <sup>2+</sup> (mM)	Cl <sup>-</sup> (mM)	AG (mM)	Lactate (mM)
0	7.35	46.87	31.49	25.04	0.27	144.22	4.26	1.31	103.87	14.65	1.71
1	7.33	50.08	33.30	25.66	-0.52	145.17	4.48	1.38	104.00	14.90	1.95
2	7.34	48.97	29.52	25.85	-0.03	145.17	4.45	1.28	105.00	15.60	1.80
3	7.37	45.95	32.58	25.73	0.38	145.00	3.98	1.31	106.33	12.88	1.42
4	7.38	44.95	32.82	25.53	0.52	142.20	4.24	1.26	103.60	13.40	1.62
5	7.35	48.55	28.23	26.08	0.33	142.50	4.40	1.28	103.67	12.80	1.72
8	7.39	44.32	30.78	25.85	0.97	143.20	4.40	1.28	105.40	11.52	1.44
10	7.37	49.57	32.75	27.77	2.02	145.50	4.38	1.28	105.50	12.47	1.78
12	7.35	45.55	36.17	24.63	-0.77	142.67	4.15	1.24	107.00	10.97	1.75
18	7.35	46.93	28.50	25.52	-0.02	141.50	4.40	1.35	103.17	12.74	1.78

\* The induction of subacute acidosis was done from day 1 to 5. The recovery period was from day 6 to 18. BE= base excess. AG= anion gap

## DISCUSSION

A mild subacute ruminal acidosis was induced in goats by feeding a wheat-barley mixed feed for 5 days, as evidenced by the ruminal pH decrease to a mean of 5.5 in the two fistulated goats. Ruminal pH in animals adapted to hay and pas-

tures (non-acidotic conditions) should not fall below 6.0 (5). Interestingly, the laboratory data showed that, in spite of this ruminal acidosis, acid-base balance parameters were not affected, suggesting the action of an efficient mechanism of compensation in the blood.

**TABLE 2.** Mean and standard deviation blood values of the acute phase proteins haptoglobin (Hp), serum amyloid A (SAA) and fibrinogen before and after the induction of subacute acidosis in goats.

Time period	Day	Hp (mg/L)	SAA (mg/L)	Fibrinogen (g/L)
Pre-induction	0	89 ± 105	2.95 ± 3.8	3.05 ± 0.91
	1	90 ± 197	3.13 ± 6.0	3.67 ± 0.82
	2	224 ± 307	7.25 ± 13.0	3.00 ± 1.10
Induction	3	180 ± 330	3.65 ± 5.68	3.00 ± 1.55
	4	306 ± 514*	6.37 ± 8.20	2.67 ± 1.03
	5	340 ± 540*	0.86 ± 2.07	3.33 ± 1.03
	8	70 ± 150	0.98 ± 1.22	2.33 ± 0.82
	10	58 ± 130	2.49 ± 2.37	2.67 ± 1.03
Recovery	12	80 ± 179	5.13 ± 1.35	2.33 ± 0.82
	18	40 ± 79	4.19 ± 2.97	2.50 ± 0.84

\* P value = 0.08 (comparison with day 0)

Experimental models to induce metabolic and ruminal acidosis include infusion of glucose (6) or sucrose solutions (1) or feeding diets rich in grains (21) for short periods of time (about 5 days). This latter method is the one that closest resembles what occurs in the field. Brossard et al. (5) induced a subacute acidosis in sheep by feeding a diet composed by 40% wheat and 60% alfalfa hay for 2 weeks. In this study, the ruminal pH ranged from 5.34 to 6.69 in the animals fed the wheat diet, while in those fed exclusively alfalfa hay ranged from 6.12 to 6.89 (5). In the present work, feeding a diet composed of 60% mixed feed and 40% alfalfa hay to goats non-adapted to mixed feed resulted in ruminal pH values ranging from 5.38 to 6.13 during the 5 days of induction, while in animals receiving only alfalfa hay the values ranged from 6.27 to 7.27 (recovery period).

Brown et al. (6) suggest that lactate, LDH, creatinine, Na, K and P would be the most sensitive blood indicators for evaluating the severity of acute acidosis in sheep. However, in subacute acidosis, plasma lactate values may be normal (11). This was the case for the present work when, during the induction period, serum lactate values did not reach 2 mmol/L, which is considered the maximum reference value for goats (22).

Acid-base status parameters were not altered, except for a slight increase in the anion gap during the induction period (15.6 mmol/L compared to the reference values of 10-12.7 mmol/L). This finding supports the opinion of Brossard et al. (5) who stated that in subacute acidosis acid-base parameters are of little help in the diagnosis of the problem. Brown et al. (6) mentioned that plasma cholesterol, non-esterified fatty acids, K and amylase could be used to differentiate ewes with

subacute acidosis from those with acute acidosis. However, in the present work, levels of K were not affected by subacute acidosis.

Acute phase proteins have been proposed as good markers of acidosis in cattle (23). The hypothesis of the present experiment was that in cases of subacute acidosis induced by feeding high levels of a mixed feed diet in goats, Hp and SAA would be useful to diagnose the problem. Fibrinogen, a classical acute phase protein, was also measured as a control parameter. However, the nutritionally-induced subacute ruminal acidosis caused only a moderate increase in Hp from 89 (day 0) to >300 mg/L (days 4 and 5) of the induction, but it had no effect on SAA concentrations, since the maximum value was 14.5 mg/L. Gozho et al. (19) found an increase in Hp and SAA in steers fed diets with more than 60% grain with levels of up to 790 mg/L and 170 mg/L, respectively. Our results indicate that Hp, but not SAA, would have the potential to be useful in the diagnosis of subacute ruminal acidosis in goats. It is possible that in acute acidosis Hp values might be higher but this needs to be investigated.

Rontved et al. (24) detected higher values of SAA in dairy cows fed 23% more energy from more contents of starch than the control diet. The authors concluded that further studies are needed to validate APPs as potential markers of ruminal acidosis. Berry et al. (25), on the other hand, did not find any changes in Hp, SAA and fibrinogen when comparing diets with different proportions of starch (34 or 48% of dietary metabolic energy from starch) in calves. The authors indicated that APP concentrations generally did not differ among dietary treatments.

The effect of subclinical acidosis on Hp levels is very difficult to study because the duration of the Hp increase prior to the clinical disease onset is variable (26). It is probable that the proportion of mixed feed used in the present study to induce acidosis was less than the threshold needed to cause a stronger response of APPs. Gozho et al. (23) mentioned that subacute ruminal acidosis in steers was induced only when the dietary mixed feed proportion was increased to 76% (23). They suggested that the acidity of the rumen lead to papillae inflammation, which caused APPs secretion, and that the response is proportional to the amount of acid.

## CONCLUSION

In spite of its moderate increase in serum values, Hp appears to be a potential indicator of acidosis in goats. It is possible that the increase in Hp might be related to the severity of the problem and the proportion of mixed feed used in non-adapted animals. SAA and fibrinogen apparently do not have as good potential as Hp for the diagnosis of ruminal acidosis in goats.

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