

Jugular versus Mammary Vein Acid-Base Balance, Blood Gases, Hematobiochemical Profiles and Inflammation Biomarkers in Goats with Acute Mastitis

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Abstract

The objectives of this study was to investigate the acid-base balance, blood gases, hematobiochemical profiles and the inflammation biomarkers fibrinogen, serum amyloid A (SAA) and haptoglobin (Hp) in the mammary and jugular veins of goats with mastitis. Blood samples were collected from the jugular and mammary veins from 26 goats with mastitis and from 10 healthy lactating goats. In diseased goats, the affected quarter had a remarkable degree of swelling, hard and sore to touch; the milk was bloody. In the jugular vein of diseased goats, the values of PCO_2 , PO_2 , base excess (BE), HCO_3 and TCO_2 were significantly lower than in the jugular vein of controls. In the mammary vein, the pH was significantly lower than in controls and the values of PCO_2 , BE, HCO_3 and TCO_2 were significantly lower than in the controls. In contrast, the PO_2 , lactic acid and anion gap values were significantly higher in the mammary vein than in controls. The RBCs was significantly lower in the jugular and mammary veins than in controls. In the jugular and mammary veins, albumin concentration was significantly lower than controls. The serum concentrations of calcium and phosphorus were significantly lower in the jugular vein than controls. The serum concentration of fibrinogen did not differ significantly in the jugular and mammary veins compared to healthy controls. In the jugular and mammary veins, the serum concentrations of SAA and Hp were significantly higher than controls. In diseased animals, the serum concentrations of SAA were significantly higher in the mammary veins than in jugular vein. In conclusion, the blood gases, acid-base, hematobiochemical profiles and inflammation biomarkers in the mammary vein goats with acute mastitis could be used as by veterinarians and researchers as reference values for investigation of goats with mastitis and healthy lactating goats.

Keywords: Acute Phase Proteins; Goat; Inflammation; Mastitis; Biomarkers

Introduction

Inflammation of the mammary gland (mastitis) usually occurs primarily in response to intramammary bacterial infection, but also to intramammary mycoplasmal, fungal, or algal infections. Mechanical trauma, thermal trauma, and chemical insult predispose the gland to intramammary infection. The incidence of mastitis depends on the interaction of host, agent, and environmental factors. Acute bovine mastitis involves an initial phase, which includes an inflammatory reaction, and a resolution phase [1].

In goats, inflammation of the mammary gland is a broad diagnosis that may be based on changes in the physical characteristics of the udder or its secretion [2]. In sheep and goats, mastitis is the most common disease in all intensive systems worldwide. The clinical manifestations of mastitis in sheep and goats ranges from peracute gangrenous mastitis with severe illness and toxemia to chronic mastitis and abscess formation without prominent signs [3]. In addition, chronic mastitis is a fundamental cause of culling in meat-producing with reduced lactation in both sheep and goats [2,3].

In biochemical analyses, the blood sampling site strongly influences the measured blood variables and therefore has to be considered in the interpretation. In cattle, the biochemical analyses of mammary vein variables versus jugular vein was reported recently that could provide valuable clinical information and facilitate appropriate medical and surgical treatments [4-6].

The acute phase proteins (APPs) are blood proteins that can be used to assess the systemic response of the innate immune system to infection, inflammation or trauma [7-9]. In ruminants, the APPs have been proposed as sensitive and rapid indicators of inflammatory disturbances [10-12]. The major APPs in ruminants are haptoglobin (Hp) and serum amyloid A (SAA) [7]. In

cattle, Hp and SAA are effective in the diagnosis and prognosis of mastitis, enteritis, peritonitis, pneumonia, endocarditis, and endometritis [7,8,13]. It has also been suggested that APPs may be useful in the assessment of animal welfare [7,8,10,14].

To the authors' knowledge, the acid-base balance, blood gases, hematobiochemical profiles as well as inflammation biomarkers were not estimated in the mammary vein of goats with acute mastitis. The purpose of this study was therefore to investigate these variables in blood samples collected from the jugular and mammary veins of goats with mastitis compared to control healthy lactating goats.

Materials and Methods

Animals and Clinical Examination

The experimental protocol was approved by the Animal Ethical Committee, Deanship for Scientific Research, Qassim University, Saudi Arabia. Twenty-six does (age; 25.1 ± 4.7 mo; weight 49.8 ± 18.6 kg) clinically affected with acute mastitis were used. Animals were referred because of anorexia, swelling and gangrene of the mammary gland, abnormal milk secretion and recumbency. Duration of the disease/symptoms ranged from 1 to 7 days. No treatment intervention was attempted. All animals underwent a thorough physical examination, which included general behavior and condition, auscultation of the heart, lungs, rumen and intestine, measurement of heart rate, respiratory rate and rectal temperature, swinging auscultation, percussion auscultation of both sides of the abdomen and rectal examination. Parallel, a control group (age; 21.1 ± 6.5 mo; weight 52.4 ± 14 kg) comprised of ten clinically healthy lactating does were used. All procedures followed were in accordance with the *Laboratory Animal Control Guidelines of Qassim University, which basically conform to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health in the USA* (NIH publications No. 86 to 23, revised 1996).

Blood Sampling

From each doe in diseased and control group, twelve mL jugular blood sample was collected just before, and a similar volume of blood was collected from the mammary vein. Of both jugular and mammary vein blood samples in the diseased and control goats, two mL were collected in EDTA tubes for hematological analyses, two mL in heparinised tubes for blood gas analyses, two mL in citrated tubes for plasma fibrinogen measurement, and the remaining six mL in plain tubes to obtain serum for the determination of the biochemical parameters and APPs Hp and SAA. Both the third and fourth blood samples were centrifuged at $1200 \times g$ for 15 min and the citrated plasma and serum samples obtained were aliquotted in tubes and immediately stored at -20°C pending the clinical chemistry analyses.

Blood gas analyses

The heparinized blood samples were used immediately to analyse the acid-base and blood gas parameter values in situ using a portable clinical veterinary analyser (i-STAT[®], Abaxis, California, USA). In this way, blood pH, partial pressure of carbon dioxide (PCO_2), oxygen partial pressure (PO_2), bicarbonate (HCO_3), total carbon dioxide (TCO_2), base excess (BE), oxygen saturation (SO_2), sodium, potassium, chloride and lactate were analysed immediately in order to prevent changes in the concentrations of these parameters.

Hematology and Serum Biochemistry

Hematological examinations [total and differential leukocyte count, red blood cells (RBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC)] were carried out using an automated analyser (VetScan HM5, Abaxis, California, USA). The serum samples were used to determine the concentrations of total protein, albumin, globulin, blood urea nitrogen (BUN), calcium, phosphorus, magnesium and glucose. The serum activity of aspartate aminotransferase (AST), γ -glutamyl transferase (GGT), alkaline phosphatase (ALP) and creatine kinase (CK) was also measured. An automated biochemical analyser (VetScan VS2, Abaxis, California, USA) was used for the measurement of the above-mentioned serum parameters.

Inflammation biomarkers assays

The APPs Hp and SAA were measured in the serum samples with validated methods for goats as previously recorded [15-18]. Serum concentrations of Hp were quantified by spectrophotometric method using a commercial kit based on the peroxidase activity of the haptoglobin-hemoglobin complex (Phase Haptoglobin Assay, Tridelata Development Ltd., Ireland). The analytical sensitivity of the assay was $0.15 \mu\text{g/mL}$, and the intra- and inter-assay CVs were 5-6% and 4-6%, respectively. Serum concentrations of SAA were determined using a commercially available enzyme-linked immunosorbant assay (ELISA) kit (Phase SAA Kit, Tridelata Development Ltd., Ireland) according to the manufacturer's instructions. The analytical sensitivity of the assay was $0.15 \mu\text{g/mL}$, with intra- and inter-assay CVs of 4.5% and 6%, respectively. Fibrinogen concentrations were measured with a commercially available ELISA kit validated for use in goats (Life Sciences Advanced Technologies Inc., FL, USA). The analytical sensitivity of the assay was 7.63 ng/mL (range 15.6 - 1000 ng/mL).

Statistical analysis

Data are presented as means \pm standard deviation, and the analysis was conducted using SPSS program software [19]. Blood gases, hematobiochemical parameters and inflammation biomarkers in the jugular and mammary blood of the diseased goats and of the controls were compared by using the Student's *T* test, and the significance was set at $P < 0.05$.

Results

Inflammation of the mammary gland occurred within the first 36 hr after parturition. There were a remarkable degree of swelling of the gland and the milk was bloody in 18 cases. There were hemorrhagic patches on the sclera in 5 cases, a severe systemic reaction with elevation of the temperature to 40-42 °C in all cases, accelerated heart rate to 110-150 bpm in 22 cases, anorexia with profound depression and absence of ruminal movements and muscular weakness in all cases and recumbency within 3-5 hr after the onset of signs in 20 cases. The onsets of local and systemic reactions were sudden. The affected quarter was grossly swollen, hard and sore to touch, and cause severe lameness on the affected side. In 15 cases a bluish discoloration of the affected gland developed as early as hours from the onset of the disease. Gangrene involved the floor of the affected quarter and the whole or part of the teat. Within 24h, the gangrenous areas became black and ooze serum freely with the formation of blisters. In 7 cases, the gland secretions were reduced to small amount of blood-stained serous fluid without odor, clots or flakes.

Table 1 summarizes the mean blood gases, acid-base and electrolyte parameters of the jugular and mammary veins in the goats with acute mastitis compared to values of same veins in healthy goats. In the jugular vein of diseased goats, the values of PCO_2 , PO_2 , base excess, HCO_3 and TCO_2 were significantly lower than in the jugular vein of controls ($P < 0.05$). Other parameters did not change significantly. In the mammary vein, the pH was significantly lower than in controls ($P < 0.05$) and the values of PCO_2 , base excess, HCO_3 and TCO_2 were significantly lower than in the controls ($P < 0.05$). In contrast, the PO_2 , lactic acid and anion gap values were significantly higher in the mammary vein than in controls ($P < 0.05$).

Parameter	Jugular vein		Mammary vein	
	Disease	Control	Disease	Control
pH	7.447 \pm 0.063 ^a	7.177 \pm 1.010 ^a	7.441 \pm 0.053 ^a	7.494 \pm 0.042 ^b
PCO_2 (mmHg)	32.9 \pm 5.8 ^a	43.0 \pm 5.6 ^b	32.8 \pm 5.7 ^a	41.0 \pm 4.8 ^b
PO_2 mmHg	30.9 \pm 2.9 ^a	33.3 \pm 3.5 ^b	37.4 \pm 9.2 ^a	25.3 \pm 5.9 ^b
Base Excess (mmol/L)	-1.3 \pm 4.7 ^a	4.0 \pm 3.7 ^b	-1.6 \pm 4.8 ^a	8.1 \pm 3.6 ^b
HCO_3 (mmol/L)	22.8 \pm 4.1 ^a	28.3 \pm 2.9 ^b	22.5 \pm 4.3 ^a	32.0 \pm 4.3 ^b
TCO_2 (mmol/L)	23.8 \pm 4.1 ^a	29.6 \pm 2.9 ^b	23.5 \pm 4.6 ^a	32.6 \pm 3.2 ^b
Lactic acid (mmol/L)	4.1 \pm 2.4 ^a	3.7 \pm 2.16 ^a	3.9 \pm 2.7 ^a	1.2 \pm 1.0 ^b
Sodium (mmol/L)	149 \pm 5 ^a	147 \pm 1.20 ^a	148 \pm 5 ^a	147 \pm 2 ^a
Potassium (mmol/L)	4.2 \pm 0.5 ^a	3.9 \pm 0.3 ^a	3.8 \pm 0.6 ^a	3.8 \pm 0.3 ^a
Chloride (mmol/L)	111 \pm 6 ^a	105 \pm 3 ^a	111 \pm 6 ^a	104 \pm 3 ^b
Anion Gap (mmol/L)	18.4 \pm 1.6 ^a	16.9 \pm 2.9 ^a	18.1 \pm 2.4 ^a	14.5 \pm 2.1 ^b

^{a,b}Differ significantly ($P < 0.05$) between diseased and control goats in the same column

Table 1: Blood gases, acid-base and electrolyte parameters (mean \pm SD) in goats with acute mastitis ($n = 26$) compared to healthy controls ($n = 10$)

Table 2 shows the mean hematological parameters in the jugular and mammary veins of the goats with acute mastitis compared to values of same veins in healthy goats. In the jugular and mammary veins of diseased goats, red blood cells count was significantly lower than in controls ($P < 0.05$), however the values of MCV and MCH were significantly higher in jugular and mammary veins than in controls ($P < 0.05$).

Table 3 shows the mean biochemical parameters in the jugular and mammary veins of the goats with acute mastitis compared to values of same veins in healthy goats. In the jugular and mammary veins of diseased goats, albumin concentration was significantly lower than in controls ($P < 0.05$), however the serum concentration of globulin was significantly higher ($P < 0.05$). The serum concentration of BUN was significantly higher in the jugular and mammary veins in diseased versus in the controls ($P < 0.05$). In the jugular vein, the serum concentrations of calcium and phosphorus were significantly lower than controls ($P < 0.05$).

Parameter	Jugular vein		Mammary vein	
	Disease	Control	Disease	Control
White blood cells ($\times 10^9/L$)	11.6 \pm 4.7 ^a	14.4 \pm 2.8 ^a	14.7 \pm 6.6 ^a	13.7 \pm 2.5 ^a
Lymphocytes ($\times 10^9/L$)	5.1 \pm 3.2 ^a	5.2 \pm 1.7 ^a	7.0 \pm 4.5 ^a	4.6 \pm 1.3 ^a
Monocytes ($\times 10^9/L$)	0.1 \pm 0.04 ^a	0.1 \pm 0.02 ^a	0.1 \pm 0.1 ^a	0.1 \pm 0.04 ^a
Neutrophils ($\times 10^9/L$)	6.4 \pm 4.7 ^a	10.0 \pm 2.5 ^a	7.6 \pm 4.4 ^a	9.0 \pm 2.2 ^a

Parameter	Jugular vein		Mammary vein	
	Disease	Control	Disease	Control
Red blood cells ($\times 10^{12}/L$)	11.5 \pm 2.7 ^a	17.6 \pm 2.2 ^b	11.9 \pm 1.6 ^a	16.2 \pm 1.8 ^b
Hemoglobin (g/dL)	10.9 \pm 1.3 ^a	10.3 \pm 1.0 ^a	10.3 \pm 1.3 ^a	9.7 \pm 1.0 ^a
Hematocrit (%)	25.6 \pm 6.0 ^a	25.8 \pm 2.5 ^a	26.8 \pm 6.3 ^a	23.8 \pm 2.6 ^a
Mean corpuscular volume (fl)	23.2 \pm 6.6 ^a	15.1 \pm 0.9 ^b	23.0 \pm 6.2 ^a	14.9 \pm 1.2 ^b
Mean corpuscular hemoglobin (pg)	9.5 \pm 2.3 ^a	5.9 \pm 0.3 ^b	8.8 \pm 1.7 ^a	6.1 \pm 0.9 ^b
Mean corpuscular hemoglobin concentration (g/dL)	41.5 \pm 5.3 ^a	38.9 \pm 0.7 ^a	40.0 \pm 9.8 ^a	40.9 \pm 8.4 ^a

^{a,b}Differ significantly ($P < 0.05$) between diseased and control goats in the same column

Table 2: Hematological parameters (mean \pm SD) in goats with acute mastitis ($n = 26$) compared to healthy controls ($n = 10$)

Parameter	Jugular vein		Mammary vein	
	Disease	Control	Disease	Control
Total protein (g/L)	74.3 \pm 11.8 ^a	76.6 \pm 3.4 ^a	74.6 \pm 11.5 ^a	73.0 \pm 2.8 ^a
Albumin (g/L)	31.7 \pm 7.2 ^a	46.5 \pm 1.9 ^b	31.6 \pm 7.6 ^a	46.1 \pm 2.0 ^b
Globulin (g/L)	42.6 \pm 14.1 ^a	29.9 \pm 2.7 ^b	43.0 \pm 13.8 ^a	27.1 \pm 2.2 ^b
Blood urea nitrogen (mmol/L)	11 \pm 7 ^a	4 \pm 1 ^b	10 \pm 7 ^a	4 \pm 1 ^b
Aspartate aminotransferase (U/L)	161 \pm 97 ^a	91 \pm 10 ^a	224 \pm 216 ^a	85 \pm 9 ^a
γ -glutamyl transferase (U/L)	63 \pm 41 ^a	43 \pm 5 ^a	63 \pm 39 ^a	41 \pm 5 ^a
Alkaline phosphatase (U/L)	78 \pm 30 ^a	70 \pm 18 ^a	76 \pm 26 ^a	67 \pm 20 ^a
Calcium (mmol/L)	2.2 \pm 0.2 ^a	2.5 \pm 0.1 ^b	2.2 \pm 0.2 ^a	2.3 \pm 0.1 ^a
Phosphorus (mmol/L)	1.6 \pm 0.4 ^a	1.2 \pm 0.1 ^b	1.7 \pm 0.4 ^a	1.4 \pm 0.2 ^a
Magnesium (mmol/L)	0.9 \pm 0.3 ^a	0.8 \pm 0.1 ^a	0.9 \pm 0.3 ^a	0.7 \pm 0.1 ^a
Creatine kinase (U/L)	396 \pm 376 ^a	240 \pm 25 ^a	381 \pm 348 ^a	229 \pm 43 ^a
Glucose (mg/dL)	73 \pm 25 ^a	100 \pm 29 ^a	63 \pm 20 ^a	67 \pm 27 ^a

^{a,b}Differ significantly ($P < 0.05$) between diseased and control goats in the same column

Table 3: Biochemical parameters (mean \pm SD) in goats with acute mastitis ($n = 26$) compared to healthy controls ($n = 10$)

Figure 1 shows the serum concentration of fibrinogen in the jugular and mammary veins of the goats with acute mastitis compared to values of same veins in healthy goats. In the jugular and mammary veins, the serum concentration of fibrinogen did not differ significantly compared to healthy controls ($P > 0.05$). In diseased animals, the serum concentrations of fibrinogen did not differ significantly in the mammary veins than in jugular vein ($P > 0.05$).

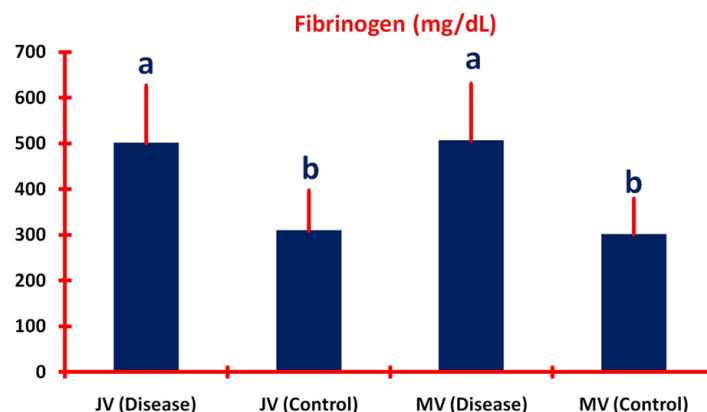


Figure 1: Serum concentrations of fibrinogen in goats with acute mastitis ($n = 26$) compared to healthy controls ($n = 10$); ^{a,b}Indicate a significant difference ($P < 0.05$)

Figure 2 illustrates serum concentration of SAA in the jugular and mammary veins of the goats with acute mastitis compared to values of same veins in healthy goats. In the jugular and mammary veins, the serum concentrations of SAA was significantly higher than controls ($P < 0.05$). In diseased animals, the serum concentrations of SAA were significantly higher in the mammary veins than in jugular vein ($P < 0.05$).

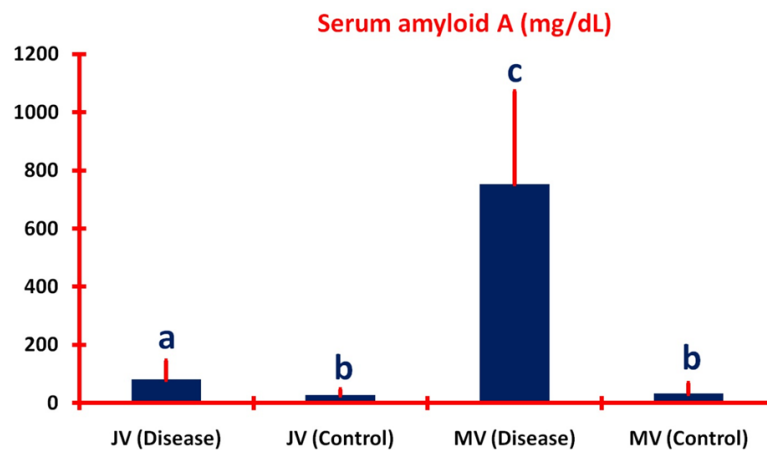


Figure 2: Concentrations of serum amyloid A in goats with acute mastitis ($n = 26$) compared to healthy controls ($n = 10$); ^{a,b,c} Indicate a significant difference ($P < 0.05$)

Figure 3 illustrates serum concentration of Hp in the jugular and mammary veins of the goats with acute mastitis compared to values of same veins in healthy goats. In the jugular and mammary veins, the serum concentrations of Hp was significantly higher than controls ($P < 0.05$). In diseased animals, the serum concentrations of Hp did not differ significantly in the mammary veins than in jugular vein ($P > 0.05$).

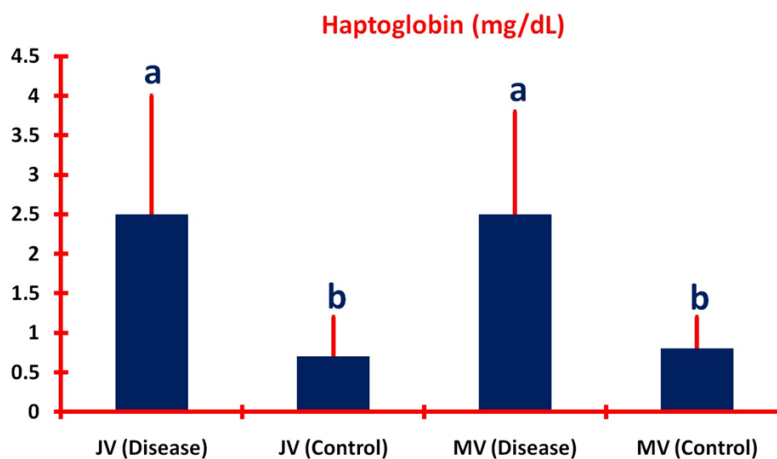


Figure 3: Concentrations of serum haptoglobin in goats with acute mastitis ($n = 26$) compared to healthy controls ($n = 10$); ^{a,b} Indicate a significant difference ($P < 0.05$)

Discussion

Here, we report, for the first time in animals, the acid-base and blood gas, hematological and biochemical parameters as well as inflammation biomarkers in the mammary vein of goats with acute mastitis compared simultaneously to values in the jugular vein. The results were compared to values in healthy lactating control goats.

In this study, PCO_2 , PO_2 , base excess, HCO_3 and TCO_2 were significantly lower in the jugular vein of diseased goats than in controls. In the mammary vein, the pH was significantly lower than in controls and the values of PCO_2 , BE, HCO_3 and TCO_2 were significantly lower than in the controls. In contrast, the PO_2 , lactic acid and anion gap values were significantly higher in the mammary vein than in controls. The decrease in the pH of the mammary vein than in controls could be justified by the decreases in HCO_3 and BE values. Other explanation may be the increased serum concentration of lactic acid. The BE represents all basic components, not just HCO_3 , and as such, is a more sensitive measure of metabolic acidosis than HCO_3 alone [20]. Due to inactivity and the recumbency of the goats with mastitis, lactic acid was decreased in the jugular blood. However, there was a markedly increased lactic acid concentration in the mammary blood compared to controls. The increase in the mammary blood lactate concentration could be due to the incapacity of the mammary cells to metabolize all the accumulated pyruvate that should be transformed into lactate, with the aim to produce NAD^+ and continue the anaerobic energy production [15].

In the present study, the red blood cells count was significantly lower in the jugular and mammary veins than in controls. The anemic diseased animal may be attributed to the peracute nature of the inflammation and bloody milk secretion [1-3]. As reported early [21], in this study, biochemical abnormalities included hypoalbuminemia, hyperglobulinemia and increased serum BUN concentrations. In the jugular vein, hypocalcemia and hypophosphatemia was noted.

The acute phase response (APR) is a rapid, nonspecific, systemic response occurring secondary to many types of tissue injury and might be a physiological protective mechanism during inflammatory events [22]. The origin of APR can be attributed to infection, inflammation, surgical trauma, or other causes [8,9]. The purpose of the APR is to restore homeostasis and to remove the cause of its disturbance [9]. The APPs are a group of blood proteins that change in concentration in animals subjected to external or internal challenges such as infection, inflammation, surgical trauma, or stress [7]. The changes in APPs due to various inflammatory and non-inflammatory conditions have been studied in many animal species [7,23-27]. In this study, the serum concentrations of the APPs SAA and Hp were significantly higher in both the jugular and mammary veins compared to values of same veins in healthy control goats. The APPs have received attention as biomarkers for APR due to the following facilitating properties: low physiological levels, a fast incline, a marked rise in concentration during APR that eases detection and a fast decline after cessation of a stimulus [9].

Conclusion

In conclusion, a significant difference was found in the values of blood gases, acid-base balance, hematobiochemical profiles and inflammation biomarkers between the mammary and jugular veins of goats with acute mastitis. It could be used by veterinarians and researchers as reference values for investigation of goats with mastitis and in healthy lactating goats. To the authors' knowledge this is the first report to elucidate the acid-base balance, blood gases, hematobiochemical profiles as well as inflammation biomarkers in the mammary and jugular veins of goats with mastitis compared to values in healthy lactating control goats.

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