

## CLINICAL RELEVANCE OF SERUM SIALIC ACIDS EVALUATION AND CORRELATION WITH HAPTOGLOBIN AND SERUM AMYLOID A IN DISEASED CATTLE

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### Summary

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The present study was conducted to evaluate the concentrations of sialic acids and their correlation with acute phase proteins (haptoglobin and serum amyloid A) in various inflammatory disorders in cattle. Data were obtained from six dairy farms in Fars province, southern Iran. Forty cows with various inflammatory diseases were examined: acute local traumatic reticuloperitonitis (TRP) (6 cases), theileriosis due to *Theileria annulata* (11 cases), acute metritis (6 cases), colisepticaemia (6 cases), ephemeral fever (5 cases) and pneumonia (6 cases). Ten clinically healthy adult cattle were selected as control group. Serum haptoglobin (Hp), serum amyloid A (SAA), total sialic acid (TSA), lipid bound sialic acid (LBSA) and protein bound sialic acid (PBSA) were measured by validated standard methods. All studied variables were statistically significantly higher in diseased animals compared to healthy ones ( $P \leq 0.001$ ); but the magnitude of increase was considerably different among various diseases. TSA, PBSA and LBSA showed relatively consistent changes in all diseases, however serum amyloid A and haptoglobin were more prominently increased in TRP and metritis. Results showed significant correlations between TSA and PBSA in healthy cattle, TRP and pneumonia groups. Significant correlations were also present between TSA and LBSA in TRP, pneumonia and metritis. No significant correlation was observed between haptoglobin and serum amyloid A as well as between either of these acute phase proteins and any other parameter.

**Key words:** cattle, inflammatory diseases, haptoglobin, serum amyloid A, sialic acid

### INTRODUCTION

Analysis of total protein concentrations and protein fractions are important in various disease states (Kaneko, 1997). Glycoproteins are defined as proteins containing glycan chains, linked glycosidically to

selected amino acid residues. Monosaccharides commonly found in the glycans of glycoproteins include N-acetylneuraminic or sialic acid (Hemming, 1991).

Sialic acids (SA), a family of over 40 neuraminic acid derivatives (Schauer, 2000), are among the most important molecules of life, since they occupy the terminal position on macromolecules and cell membranes and are involved in many biological and pathological phenomena. The majority of SA are found in either protein bound (PBSA) or lipid bound (LBSA) forms, while a little amount is in the free form. In addition, SA is localized at the end chain of many acute phase proteins (Crook, 1993; Haq *et al.*, 1993; Thougard *et al.*, 1998).

SA usually occupy exposed terminal positions on the oligosaccharide chains of glycoconjugates and frequently serve as ligands for receptors such as selectins and siglecs, which mediate a variety of cell-cell adhesion processes in inflammation and in the immune response (Malykh *et al.*, 2001). They are present in normal serum of human and animals and their content in serum has been changed in various diseases (Kloppel *et al.*, 1978; Makimura & Usui, 1990; King & Cavanagh, 1991; Ekin *et al.*, 2003; Citil *et al.*, 2004). Serum SA values are analyzed in many inflammatory and infectious diseases in cattle, such as pneumonia (Karapehlivan *et al.*, 2007), theileriosis, anaplasmosis (Ertekin *et al.*, 2000; Karagenc *et al.*, 2005), leptospirosis (Keles *et al.*, 2000), traumatic reticuloperitonitis (Citil *et al.*, 2004), keratoconjunctivitis (Gunes *et al.*, 2004), chronic tuberculosis (Carter & Martin, 1962) and bovine leukosis (Sydow *et al.*, 1988). Therefore, SA evaluation may be a valuable indicator for diagnosis and prognosis of inflammatory diseases (Motoi *et al.*, 1984). SA is also widely found in bacteria and animal tissues (Schauer, 2000). The mechanism including SA increase is not clearly understood. However, investigators have

reported that SA localized at the end chain of many acute phase proteins can be used as marker for acute phase protein concentrations (Taniuchi *et al.*, 1981; Crook, 1993; Thougard *et al.*, 1998; Enjuanes *et al.*, 2000; Ekin *et al.*, 2003), because serum acute phase proteins, especially the  $\alpha$ 1-acid glycoprotein, are sialylated glycoproteins.

The acute phase proteins (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 (Eckersall, 2004; Murata *et al.*, 2004; Gruys *et al.*, 2005). They are mainly synthesized in the liver, mediated by pro-inflammatory cytokines, and can either increase (positive APPs) or decrease (negative APPs) as a consequence of inflammatory stimuli. It has been suggested that APPs may be useful in the assessment of animal welfare (Eckersall, 2000; Murata *et al.*, 2004; Murata, 2007). Acute phase proteins and their changes due to various inflammatory and non inflammatory conditions have been studied intensively in many animal species (Kaneko, 1997; Eckersall, 2000; Murata *et al.*, 2004; Murata, 2007). Serum amyloid A (SAA) and haptoglobin (Hp) have been proposed to be markers of stress in cattle and other species (Alsemgeest *et al.*, 1995; Deak *et al.*, 1997; Hicks *et al.* 1998; Arthington *et al.*, 2003; Hickey *et al.* 2003; Pieiro *et al.*, 2007). The APPs assay may have potential for monitoring adverse environmental and/or management stressors (Murata, 2007; Pieiro *et al.*, 2007).

There are no published reports about the correlation of serum sialic acids with acute phase proteins in various inflammatory diseases of cattle. Therefore, the present study was conducted to evaluate the concentrations of sialic acids and their

correlation with haptoglobin and serum amyloid A in healthy cattle and cattle with various inflammatory disorders.

## MATERIALS AND METHODS

Data were obtained from six dairy farms in Fars province, southern Iran. Barley, corn and concentrates were used in the diet of dairy cows. All cattle were vaccinated against foot and mouth disease, brucellosis and anthrax about five months before the study. Forty cows with the following inflammatory diseases were examined: acute local traumatic reticuloperitonitis (TRP) (6 cases), theileriosis due to

*Theileria annulata* (11 cases), acute metritis (6 cases), colisepticaemia (6 cases), ephemeral fever (5 cases) and pneumonia (6 cases). Ten clinically healthy adult cattle were selected as control group for this study.

Diseased cows were thoroughly examined. Clinical signs, diagnostic criteria and the time of sampling in each disease are presented in Table 1.

All samples were taken before treatment and in the acute stage of disease. Blood samples were collected from the jugular vein into two tubes: one with and one without EDTA. The sera were separated by centrifugation at 750g for 15 min and stored at – 20°C until analyzed.

**Table 1.** Clinical signs, diagnostic criteria and time of sampling of the diseases under study

Disease	Clinical signs	Diagnostic criteria	Time of sampling*
Traumatic reticuloperitonitis	Sudden onset of reticulorumen atony, fever, pain on movement and deep palpation of ventral abdomen caudal to the xiphoid	Clinical signs, increased plasma protein concentration, neutrophilia and left shift, abdomenocentesis, radiography, ultrasonography	Within 3 days after the onset of acute disease
Theileriosis	Enlarged peripheral lymph nodes, anaemia, jaundice, dyspnea, diarrhoea, fever, petechial haemorrhages on mucous coats	Piroplasmic forms in RBCs (parasitaemia rate 5–7%), schizonts in lymphocytes and monocytes	Concurrent with the presence of fever
Pneumonia	Signs of acute pulmonary involvement, crackles and wheezes on auscultation, coughing, dyspnea, fever	Bacteriology, virus identification, <i>post mortem</i> examination	Within 3–4 days after the onset of the disease
Ephemeral fever	Lameness, muscular shivering, ocular discharge, drooling saliva, arthritis, fever	Histology, epidemiology, clinical manifestation, virology	Within 3 days after the onset of the disease
Colisepticaemia	Septic shock, loose and mucoid faeces, complications such as meningitis in some cases	Isolation of the organism from faeces, leukocytosis, neutrophilia	Within 12 hours after the onset of the disease
Metritis	Occurrence within 2–10 days after parturition, severe toxemia, copious foul smelling uterine discharge with/without foetal membrane retention, fever	Clinical signs, rectal examination, leukopenia, neutropenia, degenerative active left shift	Within 1 week after parturition

\* all samples were taken in the acute stage of disease, before treatment.

Haptoglobin was measured with a commercial colorimetric kit and SAA – by a solid phase sandwich ELISA (Tridelta Development Plc, Wicklow, Ireland). The analytical sensitivities of these tests in serum have been determined as 0.3 µg/mL for SAA and 0.0156 mg/mL for Hp by the manufacturer.

Serum total sialic acid concentration was determined by the thiobarbituric acid method as described by Warren (1959). LBSA concentration was determined by the method of Katopodis *et al.* (1982). The amount of TSA and LBSA were determined against a standard curve developed from standard N-acetyl neuraminic acid sample. Protein bound sialic acid was calculated by subtracting LBSA from TSA.

Nonparametric Kruskal-Wallis and Mann-Whitney tests were used for statistical comparisons. Spearman's rank correlation coefficients were calculated to determine relationship between variables. All statistical analyses were performed using SPSS software v. 11.5 at a level of significance  $P < 0.05$ .

## RESULTS

Summary statistics and results for studied variables are presented in Table 2. All measured variables were statistically significantly higher in diseased animals compared to healthy ones ( $P < 0.001$ ); but the magnitude of the increase varied considerably among various diseases. TSA, PBSA and LBSA showed relatively consistent changes in all diseases, whereas the increase in SAA and Hp was more prominent in TRP and metritis (Table 2).

Results showed that there were significant correlations between TSA and PBSA in TRP ( $r = 0.94$ ;  $P < 0.01$ ), pneumonia ( $r = 0.82$ ;  $P < 0.05$ ) and control ( $r = 0.86$ ;  $p < 0.01$ ) groups (Table 3). Also, significant correlations were observed between TSA and LBSA in cattle with TRP ( $r = 0.94$ ;  $P < 0.01$ ), pneumonia ( $r = 0.94$ ;  $P < 0.01$ ), colisepticaemia ( $r = 0.81$ ;  $P < 0.05$ ) and metritis ( $r = 0.94$ ;  $P < 0.01$ ) (Table 3). No significant correlation existed between Hp and SAA as well as between either of these APPs and any other parameter.

**Table 2.** Serum concentrations (mean  $\pm$  SEM) of total sialic acid (TSA), lipid bound sialic acid (LBSA), protein bound sialic acid (PBSA), haptoglobin (Hp) and serum amyloid A (SAA) in healthy cattle (control) and cattle with various diseases

	TSA (mmol/L)	LBSA (mmol/L)	PBSA (mmol/L)	Hp (g/L)	SAA (µg/mL)
Control (n=10)	2.57 $\pm$ 0.02	1.19 $\pm$ 0.02	1.38 $\pm$ 0.03	0.09 $\pm$ 0.004	4.38 $\pm$ 0.12
Traumatic reticuloperitonitis (n=6)	3.59 $\pm$ 0.07	1.94 $\pm$ 0.05	1.65 $\pm$ 0.02	1.69 $\pm$ 0.04	303.83 $\pm$ 3.37
Theileriosis (n=11)	3.52 $\pm$ 0.04	1.74 $\pm$ 0.05	1.78 $\pm$ 0.05	0.54 $\pm$ 0.04	42.36 $\pm$ 4.00
Pneumonia (n=6)	3.54 $\pm$ 0.08	1.91 $\pm$ 0.06	1.64 $\pm$ 0.03	0.73 $\pm$ 0.06	89.05 $\pm$ 3.96
Ephemeral fever (n=5)	3.33 $\pm$ 0.03	1.69 $\pm$ 0.04	1.63 $\pm$ 0.03	0.33 $\pm$ 0.03	26.51 $\pm$ 1.65
Colisepticaemia (n=6)	3.51 $\pm$ 0.05	1.90 $\pm$ 0.07	1.61 $\pm$ 0.02	0.74 $\pm$ 0.04	90.55 $\pm$ 3.10
Metritis (n=6)	3.61 $\pm$ 0.03	1.96 $\pm$ 0.03	1.65 $\pm$ 0.02	1.23 $\pm$ 0.03	249.67 $\pm$ 16.00

Note: All variables were significantly different in various diseases from control group ( $P \leq 0.001$ ).

**Table 3.** Spearman's correlations coefficients between total sialic acid (TSA), lipid bound sialic acid (LBSA), protein bound sialic acid (PBSA), haptoglobin (Hp) and serum amyloid A (SAA) in healthy cattle (control) and cattle with various diseases

	TSA	LBSA	PBSA	Hp
<i>Control (n=10)</i>				
LBSA	-0.21			
PBSA	0.86**	-0.61		
Hp	-0.50	-0.45	-0.17	
SAA	0.06	-0.14	0.22	0.33
<i>Traumatic reticuloperitonitis (n=6)</i>				
LBSA	0.94**			
PBSA	0.94**	0.83*		
Hp	-0.26	-0.25	-0.37	
SAA	-0.60	-0.66	-0.71	0.31
<i>Theileriosis (n=11)</i>				
LBSA	0.14			
PBSA	0.52	-0.76**		
Hp	0.09	-0.15	0.24	
SAA	-0.01	-0.19	0.09	-0.23
<i>Pneumonia (n=6)</i>				
LBSA	0.94**			
PBSA	0.82*	0.64		
Hp	0.66	0.60	0.76	
SAA	0.09	0.03	0.52	0.37
<i>Ephemeral fever (n=5)</i>				
LBSA	0.82			
PBSA	0.30	-0.15		
Hp	-0.20	-0.62	0.30	
SAA	0.10	0.05	-0.40	-0.10
<i>Colisepticaemia (n=6)</i>				
LBSA	0.81*			
PBSA	-0.37	-0.75		
Hp	-0.03	0.03	0.03	
SAA	0.03	-0.38	0.71	0.14
<i>Metritis (n=6)</i>				
LBSA	0.94**			
PBSA	0.03	-0.26		
Hp	-0.14	-0.09	0.09	
SAA	-0.60	-0.71	0.43	0.03

\*P<0.05; \*\* P<0.01.

## DISCUSSION

As seen from the results, serum TSA, LBSA, PBSA, Hp and SAA concentrations were significantly higher in diseased animals compared to healthy ones. Serum

sialic acid values were analyzed in many inflammatory and infectious diseases in cattle, such as pneumonia (Karapehlivan *et al.*, 2007), theileriosis, anaplasmosis (Ertekin *et al.*, 2000; Karagenc *et al.*, 2005), leptospirosis (Keles *et al.*, 2000),

traumatic reticuloperitonitis (Citil *et al.*, 2004) and the results of this study are in agreement with these reports. In contrast, Yurtseven & Uysal (2009) have observed a significant decrease in serum sialic acid levels of naturally infected one year old cattle with high parasitaemia (50–70%) of *T. annulata*. It was concluded that this contradictory result may be due to acute and chronic theileriosis with different percentage of parasitaemia rates seen in young and older cattle, which were analyzed separately in two different studies. In the research by Deger *et al.* (2007), a significant increase of serum TSA and LBSA concentrations in babesiosis was established. Infections with other parasites such as *Leishmania* spp. (Chatterjee *et al.*, 1998; Karagenç *et al.*, 2005), and *Trypanosoma* (Eslevo *et al.*, 1982; Olaniyi *et al.*, 2001) are also associated with elevated serum sialic acid concentrations. It was reported that the serum TSA and LBSA were significantly higher in cattle infected with blood parasites (*Theileria* spp. and *Anaplasma* spp.) as compared to the control group (Ertekin *et al.*, 2000).

It has been reported that, at the beginning of inflammatory reactions or in injury, serum SA concentrations increase rapidly. However, the underlying mechanism that causes increase in serum SA has not been clearly defined. Serum sialic acid may be a marker of the acute phase response, since serum concentrations were significantly related to established acute phase proteins such as alpha-1 acid glycoprotein (Taniuchi *et al.*, 1981; Stefenelli *et al.*, 1985; Haq *et al.*, 1993). Acute phase reactants influence total sialic acid concentrations because of their glycoprotein structure (Taniuchi *et al.*, 1981).

The increase in PBSA levels may be attributable to elevated serum acute phase proteins during inflammation. It is demon-

strated that SA concentration increase rapidly following the inflammatory and injury process (Citil *et al.*, 2004). The increased sialic acid level may alter receptor-ligand interactions, which are known to play an important role in inflammation and immune response (Karagenç *et al.*, 2005). On the other hand, increased TSA and LBSA during inflammation and tissue damage is attributed to liberation of sialic acid from cell membrane into circulation as sialic acid is abundantly present in all biological membranes (Haq *et al.*, 1993; Thougard *et al.*, 1998).

There were significant correlations between TSA with both PBSA and LBSA in TRP- and pneumonia-affected cattle. Also, there was significant correlation between TSA and LBSA in colisepticaemia and metritis. It should be mentioned that the correlation coefficient between TSA and LBSA was also high in the ephemeral fever group ( $r=0.82$ ); however, it was not statistically significant due to low sample size in this group.

SAA and Hp were significantly higher in diseased animals compared to healthy ones; however the magnitude of increase was considerably different among various diseases. The increase in SAA and Hp was more prominent in TRP and metritis. Both SAA and Hp are among the major positive APPs in cattle and can increase several times from baseline levels after tissue injury (Murata *et al.*, 2004; Petersen *et al.*, 2004). SAA and iron profiles reflect the course of inflammation and their levels correlate with the clinical severity of the inflammation. SAA has the greatest role in bacterial and pyogenic infections and increases in common infectious diseases such as metritis, haematologic, respiratory and digestive infections and TRP (Nazifi *et al.*, 2008a). The results of the present study are consistent

with the study of Nazifi *et al.* (2008a), which reported that the concentration of SAA in cows with inflammatory diseases such as theileriosis, TRP and acute metritis was higher compared to healthy ones. Hp is a prominent acute phase protein in most species studied. Statistically significant increase in Hp was observed between clinically healthy and diseased cows in similar researches with the same inflammatory diseases (Alsemgeest *et al.*, 1994, Katoh & Nakagawa, 1999; Ganheim *et al.*, 2003). The results of this study are in agreement with that of Nazifi *et al.* (2008b) who revealed that serum Hp was elevated in some inflammatory diseases such as acute diffuse traumatic reticuloperitonitis, theileriosis, acute respiratory infections and the previous ones. Nazifi *et al.* (2009a) observed increased Hp and SAA in bovine tropical theileriosis and introduced SAA with the highest sensitivity and specificity compared to the other APPs as a suitable indicator of inflammatory reactions in bovine tropical theileriosis for differentiating healthy cows from diseased ones. In another study by Nazifi *et al.* (2009b) on cattle with traumatic reticuloperitonitis, a significant increase and strong correlation between SAA and Hp, indicating a very similar pattern of changes for these two APPs in various internal disorders in cattle including TRP were shown.

The results of this study revealed that serum sialic acid (TSA, LBSA, PBSA) and acute phase protein (SAA and Hp) concentrations increased in diseased cattle and therefore could be suitable indicators of various inflammatory conditions in this species to differentiate diseased animals from healthy ones. However, no significant correlation was observed between acute phase proteins (SAA and Hp) and serum sialic acids (TSA, LBSA, PBSA),

which may indicate that these two groups of inflammatory markers covered the different aspects of inflammation in diseased animals. Further researches are needed for better evaluation of this point.

## REFERENCES

- Alsemgeest, S. P. M., H. C. Kalsbeek, T. Wensing, J. P. Koeman, A. M. Van Ederen & E. Gruys, 1994. Concentrations of serum amyloid A (SAA) and haptoglobin (Hp) as parameters of inflammatory diseases in cattle. *Veterinary Quarterly*, **16**, 21–23.
- Alsemgeest, S. P. M., I. E. Lambooy, H. K. Wierenga, S. J. Dieleman, B. Meerkerk, A. M. van Ederen & T. A. Niewold, 1995. Influence of physical stress on the plasma concentrations of serum amyloid A (SAA) and haptoglobin (Hp) in calves. *Veterinary Quarterly*, **17**, 9–12.
- Arthington, J. D., S. D. Eicher, W. E. Kunkle & F. G. Martin, 2003. Effect of transportation and commingling on the acute-phase protein response, growth, and feed intake of newly weaned beef calves. *Journal of Animal Sciences*, **81**, 1120–1125.
- Carter, A. & N. H. Martin, 1962. Serum sialic levels in health and disease. *Journal of Clinical Pathology*, **15**, 69–72.
- Chatterjee, M., V. Sharma, C. Mandal, S. Sundar & S. Sen, 1998. Identification of antibodies directed against O-acetylated sialic acids in visceral leishmaniasis: Its diagnostic and prognostic role. *Glycoconjugate Journal*, **15**, 1141–1147.
- Citil, M., V. Gunes, M. Karapehlivan, G. Atalan & S. Marasli, 2004. Evaluation of serum sialic acid as an inflammation marker in cattle with traumatic reticuloperitonitis. *Révue de Médecine Vétérinaire*, **155**, 389–392.
- Crook, M., 1993. The determination of plasma or serum sialic acid. *Clinical Biochemistry*, **26**, 31–38.

- Deak, T., J. L. Meriwether, M. Fleshner, R. L. Spencer, A. Abouhamze, L. L. Moldawer, R. E. Grahn, L. R. Watkins & S. F. Maier, 1997. Evidence that brief stress may induce the acute phase response in rats. *American Journal of Physiology*, **273**, 1998–2004.
- Deger, Y., H. Mert, S. Dede, F. Yur & N. Mert, 2007. Serum total and lipid-bound sialic acid concentrations in sheep with natural babesiosis. *Acta Veterinaria Brno*, **76**, 379–382.
- Eckersall, P. D., 2000. Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals. *Révue de Médecine Vétérinaire*, **151**, 577–584.
- Eckersall, P. D., 2004. The time is right for acute phase protein assays. *Veterinary Journal*, **168**, 3–5.
- Ekin, S., N. Mert, H. Gunduz & I. Meral, 2003. Serum sialic acid levels and selected mineral status in patients with type 2 diabetes mellitus. *Biological Trace Element Research*, **94**, 193–201.
- Enjuanes, L., W. J. Spaan, E. J. Snijder & D. Cavanagh, 2000. Nidovirales. In: *Virus Taxonomy. Classification and Nomenclature of Viruses*, eds M. H. V. Regenmortel, C. M. Fauquet, D. H. L. Bishop, E. B. Carsten, M. K. Estes, S. M. Lemon, D. J. McGeoch, J. Maniloff, M. A. Mayo, C. R. Pringle & R. B. Wickner, Academic Press, New York, pp. 827–834.
- Ertekin, A., I. Keles, S. Ekin, M. Karaca & H. A. Akkan, 2000. An investigation on sialic acid and lipid-bound sialic acid levels in animals with blood parasites. *Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi*, **11**, 34–35.
- Eslevo, K., D. I. Saror, A. A. Ilemobade & M. H. Hallaway, 1982. Variation in erythrocyte surface and free serum sialic acid concentrations during experimental *Trypanosoma vivax* infection in cattle. *Research in Veterinary Science*, **32**, 1–5.
- Ganheim, C., C. Hulten, U. Carlsson, H. Kindahl, R. Niskanen & K. P. Waller, 2003. The acute phase response in calves experimentally infected with bovine viral diarrhoea virus and/or *Mannheimia haemolytica*. *Journal of Veterinary Medicine B*, **50**, 183–190.
- Gruys, E., M. J. M. Toussaint, T. A. Niewold & S. J. Koopmans, 2005. Acute phase reaction and acute phase proteins. *Journal of Zhejiang University Science B*, **6**, 1045–1056.
- Gunes, V., M. Karapehlivan, M. Citil, G. Atalan & S. Marasli, 2004. Relationship between serum sialic acid levels and eye lesions in calves with infectious bovine keratoconjunctivitis. *Révue de Médecine Vétérinaire*, **155**, 508–510.
- Haq, M., S. Haq, P. Tutt & M. Crook, 1993. Serum total sialic acid and lipid-associated sialic acid in normal individuals and patients with myocardial infarction and their relationship to acute phase proteins. *Annals of Clinical Biochemistry*, **30**, 383–386.
- Hemming, F. W., 1991. Glycoproteins of mammalian and avian cells. In: *Post-translational Modifications of Proteins*. 1<sup>st</sup> edn, eds J. J. Harding & M. J. C. Crabbe, CRC Press, London, pp. 217–253.
- Hickey, M. C., M. Drennan & B. Early, 2003. The effect of abrupt weaning of suckler calves on the plasma concentrations of cortisol, catecholamines, leukocytes, acute phase proteins and *in vitro* interferon gamma production. *Journal of Animal Sciences*, **81**, 2847–2855.
- Hicks, T. A., J. J. Mcglone, C. S. Whisnant, H. G. Kattesh & R. L. Norman, 1998. Behavioral, endocrine, immune and performance measures for pigs exposed to acute stress. *Journal of Animal Sciences*, **76**, 474–483.
- Kaneko, J. J., 1997. Serum proteins and dysproteinemias. In: *Clinical Biochemistry of Domestic Animals*, 5<sup>th</sup> edn, eds J. J. Kaneko, J. W. Harvey, M. L. Bruss, Academic Press, San Diego, pp. 117–138.
- Karagenc, T. I., F. K. Kiral, K. Seyrek, A. Bildik & H. Eren, 2005. Detection of serum total sialic acid in cattle with natural



- tropical theileriosis. *Révue de Médecine Vétérinaire*, **156**, 578–582.
- Karapehlivan, M., E. Atakisi, M. Cital, O. Kankavi & O. Atakisi, 2007. Serum sialic acid levels in calves with pneumonia. *Veterinary Research Communications*, **31**, 37–41.
- Katoh, N. & H. Nakagawa, 1999. Detection of haptoglobin in the high-density lipoprotein fractions from sera of calves with experimental pneumonia and cows with naturally occurring fatty liver. *Journal of Veterinary Medical Science*, **61**, 119–124.
- Katopodis, N., Y. Hirshaut, N. L. Geller & C. C. Stock, 1982. Lipid-associated sialic acid test for the detection of human cancer. *Cancer Research*, **42**, 5270–5275.
- Keles, I., A. Ertekin, M. Karaca, S. Ekin & H. A. Akkan, 2000. An investigation on serum sialic acid and lipid-bounded sialic acid levels in cattle with leptospirosis. *Journal of the Faculty of Veterinary Medicine, University of Yuzuncu Yil*, **11**, 121–122.
- King, D. J. & D. Cavanagh, 1991. Infectious bronchitis. In: *Diseases of Poultry*, 9<sup>th</sup> edn, eds B. W. Calnek, H. J. Barnes, W. M. Reid & H. W. Yoder, Iowa State University Press, Ames. pp. 471–484.
- Kloppel, T. M., C. P. Franz, D. J. Morre & R. C. Richardson, 1978. Serum sialic acid levels increased in tumor bearing dogs. *American Journal of Veterinary Research*, **39**, 1377–1380.
- Malykh, Y. N., R. Schauer & L. Shaw, 2001. N-glycolylneuraminic acid in human tumors. *Biochimie*, **83**, 623–634.
- Makimura, S. & M. Usui, 1990. Correlation between haptoglobin and sialic acid or mucoprotein in diseased bovine serum. *Japanese Journal of Veterinary Sciences*, **52**, 1245–1250.
- Motoi, Y., Y. Kimura, H. Wakamatsu & K. Shimbayashi, 1984. Determination and clinical evaluation of sialic acid and mucoprotein in bovine blood. *National Institute of Animal Health*, **37**, 643–649.
- Murata, H., 2007. Stress and acute phase protein response: An inconspicuous but essential linkage. *The Veterinary Journal*, **173**, 473–474.
- Murata, H., N. Shimada & M. Yoshioka, 2004. Current research on acute phase proteins in veterinary diagnosis: An overview. *The Veterinary Journal*, **168**, 28–40.
- Nazifi, S., A. Khoshvaghti & H. R. Gheisari, 2008a. Evaluation of serum and milk amyloid A in some inflammatory diseases of cattle. *Iranian Journal of Veterinary Research*, **9**, 222–226.
- Nazifi, S., A. Rezakhani, M. Koohimoghadam, M. Ansari-Lari & Z. Esmailnezhad, 2008b. Evaluation of serum haptoglobin in clinically healthy cattle and cattle with inflammatory diseases in Shiraz, a tropical area in southern Iran. *Bulgarian Journal of Veterinary Medicine*, **11**, 95–101.
- Nazifi, S., S. M. Razavi, Z. Esmailnejad & H. Gheisari, 2009a. Study on acute phase proteins (haptoglobin, serum amyloid A, fibrinogen, and ceruloplasmin) changes and their diagnostic values in bovine tropical theileriosis. *Parasitology Research*, **105**, 41–46.
- Nazifi, S., M. Ansari-Lari, J. Asadi-Fardaqi & M. Rezaei, 2009b. The use of receiver operating characteristic (ROC) analysis to assess the diagnostic value of serum amyloid A, haptoglobin and fibrinogen in traumatic reticuloperitonitis in cattle. *The Veterinary Journal*, **182**, 315–319.
- Olaniyi, M. O., V. O. Taiwo & A. O. Ogunsanmi, 2001. Haematology and dynamics of erythrocyte membrane sialic acid concentration during experimental *Trypanosoma congolense* and *T. brucei* infection of sheep. *Journal of Applied Animal Research*, **20**, 57–64.
- Petersen, H. H., J. P. Nielsen & P. M. H. Heegaard, 2004. Application of acute phase protein measurements in veterinary clinical chemistry. *Veterinary Research*, **35**, 163–178.
- Pieiro, M., C. Pineiro, R. Carpintero, J. Morales, F. M. Campbell, P. D. Eckersall, M. J. Toussaint & F. Lampreave, 2007. Cha-

- racterization of the pig acute phase protein response to road transport. *The Veterinary Journal*, **173**, 669–674.
- Schauer, R., 2000. Achievements and challenges of sialic acid research. *Glycoconjugate Journal*, **17**, 485–499.
- Stefenelli, N., H. Klotz, A. Engel & P. Bauer, 1985. Serum sialic acid in malignant tumours. Bacterial infections and chronic liver diseases. *Journal of Cancer Research and Clinical Oncology*, **109**, 55–59.
- Sydow, G., W. Wittmann, E. Bender & E. Starick, 1988. Der Sialinsäuregehalt im Serum von mit bovinem Leukosevirus infizierten Rindern. *Archiv für experimentelle Veterinärmedizin*, **42**, 194–197.
- Taniuchi, K., K. Chifu, N. Hayashi, Y. Nakamachi, N. Yamaguchi, Y. Miyamoto, K. Doi, S. Baba, Y. Uchida, Y. Tsukada & T. Sugimori, 1981. A new enzymatic method for the determination of sialic acid in serum and its application for a marker of acute phase reactants. *Kobe Journal of Medical Sciences*, **27**, 91–102.
- Thougaard, A. V., E. Hellmen & A. L. Jensen, 1998. Total serum sialic acid is a general disease marker rather than a specific tumor marker in dogs. *Journal of Veterinary Medicine A*, **45**, 471–479.
- Warren, L., 1959. The thiobarbituric acid assay of sialic acids. *Journal of Biological Chemistry*, **234**, 1971–1975.
- Yurtseven, S. & H. Uysal, 2009. Decreased serum sialic acid, albumin-globulin ratio and total protein levels in cattle heavily infected with *Theileria annulata*. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, **56**, 141–144.

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