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Evaluation of Inflammatory Mediators (Sialic Acid, Tumor Necrosis Factor-a and Interferon-Γ) and Acute-Phase Proteins (Haptoglobin, Serum Amyloid a and A_1-acid Glycoprotein) and Relationship Between These Parameters in Healthy Ostriches

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Abstract: Measurement of acute phase proteins has important in disease diagnosis and investigation of treatment course in human and animals, because positive acute phase proteins increase immediately after disease appearing and decrease level of these proteins with disease severity subtraction .In recent years, ostriches training has considered in Iran but reference values of acute phase proteins and inflammatory mediators in healthy ostriches aren't known. In the present study reference values of haptoglobin (Hp),serum amyloid A (SAA), $\alpha_1$acid glycoprotein (AGP), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), total sialic acid (TSA), lipid bound sialic acid (LBSA) and protein bound sialic acid (PBSA) and relationships between these parameter were investigated. Blood samples were collected from the jugular vein of one hundred apparently healthy adult male ostriches and the concentrations of Hp, SAA, AGP, TNF-α, INF-γ, TSA, LBSA, PBSA were measured. The mean concentration of Hp, SAA, PBSA, LBSA, TSA, TNF-α and INF-γ were 0.075±0.0008 mg/ml, 1.76±0.014 µg/ml, 0.0002±0.000 mmol/L, 0.0003±0.0000 mmol/L, 0.0005±0.000mmol/L, 16.5±0.165 pg/dl and 9.827±0.116 pg/dl, respectively. AGP wasn't detectable in serum of these ostriches. No correlation was observed between Hp with any other parameters, however statistically tests shown that there is positive significant correlations between INF-γ and PBSA (r= 0.220, P = 0.044) also between PBSA and SAA (r= 0.203, P =0.028). There was a negative correlation between LBSA and PBSA (r=-0.286, P =0.004) and a significant positive correlation between LBSA and TSA (r= 0.887, P =0.000). The conclusions s of this study reveal the normal values of sialic acid, tumor necrosis factor-α, Interferon-γ, haptoglobin, serum amyloid A and α_1-acid glycoprotein in ostriches are very lower than the normal values of these parameters in mammalians but are rather equal with those of other birds.

Key words: Acute Phase Proteins · Tumor Necrosis Factor-A · Interferon-Γ · Total Sialic Acid · Lipid Bound Sialic Acid · Protein Bound Sialic Acid · Ostriches

Abbreviations

APPs	Acute-phase proteins
TSA	Total sialic acid
LBSA	Lipid-bound sialic acid
PBSA	Protein-bound sialic acid
Hp	Haptoglobin
SAA	Serum amyloid A
AGP	$\alpha_1$acid glycoprotein
TNF-α	Tumor necrosis Factor-α
Interferon-γ	IFN-γ

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INTRODUCTION

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 [1-3]. SAA and Hp as well as other APPs have been proposed to be markers of stress in cattle and other species [4-7]. The APPs assay may have potential for monitoring adverse environmental and/or management stressors, thus enabling better control of animal welfare [8, 9]. They are mainly synthesized in the liver, mediated by pro-inflammatory cytokines and their concentration can 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 [2,9]. APPs and their change due to various inflammatory and non-inflammatory conditions have been studied intensively in many animal species [2, 9, 10]. Glycoproteins are defined as proteins which contain glycan chains linked glycosidically to selected amino acid residues. Monosaccharides commonly found in the glycans of the glycoproteins including N-acetylneuraminic acid, sialic acid [11]. Sialic acids as monosaccharides are linked to the terminal galactose, N-acetylgalactosamine, or to other sialic acids in carbohydrate chains attached to glycoproteins and glycolipids [12]. Sialic acids are often involved in important cell surface communications and infection processes and are present in normal serum in humans and animals; their content in serum is changed in various diseases [13-17] Sialic acids are also found in bacteria and animal tissues [12]. High serum sialic acid level is an important factor for certain diseases. Sialic acid concentration increases rapidly following the inflammatory and injury process [16]. The mechanism underlying the induction of sialic acid increase is not clearly understood. However, investigators have reported that sialic acid localized at the end chain of many acute-phase proteins can be used as marker for the determination of acute-phase protein concentrations [16, 18-21] because serum acute-phase proteins, especially α1-acid glycoprotein are sialyted glycoproteins.

Tumor necrosis factor is a cytokine involved in systemic inflammation and is a member of group of cytokines that stimulate the acute phase reaction. The primary role of TNF is in the regulation of immune cells. TNF is able to induce apoptotic cell death, to induce inflammation and to inhibit tumorigenesis and viral replication. TNF was thought to be produced primarily by macrophages, but it is produced also by a broad variety of cell types including lymphoid cells, mast cells, endothelial cells, cardiac myocyte, adipose tissue, fibroblast and neural tissue. Large amounts of TNF are released in response to lipopolysaccharide, other bacterial products and Interleukin-1 .In the skin, mast cells appear to be the predominant source of pre-formed TNF, which can be released upon inflammatory stimulus [22]. Amylin injection induced significant increase in haptoglobin, C-reactive protein and nitrus oxide secretion [23].

There are no studies on APPs, sialic acids, TNFα and INFγ in healthy ostriches, also there is no published study on the relationship between APPs, sialic acids, TNFα and INFγ in healthy ostriches.

The aim of the present study was to find and compares the concentrations of Hp, SAA, sialic acid (total, lipid- and protein-bound), TNFα and INFγ in healthy ostriches.

MATERIALS AND METHODS

Birds: After clinical examinations and laboratory tests, one hundred clinically healthy adult male ostriches were selected from two ostrich's farms around Shiraz and Kerman provinces.

Blood Sampling and Processing: Blood samples were collected from the jugular vein of healthy ostriches into tubes without anticoagulant. The sera were separated by centrifugation at 750*g* for 15 min and stored at – 20°C until used. In the serum of ostriches, acute phase proteins and inflammatory mediators were measured using validated standard procedures.

Haptoglobin Determination: Haptoglobin (Hp) was measured according to prevention of the peroxidase activity of hemoglobin, which is directly proportional to the amount of Hp. The analytical sensitivity of this test in serum has been determined as 0.0156 mg/ml for Hp by the manufacturer (Tridelta Development Plc, Wicklow, Ireland).

Serum amyloid A determination: Serum amyloid A (SAA) was measured by a solid phase sandwich ELISA. The analytical sensitivity of this test in serum has been determined as 0.3 μg/ml for SAA by the manufacturer (Tridelta Development Plc, Wicklow, Ireland).

Serum α1-acid Glycoprotein Determination: Serum α1-acid glycoprotein was measured by Radial-immunodiffusion method (Tridelta Development Plc, Wicklow, Ireland).

Total Sialic Acid Determination: Serum total sialic acid concentration was determined by the thiobarbituric acid method previously described by Warren The amount of total sialic acid was determined by use of a standard curve developed from a standard sample of N-acetyl neuraminic acid [24].

Lipid Bound Sialic Acid Determination: Lipid bound sialic acid concentration was determined by the method described by Katopodis *et al.* The amount of lipid bound sialic acid was determined by use of a standard curve developed from a standard sample of N-acetyl neuraminic acid [25].

Protein Bound Sialic Acid Determination: Protein bound sialic acid concentration was measured by subtracting serum total sialic acid from lipid bound sialic acid.

INF-γ and TNF-α Determination: INF-γ and TNF-α were measured by a solid phase sandwich ELISA (AbC 606 and AbC 607, respectively; Votre fournisseur AbCys S.A. Paris, France).

Statistical Analysis: Descriptive statistics including mean and standard error were calculated for all variables. Association between the studied variables was investigated using Spearman's correlation coefficients and only statistically significant correlations were reported. Data were analyzed by SPSS software, version 11.5. A P-value less than 0.05 were considered as statistically significant.

RESULTS

The mean ±SE of acute phase proteins and inflammatory mediators (Hp, SAA, TNF-α, IFN-γ, TSA, LBSA and PBSA) in 100 clinically healthy ostriches was shown in Table 1. The relationship between different parameters was shown in Table 2. There were positive significant correlations between PBSA and SAA (r= 0.203, P=0.044), also between PBSA and INFγ (r=0.220, P =0.028).There is a severe positive significant correlation between LBSA and TSA (r= 0.887, P=0.000) and observed a negative significant correlation between PBSA and LBSA (r=-0.286, P =0.004). Statistical evaluations showed that there were no correlations between other parameters.

DISCUSSION

The concentration of serum Hp in clinically healthy ostriches was 0.0752±0.0008mg/ml, which is in agreement to the concentration of serum Hp in clinically healthy avian that reported by Nazifi *et al.* [26], but the mean concentration of serum Hp in clinically healthy ostriches is lower than the normal value of cattle serum Hp that have been reported in other studies [27, 28].

Table 1: Mean±SE serum concentration of HP, SAA, PBSA, LBSA, TSA, TNF-α, INF-γ in 100 clinically healthy ostriches

	Mean±SE						
Parameter	Hp (µg/ml)	SAA (mg/ml)	PBSA (mmol/L)	LBSA (mmol/L)	TSA (mmol/L)	TNF-α (pg/dl)	INF-γ (pg/dl)
Healthy ostriches	0.0752±0.0008	1.7506±0.014	0.0002±0.000	0.0003±0.000	0.0005±0.000	16.500±0.165	9.827±0.116

Table 2: Correlation between serum concentrations of Hp, SAA, PBSA, LBSA, TSA, TNF-α, INF-γ in 100 clinically healthy ostriches

Parameter	Hp (µg/ml)	SAA (mg/ml)	TSA (mmol/L)	PBSA (mmol/L)	LBSA (mmol/L)	TNF-α (pg/dl)	INF-γ (pg/dl)
Hp (µg/ml)	1	0.149	0.037	0.061	-0.004	-0.067	-0.069
SAA (mg/ml)	0.149	1	-0.042	0.203*	-0.116	-0.121	0.120
PBSA (mmol/L)	0.061	0.203*	0.110	1	-0.286*	-0.025	0.220*
LBSA (mmol/L)	0.004-	-0.116	0.887**	0.225*	1	0.079	0.068
TSA (mmol/L)	0.037	-0.042	1	-0.053	0.887**	.059	0.157
TNF-α (pg/dl)	-0.067	-0.121	-.035	0.072	0.079	1	0.045
INF-γ(pg/dl)	-0.069	o.120	0.157	0.220*	0.068	0.045	1

*Significant in P<0.05 **Significant in P<0.01

The concentration of serum amyloid A in this study was 1.76±0.014ig/ml that is in agreement with normal value of avian that was observed by Nazifi *et al.* [26], but is very lower in comparison to normal value of serum amyloid A in other animals, for example the reference value for SAA in apparently healthy cows was determined as <8.8 mg/ml [29]. In this study, Hp to SAA ratio was 0.0423±0.000 that is in agreement that was reported by Nazifi *et al.* in chicks [26].

In this study was observed that α1- acid glycoprotein wasn't detectable in serum of ostriches, but this protein is an important acute phase protein in avian diseases diagnosis [30].

The concentration of acute phase proteins is generally low to no detectable in healthy animals and elevations are used to diagnose and monitor inflammatory diseases [31].

The serum APPs have been used as nonspecific clinical markers of health problems in humans and other mammalian species [32]. APPs can be used for prognosis and diagnosis of disease. APP may provide a similar use in identifying poultry health problems. The acute phase response is mediated by the pro-inflammatory cytokines, which induce the synthesis of acute phase proteins by the liver, particularly interleukin-1(IL-1), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) [33,34]. The pro-inflammatory cytokines induce the synthesis of acute phase proteins by the liver. There are major differences between various animal species in the APP response in disease [35]. Several acute phase proteins have been analyzed in chicken in association with common poultry diseases. Most of these APPs do not change to the same level as mammalian APPs and could not be analyzed in natural infections. In mammals, the increased synthesis of acute phase proteins in response to an immune challenge varies in magnitude from a 50% increase in ceruloplasmin to a several hundred-fold increase in C-reactive protein [36]. In chickens, only a few APPs have been described so far. Of these the plasma α1-acid glycoprotein, SAA, transferrin, TSA, LBSA, PBSA and Ovotransferrin can be mentioned [30, 37-41].

Inflammation or tissue injury causes the release of pro-inflammatory cytokines such as IL-1, IL-6 and tumour necrosis factor which alter the blood concentration of a variety of proteins that are produced primarily in liver. α- and/or β-regions by serum protein electrophoresis.

Tracheitis, bronchitis, tracheal edema, bronchial caseous plugs and air sacculitis indicates widespread inflammatory reaction in IBV this causes release and elevation of SAA and Hp concentrations. Kovacs *et al.* (showed a mild increase in SAA in the goose by administration of a fowl cholera vaccine containing inactivated *Pasteurella multocida.* Vaccination was an inflammatory factor and produced increased SAA levels [42].

Nazifi *et al.* showed a significant increase in SAA levels in chicks by gumboro [39]. Chamanza *et al.* reported that administration of terpentin to pullet and *Staphylococcus aureus* infection in chicks cause the elevation in SAA and transferrin concentration [37].

The concentrations of serum LBSA, PBSA, TSA in ostriches were 0.0003±0.0000, 0.0002±0.0000, 0.0005±0.0000 mmol/L respectively that is lower than the normal value of cattle serum LBSA, PBSA, TSA that have been reported in other studies [41], that is apparently in agreement with normal value of avian that was showed by Nazifi *et al.* [26]. LBSA to PBSA ratio in ostriches was 1.5±0.000, however this ratio in avian was reported 0.98±0.27 [26].

In this study the concentrations of serum TNFα and INFγ in ostriches were 16.50± 0.165 and 9.827±0.116 pg/dl, respectively.

They are capable of stimulating mononuclear phagocytes and endothelial cells to release immune modulators such as TNF-α, members of the interleukin family (IL-1, IL-6, IL-8, IL-12) and interferon-α [43].

There were positive significant correlations between PBSA and SAA (r=0. 203, P=0.044),also between PBSA and INFγ (r= 0.220, P=0.028).There is a sever positive significant correlation between LBSA and TSA (r=0.887, P= 0.000) and there is a negative significant correlation between PBSA and LBSA (r= -0.286, P= 0.004).

Statistical evaluations showed that there were no correlations between other parameters that were investigated in present study.

Nazifi *et al.* showed significant associations between Hp and INF-γ, fibrinogen with TSA, fibrinogen with PBSA, TSA with PBSA and TNF-α with ceruloplasmin in serum of normal cattle[40]. The previously studies on chicks showed positive significant correlations between TSA, LBSA and PBSA, however, there was significant negative correlation between Hp and SAA in chick serum [26].

The concentrations of TSA, LBSA and PBSA are significantly higher in diseased birds than healthy avian [26, 44-47].

In conclusion, serum concentrations of Hp, SAA, α1-acid glycoprotein, LBSA, PBSA, TSA, TNFα and INFγ in ostriches are as same as these parameters concentrations in avian serum, but their concentrations are very lower in comparison of serum concentrations of Hp, SAA, α1-acid glycoprotein, LBSA, PBSA, TSA, TNFα and INFγ in mammalians.

REFERENCES

1. Eckersall, P.D., 2004. The time is right for acute phase protein assays.Vet. J., 168: 3-5.
2. Murata H., N. Shimada and M. Yoshioka, 2004. Current research on acute phase proteins in veterinary diagnosis: an overview. Vet. J., 168: 28-40.
3. Gruys, E., M.J.M. Toussaint, T.A. Niewold and S.J. .Koopmans, 2005. Acute phase reaction and acute phase proteins. J. Zhejiang. Univ. Sci .B 6: 1045-1056.
4. Alsemgeest, S.P.M. Lambooy, I.E. Wierenga, H.K. Dieleman, S.J. Meerkerk, B. van A.M. Ederen and T.A. Niewold, 1995. Influence of physical stress on the plasma concentrations of serum amyloid A (SAA) and haptoglobin (Hp) in calves. Vet. Q, 17: 9-12.
5. Deak, T., J.L. Meriwether, M. Fleshner, R.L. Spencer, A. Abouhamze, L.L. Moldawer, R.E. Grahn, L.R. Watkins and S.F. Maier, 1997. Evidence that brief stress may induce the acute phase response in rats. Am. J. Physiol., 273: 1998-2004.
6. Hicks, T.A., J.J. Mcglone, C.S. Whisnant, H.G. Kattesh and R.L. Norman, 1998. Behavioral, endocrine, immune and performance measures for pigs exposed to acute stress. J. Anim. Sci., 76: 474-483.
7. Hickey M.C., M. Drennan and 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. J. Anim. Sci., 81: 2847-2855.
8. Pieiro, M., C. Pineiro, Carp R. Intero, J. Morales, F.M. Campbell, P.D. Eckersall, M.J. Toussaint and F. Lampreave, 2007.Characterization of the pig acute phase protein response to road transport. Vet. J., 173: 669-674.
9. Murata, H., 2007. Stress and acute phase protein response: an inconspicuous but essential linkage. Vet. J., 173: 473-474.
10. Kaneko, J.J., 1997. Serum proteins and dysproteinemias. In: Kaneko, J.J., J.W. Harvey and M.L. Bruss, (Eds) Clinical biochemistry of domestic animals, 5th edn. SanDiego, Academic, pp: 117-138.
11. Hemming, F.W., 1991. Glycoproteins of mammalian and avian cells. In:Harding JJ, Crabbe MJC (eds) Post-translational modifications of proteins, 1st edn. CRC Pres, London, pp: 217-253.
12. Schauer, R., 1985. Sialic acids and their role as biological markers. Trends. Biochem. Sci., 10: 357-360.
13. Kloppel, T.M., C.P. Franz, D.J. Morre and R.C. .Richardson, 1978. Serum sialic acid levels increased in tumor bearing dogs. Am. J. Vet. Res., 39: 1377-1380.
14. Makimura, S. and M. Usui, 1990. Correlation between haptoglobin and sialic acid or mucoprotein in diseased bovine serum. Jpn. J. Vet. Sci., 52: 1245-1250.
15. King, D.J. and D. Cavanagh, 1991. Infectious bronchitis. In: Calnek, B.W., H.J. Barnes, W.M. Reid and H.W. Yoder, (eds), Diseases of poultry, 9th edn. Iowa State University Press, Ames, pp: 471-484.
16. Ekin S., N. Mert, H. Gunduz and I. Meral, 2003. Serum sialic acid levels and selected mineral status in patients with type 2 diabetes mellitus. Biol. Trace. Elem. Res., 94: 193-201.
17. Citil, M., V. Gunes, M. Karapehlivan, G. Atalan and S. Marashı, 2004. Evaluation of serum sialic acid as an inflammation marker in cattle with traumatic reticuloperitonitis. Rev. Med. Vet., 155: 389-392.
18. Taniuchi, K., K. Chifu, N. Hayashi, Y. Nakamachi, N. Yamaguchi, Y. Miyamoto, K. Doi, S. Baba, Y. Uchida, Y. Tsukada and 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. J. Med. Sci., 27: 91-102.
19. Crook, M., 1993. The determination of plasma or serum sialic acid. Clin. Biochem., 26: 31-38.
20. Thougaard, A.V., E. Hellmen and A.L. Jensen, 1998. Total serum sialic acid is a general disease marker rather than a specific tumour marker in dogs. J .Vet. Med. A., 45: 471-479.
21. Enjuanes, L., W.J. Spaan, E.J. Snijder and D. Cavanagh, 2000. Nidovirales. In: Regenmortel MHV, Fauquet, C.M., D.H.L. Bishop, E.B. Carsten, M.K. Estes, J. Lemon, M.A. Mayo, C.R. Pringle and R.B. Wickner, (eds) Virus taxonomy. Classification and nomenclature of viruses. Academic Press, New York, pp: 827-834.
22. Walsh, L.J., 1991. Tumor necrosis factor alpha. Proc. Acad. Sci., 88(10): 4220-4224.
23. Soliman, W.N., A.D. Abdel Majid, O.M. Abdel Hamid and Z.S. Ibrahim, 2011. Acute phase proteins immunoglobulins and cytokines responses to amylin injection in wistar Rat.Global J. Biotechnology and Biochemistry, 6(3): 112-118.

24. Warren, L., 1959. The thiobarbituric acid assay of sialic acids. J. Biol. Chem., 234: 1971.
25. Katopodis, N., Y. Hirshaut, N.L. Geller and C.C. Stock, 1982. Lipid-associated sialic acid test for the detection of human cancer. Cancer Res., 42: 5270-5275.
26. Nazifi, S., M.R. Tabande, S.A. Hoseinian, M. Ansari-Lari and H. Safari, 2011. Evaluation of sialic acid and acute-phase proteins (haptoglobin and serum amyloids A) in healthy and avian infection bronchitis virus-infected chicks. Comp. Clin. Pathol., 20: 69-73.
27. Nazifi, S., M. Haghkhah, Z. Asadi, M. Ansari-Lari, M.R. Tabandeh, Z. Esmailnezhad and M. Aghamiri, 2010. Evaluation of sialic acid and acute phase proteins (haptoglobin and serum amyloid A) in clinical and subclinical bovine mastitis. Pakistan. Vet. J., 31: 55-59.
28. Nakagama, T., S. Sonada, T. Urano, T. Yamada and M. Okada, 1993. Monitoring both serum amyloid protein A and C-reactive protein as inflammatory markers in infectious disease, Clin. Chem., 39: 293-297.
29. Horadagoda, N.U., K.M.G. Knox, H. Gibbs, S.W.J. Reid, A. Horadagoda, S.E.R. Edwarda and P.D. Eckersall, 1999. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. Vet. Rec., 144: 437-441.
30. Inoue, M., W. Satoh and H. Murakami, 1997. Plasma α1-acid glycoprotein in chickens infected with infectious bursal disease virus. Avian Dis., 41: 164-170.
31. Feldman, B.V., J.G. Zinkl and N.C. Jain, 2000. Schalm's Veterinary Hematology. 5th edn. Williams & Wilkins, Philadelphia, pp: 1152.
32. Gaby, C. and I. Kushner, 1999. Acute-phase proteins and other systemic responses to inflammation. New England J. Med., 340: 448-454.
33. Rivier, C., R. Chizzonite and W. Vale, 1989. In the mouse, the activation of hypothalamic_pituitary-adrenal axis by a lipopolysaccharide endotoxin is mediated through interleukin-1. Endocrinol., 125: 2800-2805.
34. Zetterstrom, M., A.K. Sundgren-Andersson, P. Ostlund and T. Bartfai, 1998. Delineation of the proinflammatory cytokine cascade in fever induction. Annals. New York Acad. Sci., 856: 48-52.
35. Eckersall, P.D., 2006. Acute phase proteins as biomarkers of disease in companion and laboratory animals. Proceedings of the 57th Annual Meeting of the American College of Veterinary Pathologists and 41st Annual Meeting of American Society for Veterinary Clinical Pathology, Tucson, Arizona, pp: 47-52.
36. Barnes, D.M., Z. Song, K.C. Klasing and W. Bottje, 2001. Protein metabolism during an acute phase response in chickens. Amino acid., 22: 15-26.
37. Chamanza, R., M.J. Toussaint, A.M. van Ederen, L. van Veen, C. Hulskamp Koch and T.H. Fabri, 1999. Serum amyloid A and transferrin in chicken. A preliminary investigation of using acute-phase variables to assess diseases in chickens. Vet. Q., 21: 158-162.
38. Holt, P.S. and R.K. Gast, 2002. Comparison of the effects of infection with salmonella enteritidis, in combination with an induced molt, on serum levels of the acute phase protein α1-acid grycoprotein, in hens. Poult. Sci., 81: 1295-1300.
39. Xie, H., G.R. Huff, J.M. Balog, P. Holt and N.C. Rath, 2002. Identification of ovotransferrin as an acute phase protein in chickens. Poult. Sci. 81: 112-120.
40. Nazifi, S., H. Dadras, S.A. Hoseinian, M. Ansari-Lari and M. Masoudian, 2010. Measuring acute phase proteins (haptoglobin, ceruloplasmin, serum amyloid A and fibrinogen) in healthy and infectious bursal disease virus-infected chicks. Comp. Clin. Pathol., 19: 283-286.
41. Nazifi, S., M. Ansari-Lari, N. Ghafari, S. Mohtarami, A. Ghezelbash and M.R. Tabandeh, 2010. Evaluation of sialic acids, TNF-α, INF-γ and acute- phase proteins in cattle infected with foot-and-mouth disease. Comp .Clin. Pathol (In Press).
42. Kovacs, B.M., M.J. Toussaint, E. Gruys, I.B. Fabian, J.J. Szilagyil and P. Rudas, 2007. Evaluation of goose serum amyloid A acute phase response by enzyme-linked immunosorbent assay. Acta. Vet. Hung., 55: 349-357.
43. Hamann, L., V. EL-Samalouti, A.J. Ulmer, H.D. Flad and E.T. Rietschel, 1998. Components of gut bacteria as immunomodulators. Inter. J. Food Microbiol., 41: 141-154.
44. Tsolov, V., P. Tsonev, G. Galbinowv and G. Kamburov, 1973. Untersuchungen überdie veränderungen und den diagnostischen wert der sialinsäure in Kuhmilch beisubklinischen mastitis formen. Zbl. Vet. Med. B, 20: 577-583.

45. Ertekin, A., I. Keles, S. Ekin, M. Karaca and H.A. Akkan, 2000. Kan parazitli hayvanlarda sialik asit ve lipidbagli sialik asitd, zeyle- rinin arastirilmasi. YYU Vet Fak Derg., 11: 34-35.
46. Keles, I., A. Ertekin, M. Karaca, S. Ekin and H.A. Akkan, 2000. Sigirlarin,leptospirozisinde serum sialik asit ve lipid-bagli sialik asit düzeyleri üzerine arastirma. YYU. Vet. Fak. Derg., 11: 121-122.
47. Farsang, A., C. Ros, L.H.M. Renstrom, C. Baule, T. Soos and S. Belak, 2002. Molecular epizootiology of infectious bronchitis virus in Sweden indicating the involvement of a vaccine strain. Avian Pathol., 31: 229-236.