Bulgarian Journal of Veterinary Medicine (2006), **9**, No 3, 189–192

CHANGES IN THE PROTEIN PROFILE IN BIRDS WITH EXPERIMENTAL ACUTE FOWL TYPHOID

T. KOKOSHAROV
Regional Veterinary Medical Service, Haskovo, Bulgaria

Summary

Kokosharov T., 2006. Changes in the protein profile in birds with experimental acute fowl typhoid. *Bulg. J. Vet. Med.*, **9**, No 3, 189–192.

It was observed that the total protein in liver and blood serum decreased significantly during the course of experimental oral infection with *Salmonella gallinarum* in 6-month old chickens. Six serum protein fractions were identified. Albumin, a liver-synthesized fraction, followed the tendency of total protein change; α_2 globulins were increased, β- and γ_2 globulins – considerably reduced compared to non-infected control birds. The combined effect of these changes resulted in lower albumin/globulin ratio (0.14). The development of the experimental infection was accompanied by hypoproteinaemia and dysproteinaemia.

Key words: chickens, experimental infection, protein fractions, *Salmonella gallinarum*, total protein

INTRODUCTION

Proteins are high-molecular nitrogen containing organic compounds that are responsible for the membrane transport, form the colloid osmotic pressure, maintain blood pH in a narrow range, participate in blood clotting and have a structural role (Griwinger & Scanes, 1986; Saier, 1996).

It is known that changes in ceruloplasmin, haptoglobin, C-reactive protein, albumin, are observed in the early stages of infections in all animal species. During the study of this phenomenon, the respective proteins were classified as acute phase proteins (APP) and their response became an exceptionally interesting field of study. This interest was also enhanced by the utilization of APPs by clinicians as a reliable sign for the presence of any form of inflammation (Kaneko, 1997). Sepsis is accompanied by a change in acute phase proteins (Vary & Kimball, 1992). Serum proteins' electrophoresis is one of diagnostic techniques that provide information about the healthy status of animals. If correctly interpreted, it could give information about the systemic health and the relationship between the biological response and each serum protein (Tohio *et al.*, 1995).

It is accepted that serum protein profile and the absolute values of individual fractions are an excellent basis for a tentative diagnosis (Kaneko, 1997). Relatively few is however known about the changes in the chemical composition of blood, including APPs during a *Salmonella gallinarum* infection, one of the most important infectious poultry diseases, causing a high death rate and economical losses (Shivaprasad, 1997). This motivated our interest to study the effect of acute *S. gal-*

linarum infection in fowl upon the changes in some proteins.

MATERIALS AND METHODS

Experimental animals

The study was performed in 28 6-month-old female *Salmonella*-free New Hampshire chickens divided into 2 groups: control (non-infected, n=8) and experimental (infected, n=20). The chickens were housed in standard conditions and received food and water without antibiotic supplements.

Experimental design

The *S. gallinarum* strain was isolated from a dead hen and was maintained on Dorset's medium at 4 ^{o}C. During the experiment, the strain was subcultivated for 5 days on 5% sheep blood agar for maintenance of its virulence. A bacterial suspension in physiological saline, containing 1.5×10^{9} colony forming units in 1 mL was prepared and 1 mL of it was introduced in the crop of experimental birds. Between post infection days 4 and 7, half of the chickens died and the rest infected and control birds were euthanized by exsanguination.

Parameters and methods of determination

The concentration of total protein in liver and blood serum was determined by the biuret reaction (Gornall, 1946). All reagents were prepared in the laboratory. Serum albumin and globulins were assayed by agar electrophoresis[1].

[1] Special thanks to Assoc. Prof. Petko Ivanov from the Department of Veterinary Microbiology, Infectious and Parasitic Disease at the Faculty of Veterinary Medicine, Trakia University, Stara Zagora, for his assistance.

Statistical analysis

The statistical analysis was done by the Student's t-test at a level of significance of $P<0.05$.

RESULTS

Total protein concentrations in the liver as well as blood protein fractions in *S. gallinarum*-infected and non-infected birds are presented in Table 1. It is seen that liver total protein in infected chickens was statistically significantly lower compared to controls (69.0 ± 20.3 g/L in euthanized and 64.1 ± 11.9 g/L in dead infected chickens; $p<0.001$ vs control values: 86.6 ± 9.8 g/L). The same tendency was exhibited by blood serum total protein in infected birds ($P<0.001$). Albumin concentrations decreased in the same way as total protein levels ($P<0.001$). Alpha$_1$ and γ_1 globulins were not altered whereas α_2 levels increased and reached 7.98±0.92 g/L in the experimental group vs 5.89 ± 0.68 g/L in controls ($P<0.001$). The concentrations of β and γ_2 globulins were considerably lower ($P<0.001$). The albumin/globulin ratios were 0.42 and 0.14 in the control and experimental groups, respectively.

DISCUSSION

The infection of 6-month-old female chickens with *S. gallinarum* produced a significant decrease in liver and blood serum total protein concentrations in euthanized and dead birds. This corresponds to the findings of Vary & Kimball (1992), Dickerson *et al.* (1990) and Chiolero *et al.* (1997) reporting inhibited synthesis of protein in animals with sepsis. The systemic reaction after the infection was characterized by numerous changes in protein synthesis. Regardless of the non-

Table 1. Total protein and protein fractions (g/L) in healthy chickens and chickens, infected with *Salmonella gallinarum* (mean ± SD)

Parameter	Infected chickens		Control (non-infected chickens) (n=8)
	diseased (n=7)	dead (n=7)	
Total protein:			
in liver	69.00 ± 20.30 **	64.10 ± 11.90 *	86.60 ±9.80
in blood serum	37.80 ± 3.50 **	NA	55.00 ± 12.30
Serum protein fractions			
Albumin	6.57 ± 0.47 **	NA	15.17 ± 1.71
α_1 globulin	3.53 ± 0.88	NA	4.63 ± 1.87
α_2 globulin	7.98 ± 0.92 **	NA	5.89 ± 0.68
β globulin	3.26 ± 1.14 **	NA	7.23 ± 2.18
γ_1 globulin	10.00 ± 0.34	NA	10.45 ± 1.58
γ_2 globulin	6.46 ± 0.85 **	NA	11.43 ± 1.28
Albumin/globulin ratio	0.14	NA	0.42

Statistically significant differences: * P<0.001; ** P<0.05 vs controls; NA = not available.

specificity of changes, they provided valuable information about the severity of the pathological processes. These alterations contributed at a great extent to the elucidation of pathogenetic mechanisms of the acute phase of experimental *S. gallinarum* infection, observed also in previous studies of ours (Kokosharov & Goranov, 1997). We found also decreased cholinesterase activity during the acute infection that was accepted as a positive sign for impaired liver function. Also, degenerative lesions of the liver were observed (Kokosharov *et al.,* 1997). The impaired liver morphology was considered responsible for the reduced synthesis of protein. These data corresponded to the decreased albumin concentrations in the present study and are in accordance with the view that hypoalbuminaemia is an important parameter for liver damage as liver is the only place where albumin is synthesized. According to Kaneko (1997), during sepsis, acute phase proteins are preferentially produced in liver and thus, albumin synthesis is inhibited. The latter in our experiments is a bad prognostic sign because serum albumin sharply declines prior to occurrence of death (Goldwasser & Feldman, 1997). Kaneko (1997) and Lumeij (1997) conclude that due to its small size molecule and osmotic sensitivity, albumin is selectively lost in renal and intestinal diseases, such as acute fowl typhoid (Kokosharov *et al.*, 1997).

In our experiments, hypoalbuminaemia was accompanied by hypoproteinaemia in the advanced stage of the experimental infection.

The parallel interpretation of total protein and protein fraction changes is informative about the course of the infection. The increased values of α_2 globulins and the decrease in β and γ_2 globulins could be considered as a sign of destructive processes in infected birds. The data about sialic acids (neuraminic acid derivatives) concentrations are further confirming this thesis (Kokosharov, 2000). Despite the fact that the evaluation of changes in the acute phase has no diagnostic specificity, it is useful for clinicians because these

changes reflect the intensity of the inflammation (Epstein, 1999). We observed a maximum mortality in infected chickens by post infection days 4–7 and that is why the determined liver protein alterations should be interpreted as typical for this period of disease's development. The acute *S. gallinarum* infection caused reduction in albumin whereas globulin factions increased. The combined effect of these changes was the reduced albumin/globulin ratio. This dysproteinaemia has a bigger diagnostic value than the determination only of total protein (Lumeij, 1997).

REFERENCES

Chiolero, R., J. P. Revelly & L. Tappy, 1997. Energy metabolism in sepsis and injury. *Nutrition*, **13**, No 9 (Suppl.), 45–51.

Dickerson, R., P. Guenter, T. Gennarelly, D. Mullen & J. Dempsey, 1990. Increased contribution of protein oxidation to energy expenditure in heat-injured patients. *Journal of the American College of Nutrition*, **9**, 86–92.

Epstein, G. H., 1999. Acute-phase proteins and other systemic responses to inflammation. *New England Journal of Medicine*, **340**, No 6, 448–454.

Goldwasser, P. & H. Feldman, 1997. Association of serum albumin and mortality risk. *Journal of Clinical Epidemiology*, **50**, No 6, 693–703.

Gornall, A. G., 1946. Biuret method (Weichselbaum). *American Journal of Clinical Pathology*, **16**, 40–44.

Griwinger, P. & C. G. Scanes, 1986. Protein metabolism. In: *Avian Physiology*, 8th edn., ed. P. D. Sturkie, Springer, Verlag, N.Y., Berlin, Heidelberg, Tokyo, pp. 326–345.

Kaneko, J. J., 1997. Serum proteins and the dysproteinemias. In: *Clnical Biochemistry of Domestic Animals,* 5th edn, eds J. Kaneko, J. Harvey & M. Bruss, Academic Press, San Diego, CA, pp. 117–138.

Kokosharov, T. & H. Goranov, 1997. Enzyme activities and lipid levels in the serum of poultry with experimental acute *Salmonella gallinarum* infection. *Veterinarski Arhiv*, **67**, No 2, 53–58.

Kokosharov, T., H. Hristov & L. Belchev, 1997. Clinical, bacteriological and pathological studies on experimental fowl typhoid. *Indian Veterinary Journal*, **74**, 547–549.

Kokosharov, T., 2000. Sialic acids in the serum of poultry with experimental acute fowl typhoid. *Indian Veterinary Journal*, **77**, 1–3.

Lumeij, J. T., 1997. Avian clinical biochemistry. In: *Clinical Biochemistry of Domestic Animals*, 5th edn, eds J. Kaneko, J. Harvey & M. Bruss, Academic Press, San Diego, CA, pp. 857–883.

Saier, M. H. Jr., 1996. Phylogenetic approaches to the identification and characterization of protein families and superfamilies. *Microbial and Comparative Genomics*, **1**, 129–150.

Shivaprasad, H. L., 1997. Pullorum disease and fowl typhoid. In: *Diseases of Poultry,* 10th edn, Iowa State University Press, pp. 82–95.

Tohio, H., F. Miyoshi, E. Uchida & M. Niiyawa, 1995. Polyacrylamide gel electrophoresis patterns of chicken serum in acute inflammation induced by intramuscular injection of turpentine. *Poultry Science*, **74**, 648–655.

Vary, T. C. & S. T. Kimball, 1992. Sepsis-induced changes in protein synthesis: Differential effects on fast- and slow-twitch muscles. *American Journal of Physiology: Cell Physiology,* **31**, No 6, 1513–1519.

Paper received 28.09.2005; accepted for publication 04.07.2006

Correspondence:

Assoc. Res. Scientist Dr. T. Kokosharov,
10, General Radetski str.,
6300 Haskovo, Bulgaria

Bulgarian Journal of Veterinary Medicine (2000),**3**, No 14, 1