

EFFECT OF EXPERIMENTAL YOLK SAC INFECTION WITH *STAPHYLOCOCCUS AUREUS* ON IMMUNE STATUS OF BROILERS

M. F. Rai, S. A. Khan, A. Aslam, K. Saeed,¹ K. A. Khan and M. S. Shah.

Department of Pathology, ¹Department of Parasitology, University of Veterinary and Animal Sciences, Lahore-54000, Pakistan

ABSTRACT

The present project was carried out to study the effect of experimental yolk sac infection with *Staphylococcus aureus* on immune status of broiler chicks. For this purpose, one hundred day-old chicks were divided into two groups A and B, having fifty chicks each. Confirmed pathogenic isolate of *Staphylococcus aureus* was inoculated into the yolk of each chick of group A, while group B acted as control group. Parameters studied included pathological examination of yolk sac, yolk sac weight/body weight ratio, antibody titres against Newcastle disease virus (NDV) in serum and yolk and analysis of fractional serum proteins. Results showed that yolk sac weight/body weight ratio was higher with marked pathological changes (abnormal colour, consistency and odour) were also observed in unabsorbed yolks of the infected group. Geometric mean titres of maternal antibodies against NDV were significantly lower in serum but higher in unabsorbed yolks of infected group than in control one. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used for serum protein analysis and percent area covered by each protein fraction was calculated. The percent area covered by most serum protein fractions was lower in infected chicks than the control chicks. It was therefore concluded that yolk sac infection (omphalitis) with *S. aureus* led to decline in the immunity.

Key words: Yolk sac infection, omphalitis, SDS-PAGE, maternal immunity, *Staphylococcus aureus*, serum proteins, broiler chicks.

INTRODUCTION

The rapid expansion of poultry industry has presented many disease problems and among these, the early chick mortality is becoming more important and significant (Suneja *et al.* 1983). The first two weeks of age are very important in the life of a broiler chick, as about 30–50% mortality occurs in this period. Major problems during this period are omphalitis, brooder pneumonia, avian encephalomyelitis, spiking mortality, dehydration, ammonia burns and pullorum disease (Charlton, 1996). The most prevalent and the commonest cause of early chick mortality in Pakistan is yolk sac infection (Anjum, 1997).

The nutrient substances and immunoglobulins present in the yolk sac are used by the chick during first few days of development. Yolk sac is also an important mean of providing passive immunity. Passive immunity present in the yolk represents defenses that are determined from maternal experiences (Stone *et al.*, 1992). Immunoglobulins are serum proteins and play an important role in immune status (Tizard, 1987). Infections alter protein structure and thus lower the immune status of animals (Hegazi, 1990; Segarra *et al.*, 1991).

Because of the importance of maternal immunity and potential of bacterial contamination of the yolk sac to alter protein structures, yolk sac infection adversely affecting passive protection and serum proteins is of great

concern. The present project was designed to study the effect of experimentally induced yolk sac infection with *Staphylococcus aureus* on immune status of broiler chicks.

MATERIALS AND METHODS

The effect of experimental yolk sac infection with *Staphylococcus aureus* on immune status of broiler chicks was studied.

Preparation of inoculum

Staphylococcus aureus was isolated from the bird suspected for staphylococcosis. Identification and confirmation of isolated organisms was made on the basis of cultural, morphological and staining characters, and biochemical tests, as described by Buxton and Fraser (1977) and Rehman *et al.* (1996). Pathogenicity test was ascertained by the method described by Harry (1957). Viable count of the isolated organisms was determined by Miles and Misra technique (Quinn *et al.*, 1994).

Experimental design

One hundred, day-old broiler chicks were procured from the local hatchery and were randomly divided, on day one, into two groups A and B, containing 50 birds each. Chicks of group A received *Staphylococcus aureus* broth inoculations into yolk sac (0.1 ml per chick containing 10^2 c.f.u) on day-1 of the experiment (Harry,

1957). Sterilized insulin syringe was used for this purpose. Chicks in group B received no bacteria and acted as control. Sterile nutrient broth (0.1 ml/chick) was injected into yolk sac of the chicks of this group with sterilized insulin syringe, on day-1 of the experiment.

Ten birds from each group were slaughtered at interval of 48 hours i.e. on 3rd, 5th, 7th and 9th day of the experiment to study the experimental parameters. Before slaughtering, 1-2 ml blood was collected from each chick, on all sampling days in sterilized disposable syringes and serum was obtained from these samples. The yolks were also collected from these birds after slaughtering.

Experimental parameters

The following experimental parameters were studied:

- 1. Pathological examination of yolk sac:** The yolk sacs of the chicks were thoroughly examined to record any gross pathological changes at the time of slaughtering.
- 2. Yolk sac weight/body weight ratio:** Body weight and yolk sac weight were determined and yolk sac weight/body weight ratio was calculated by using following formula (Khan, 2002).

$$\text{Yolk sac weight/body weight ratio} = \frac{\text{Yolk sac weight}}{\text{Body weight}} \times 100$$

- 3. Antibody titre against Newcastle Disease Virus:** Antibody titers against NDV in serum and yolk were determined by Haemagglutination Inhibition (HI) test, as described by Silim and Venne (1989).
- 4. Fractional serum proteins:** The banding patterns of different fractions of serum proteins were determined by using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) method, as described by Youssef *et al.* (1988) and Sadaf (1999). Mean percent area covered by each protein fraction was measured and compared with control group to find the enhancement or reduction and appearance or disappearance of particular protein fractions.

Statistical analysis:

Data thus collected was statistically analyzed by applying unpaired t-test (Steel and Torrie, 1982).

RESULTS

The birds of group A infected with *Staphylococcus aureus* seemed weak, huddled together and had a watery diarrhea. The umbilicus was open, infected and discoloured to bluish black. There was pungent odour from the chicks. The abdomen felt soft, mushy, flabby and enlarged. The birds were off-feed and water also. Out of total 50 birds one died on day -2, two

died on day-4 and day-7 each and three died on day-8 of the trial. All chicks in control group maintained a normal healthy appearance and feed consumption, and no mortality was recorded in this group.

Results of different parameters are shown in Tables 1-4 and the banding patterns of different fractions of serum proteins are shown in Figs. 1-4.

DISCUSSION

Omphalitis or yolk sac infection occurs frequently in commercial poultry. The most prevalent bacteria causing yolk sac infection is *Escherichia coli* (Deeming, 1995; Rehman *et al.*, 1996; Anjum, 1997; Sharada *et al.*, 1999). Besides *E. coli*, *Staphylococcus aureus* is next most important bacterium associated with yolk sac infection (Choudhury *et al.*, 1993; Deeming, 1995; Rehman *et al.*, 1996).

The infected yolks were large in size, having yellowish brown and green to yellowish red appearances, offensive pungent smell and watery to caseous consistency. Jordan (1990), Skeeles (1991), Sainsbury (1992) and Anjum (1997) reported similar observations.

Yolk sac weight/body weight ratio was higher in infected group as compared to control group. Similar findings are reported by Sander *et al.* (1998) and Khan (2002). This observation can be explained as the weight of the unabsorbed yolk was higher and body weight was lower in chicks infected with *Staphylococcus aureus* than that of control chicks. Deeming (1995), Sander *et al.* (1998) and Khan (2002) observed high yolk weight in chicks infected with yolk sac infection. Lower body weight in infected chicks was reported by Khan (2002).

Haemagglutination inhibition (HI) titres of serum and yolk against Newcastle Disease Virus (NDV) decreased with age, being highest at day 3 and lowest at day 9. These results are favoured by the findings of Saeed *et al.* (1988), who reported that HI titres were log mean 2⁷ on first day, log mean 2⁶ on 5th day and log mean 2⁵ on 10th day of age. Results are also in accordance with the findings of Mitra *et al.* (1998).

HI antibody titres of serum against NDV were significantly lower in infected chicks than the control chicks. Sander *et al.* (1998) also reported that the geometric mean serum titre (log₂) for Newcastle disease antibody was significantly lower in chicks infected with *Streptococcus faecalis* than in control chicks. This observation may be justified as in infected chicks maternal antibodies against NDV are not absorbed from the yolk and their concentration in serum increases. On the other hand, HI antibody titres of unabsorbed yolk samples against NDV were significantly higher in infected group as compared to yolks of control group. Low serum and

Table 1: Gross pathological changes in yolk sac of chicks of control (B) and infected (A) groups

Day	Group	Discolouration	Offensive odour	Consistency		
				Watery	Caseous	Hard
3	A	++	++	++	-	-
	B	-	+	+	-	+
5	A	++	++	+	++	-
	B	-	-	-	-	++
7	A	+++	+++	+++	-	-
	B	+	-	-	-	++
9	A	++++	++++	+++	+	-
	B	-	-	-	-	+++

Table 2: Mean yolk sac weight/body weight ratio of chicks of control (B) and infected (A) groups

Groups	Sampling days			
	Day 3	Day 5	Day 7	Day 9
A	11.63	8.34	6.71	5.78
B	4.06	1.58	0.55	.03

*Significance difference (P < 0.05)

Table 3: HI titer against NDV in serum and yolk samples of chicks of control (B) and infected (A) groups

Groups	Serum samples				Yolk samples			
	Day 3	Day 5	Day 7	Day 9	Day 3	Day 5	Day 7	Day 9
A	147.0	111.4	32.0	19.7	477.7	415.9	157.6	90.5
B	294.1	256.0	128.0	21.1	181.0	147.0	39.9	21.1

Table 4: Mean percent area covered by different serum protein fractions

Fraction No.	1	2	3	4	5	6	7	8	9	10	11	12	13	
Mol. Wt. (kD)	126	112	89	71	64	56	50	45	32	28	25	22	20	
Days														
Day														
3	A	3.57	Absent	Absent	4.15	Absent	5.01	Absent	Absent	2.15	3.22	4.29	Absent	3.72
	B	3.92	Absent	Absent	4.33	Absent	5.00	Absent	Absent	2.58	3.50	4.44	Absent	4.37
5	A	Absent	4.14	3.07	Absent	Absent	1.86	Absent	Absent	3.28	3.78	4.28	3.71	Absent
	B	Absent	4.57	3.36	Absent	Absent	2.14	Absent	Absent	3.57	3.64	4.72	4.43	Absent
7	A	Absent	3.87	Absent	Absent	Absent	Absent	4.46	2.37	4.74	Absent	3.30	Absent	Absent
	B	Absent	4.16	Absent	Absent	Absent	Absent	4.60	2.16	4.45	Absent	3.66	Absent	Absent
9	A	Absent	3.33	Absent	Absent	4.62	2.31	Absent	Absent	Absent	4.34	4.47	Absent	Absent
	B	Absent	3.91	Absent	Absent	4.91	2.67	Absent	Absent	Absent	5.13	4.98	Absent	Absent

high yolk antibody titre may be justified as bacterial contamination of yolk sac decreased passive antibodies absorption either by protein alteration, binding or decrease in the ability of yolk sac membrane to absorb proteins (Sander *et al.*, 1998).

SDS-PAGE was used to evaluate the effect of yolk sac infection on serum protein fractions, which could be used in diagnosis of immune status of the chicks. Youssef *et al.* (1988), JengShang *et al.* (1999), Xie *et al.* (2001) and Yigit *et al.* (2001) used this technique for serum protein analysis. Campbell (1992) reported that among serum protein electrophoresis, SDS-PAGE is a powerful and versatile technique and could be used to study the serum protein contents.

The mean percent area covered by serum protein fractions of molecular weights 126, 112, 89, 71, 64, 50, 25 and 22 kD was lower and of molecular weights 45 and 20 kD was higher in infected than in control group while mean percent area covered by protein fractions of

molecular weights 56, 32 and 28 kD was variable. On day 3, the infected group showed decreased protein fractions of molecular weight 126 (hepatoglobin), 71 (alpha-fetoprotein), 32 (alpha-acid glycoprotein) and 28 kD, and increased protein fractions of molecular weight 56 (prealbumin) and 20 kD as compared to control group.

On day 5, protein fractions of molecular weight 112 (hepatoglobin), 89 (transferrin), 56 (prealbumin), 32 (alpha-acid glycoprotein), 25 and 22 kD were decreased while protein fraction of molecular weight 28 kD was increased in infected group as compared to the control group. On day 7, the infected group showed decreased protein fractions of molecular weight 112 (hepatoglobin), 50 (prealbumin) and 25 kD and increased protein fractions of molecular weight 45 and 32 kD (alpha-acid glycoprotein) than control group. On day 9, the infected group showed decreased protein fractions of molecular weight 112 (hepatoglobin), 64 (albumin), 56 (prealbumin) and 25 kD and increased protein fractions of molecular

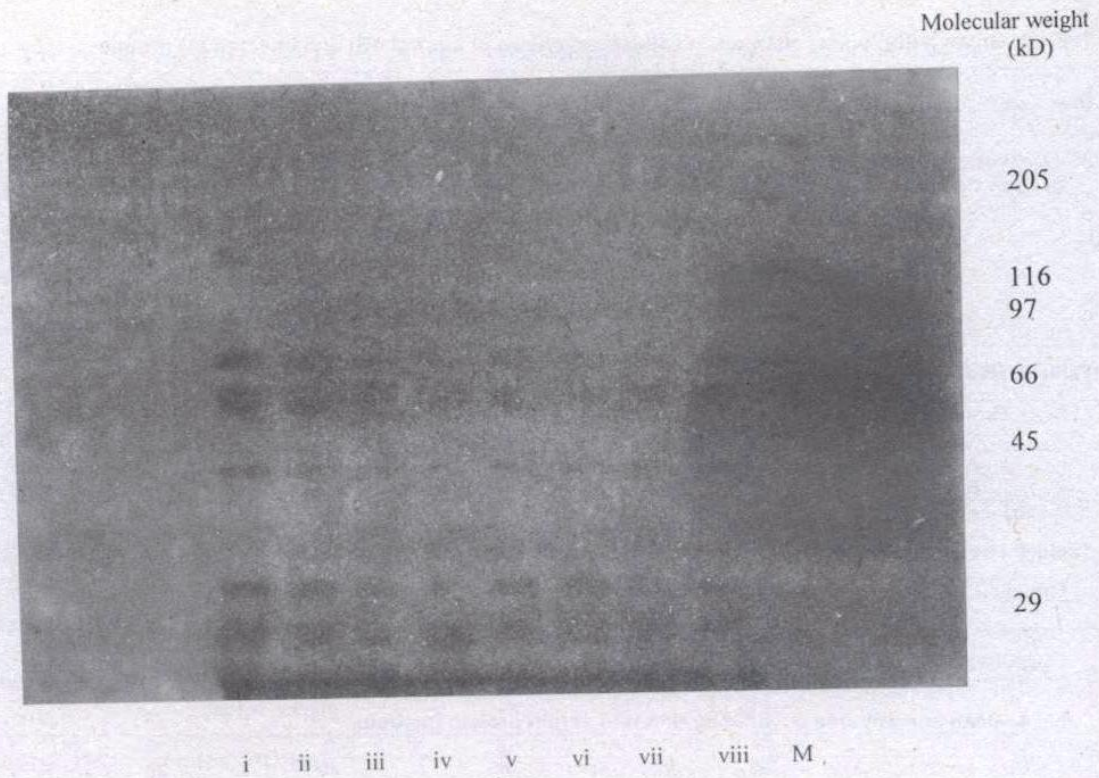


Fig. 1. 10% SDS-PAGE of serum samples of infected and control groups on day 3

Lanes i-vi = Serum protein profiles of infected group. Lanes v-viii = Serum protein profiles of control group.
M = Molecular weight marker

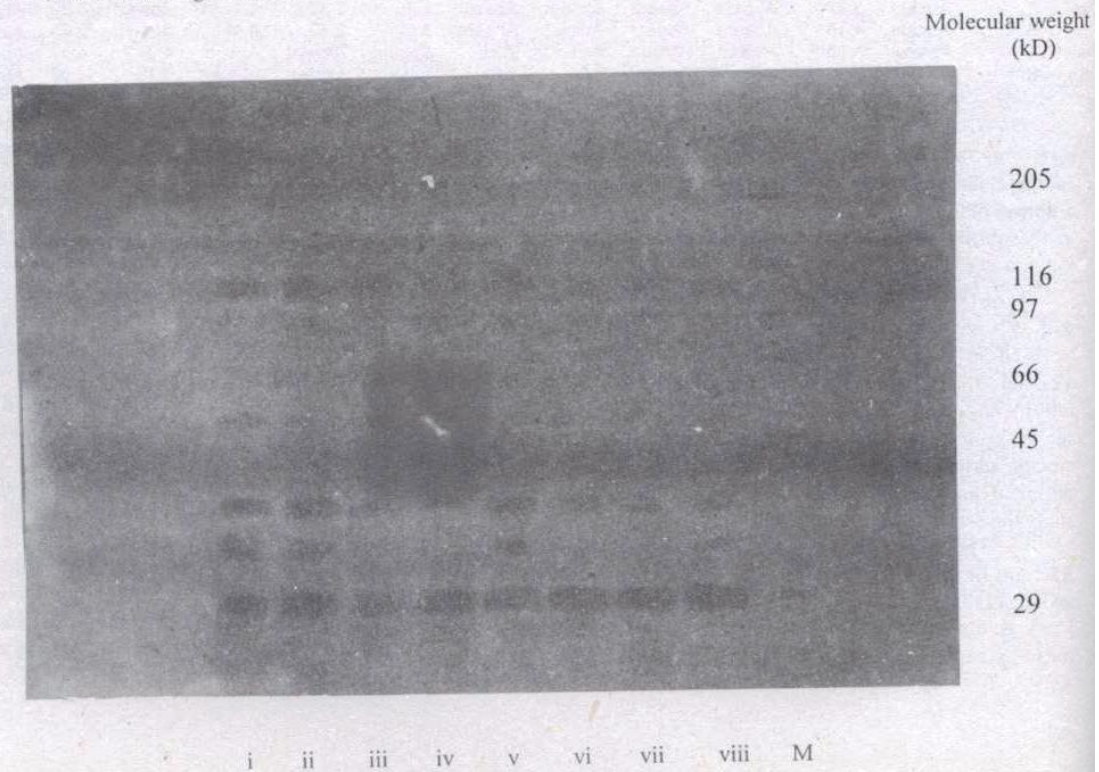


Fig. 2. 10% SDS-PAGE of serum samples of infected and control groups on day 5

Lanes i-vi = Serum protein profiles of infected group. Lanes v-viii = Serum protein profiles of control group
M = Molecular weight marker

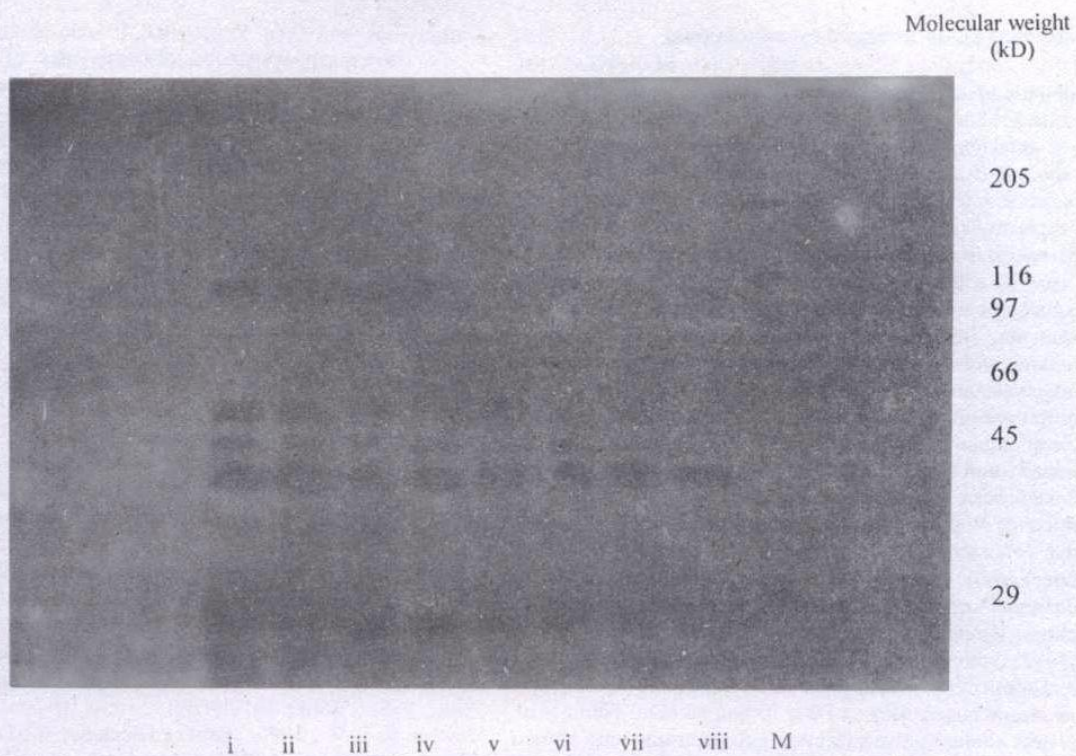


Fig. 3. 10% SDS-PAGE of serum samples of infected and control groups on day 7

Lanes i-vi = Serum protein profiles of infected group. Lanes v-viii = Serum protein profiles of control group.
M = Molecular weight marker

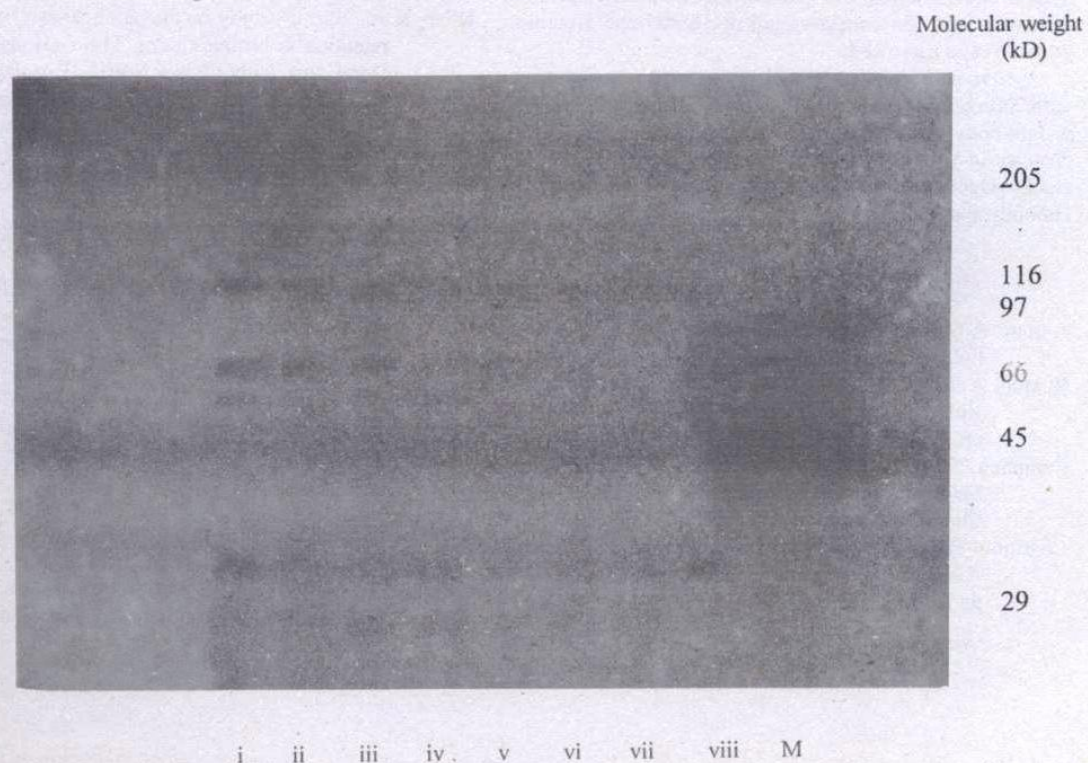


Fig. 4. 10% SDS-PAGE of serum samples of infected and control groups on day 9

Lanes i-vi = Serum protein profiles of infected group. Lanes v-viii = Serum protein profiles of control group.
M = Molecular weight marker

weight 28 kD as compared to control group.

From the above results it can be inferred that albumin values were decreased in infected group. Youssef *et al.* (1988) reported a decrease in albumin value after S/C infection with *Salmonella arizonae*. Pearson (1990) reported decreased values of albumin in horses with parasitic infestation, chronic infection, inflammation and neoplasm. Jain (1993) reported that serum transferrin decreased in acute and chronic infections and leukemia.

In this study, infected group showed decreased hepatoglobin concentration but this finding is not in line with the findings of Kent and Goodall (1991), who reported that hepatoglobin concentrations were elevated in infections and inflammatory conditions in horses. This difference might be due to species variation. In our results, the prealbumin values were elevated in infected group initially and then decreased. Youssef *et al.* (1988) reported a significant increase in pre-albumin values after S/C infection with *Salmonella arizonae*. Jain (1993) reported that concentration of prealbumin was elevated with acute infections, laminitis and malignant tumours. Such decrease in certain proteins and increase in others is in partial agreement with the findings of Ching and Yeh (1992), who reported that in acute heat stress in chickens and broilers the serum proteins of 30, 40, 83 and 94 kDa increased but not those of 24, 70 and 90 kDa. Tohjo *et al.* (1995) studied polyacrylamide gel electrophoretic pattern of chicken serum in acute inflammation and reported that the electrophoretic patterns were divided in to 21 segments but only two of these segments increased markedly. Sefer *et al.* (1999) reported that in T-2 toxin treated chicks, alpha and gamma globulin concentration increased, albumin concentration decreased and albumin: globulin ratio narrowed.

It is therefore concluded that yolk sac infection with *Staphylococcus aureus* not only increases yolk sac weight/body weight ratio with marked pathological changes in yolk but also results in low immune status of chicks which was the consequence of decreased maternal antibody absorption.

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