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Alteration in Serum Protein Profile in White Leg Horn Chicks with Experimental different doses of infection of *Ascaridia galli*

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Abstract

The enteric nematode parasite *Ascaridia galli* modulate the immune system, of the hosts and eventually leads to malnutrition in the chicks which eventually results in the decreased return of products derived from poultry. The accurate interpretation of avian plasma proteins and the dramatic changes in protein fraction are important events in several diseases and may help in procuring a diagnosis .The present work was carried out to investigate the immunological alteration of serum protein profiles, induced along with different doses of *A. galli* infective eggs. The albumin concentration was found to be highly significantly (p<0.005) decreased in comparison to control group. The statistical analysis revealed highly significant (p<0.005) fall in IgA^1 antibodies as compared to the control group. The IgA^2 antibodies were found to be significantly (p<0.005) increased as compared to control group, depicting increased humoral immune response. The concentration of beta globulins were observed to be significantly (p<0.005) decreased as compared to control group. The IgG antibodies showed a significant (p<0.005) elevation in humoral immunity as compared to control group. The possible influence of the parasite induced alteration in immune responses in WLH Chicks is discussed in this paper.

Key-Words: *Ascaridia galli*, WLH chicks, different infective doses, serum protein profile, humoral immune response

Introduction

Parasitic infections account for hundred of millions of dollars in annual losses and medicated cost sin the livestock and poultry industry throughout the world. The most costly parasites in terms of production losses are the geastrointestinal nematodes in ruminants and poultry (Gamble and Zarlenga, 1986).The parasites found in the small intestine of poultry belong to the genera Ascaridia galli of these Ascaridia galli is the most common and most important parasite of chicks. Ascarridiasis is a gastrointestinal disease and is caused by an enteric nematode parasite, Ascaridia galli (Schrank, 1788). Studies have suggested that A. galli is the most common nematode in all types of production systems and has a worldwide distribution (Permin et al., 1997, Martin-Pacho et al., 2005; Rabbi et al., 2006; Abdelqader et al., 2008). Ascaridia galli population dynamics in chickens reflects the population studies of parasite turnover within the animal host (Kiran Kumar Katakam et al., 2010).

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Hartmann (2003) studied the triggered modulation of host immune responses by nematode that possess multiple specific capacities for immuno-modulation, acting in parallel and have different immune effect or mechanism. Intestinal nematodes affect the productivity in adulthood (Guyatt, 2000).Concurrent infections with Ascaridia galli and Escherichia coli in chickens raised for table egg production, Characteristic pathological lesions including airsacculitis, peritonitis and/or polyserositis were seen in all groups infected with E. coli (A Permin , JP Christensen and M Bisgaard 2006).Parasitic infections account for hundred of millions of dollars in annual losses and medicated cost sin the livestock and poultry industry throughout the world. The most costly parasites in terms of production losses are the geastrointestinal nematodes in ruminants and poultry (Gamble and Zarlenga, 1986). *Ascaridia galli* is an intra intestinal worm found in chickens, turkeys, geese and a number of wild birds with direct life cycle, they develop to the next infective stage containing a second stage of larva in just 8-14 days under ordinary conditions but they may take a longer and shorter time depending on temperature availability. Reid (1960) found that *A.*

galli reached the infective stage in 5 days at the optimum temperature of 30-34^{0}C.

The main objective is to investigate the immunological changes in Male White Leg Horn chicks due to nematode infection of *Ascaridia galli* parasite, enumerating their parasitological characteristics.

Material and Methods

Collection of Parasites and culturing of eggs

These female parasites were kept in petridish containing saline water for egg laying at 36^{0}C in incubator. After 24-36 hours females layed large number of eggs which were collected in petridish having sterile solution. Eggs were also obtained by squeezing the uterus after dissecting the female parasites. The eggs were kept in normal saline solution at the 34^{0}C for embryonation for 20 days. These embryonated infective eggs were used for given challenged infection in male WLH chicks.

Preparation of doses and counting of eggs

The fully embryonated infective eggs were prepared for inoculation at the time of infection. The dilution method was used for the counting of eggs. The eggs were suspended in known volume of normal saline solution. With the graduated pipette the 0.2 ml of the suspended solution was sucked and placed on the clean and dry counting slide. The embryonated and infective eggs were counted with the help of stereoscopic binocular microscope. Three values were taken by repeating the process. Mean of these three values was used for calculating the number of embryonated infective eggs of *A. galli.*

Administration of the embryonated infective eggs to the experimental male White Leg Horn (WLH) chicks

The male WLH chicks were grouped and labeled properly. The inocula with desired number of embryonated infective eggs (500 embryonated eggs as low dose and 1500 embryonated eggs as high dose) were administered orally to male white leg horn chicks. After infection, the male GLH chicks were kept separately in spacious wooden cages in the animal house. The food and water were given after four hour of administering the infection. The life cycle of *Ascaridia galli* was observed to be completed between 28-30 days and experimental results were taken after 25 days and 50 days. Four WLH chicks from each of the control group,infected with low dose(500 embryonated eggs),and high dose(1500embryonated dose) groups were autopsied after 25 and 50 days for studying immunological study.

Estimation of serum protein profile of White Leg Horn Chicks (WLH)

The serum protein profiles were analyzed by agarose gel electrophoresis

Collection of blood and Preparation of serum

For the collection of blood, the male WLH chicks were sacrificed after 25 and 50 days of post infection. Blood was collected from the heart with the sterilized dry glass syringe by the cardiac puncture. Blood was taken in the vials that were sterilized by boiling in water and dried before using. This blood was used for the separation of T and B-lymphocytes and haematological studies.

The blood was taken in the clean and dry centrifugal tube and centrifuged at 3000 rpm for 15 minutes. After centrifugation, the pale yellow serum was obtained and stored in deep freezer for analysis of serum protein profiles and further studies.

Analysis of serum protein profile (by Agarose gel electrophoresis)

Agarose gel electrophoresis was used for the analysis of various protein profiles of the serum of the control and different experimental group of male White Leg Horn chicks. Gel was prepared with the Agarose. The stock solution was made by dissolving barbituric acid (2.095 gms) and sodium barbitone (11.380 gms) in distilled water (11.5 litre). The electrophoretic apparatus were filled with the diluted buffer. Gel slabs were prepared using agarose (200 mg) dissolved in buffer solution (20 ml) for 6 sterilized microscopic slides. Agarose and buffer were taken in a clean glass tube for the preparation of gel. This tube was kept in beaker having the water and heated on the burner of gas. The solution of agarose and buffer was stirred until it became transparent. 2.5 ml of this transparent solution was poured per slide and spreaded very carefully and allowed to settle. These slides were kept in refrigerator at 4^{0}C for proper setting of gel. The end point of each slide was connected to buffer chamber by strips of Whatman filter paper. Initially the apparatus was kept for one hour to set equilibrium at 350V voltage and 6 mA current. The serum was loaded on the slides about 1.5 cm from the cathode end. The 6 mA current was supplied for each slide for 90 minutes. After electrophoresis these slides were stained in freshly prepared 0.1 percent amido block (0.1 gm amido black in 7 ml glacial acetic acid and 93 ml distilled water) for 10 minutes. After 10 minutes these slides were kept in destain solution (15 ml methanol + 5 ml acetic acid + 80 ml distilled water) for 24 hours. The slides were kept in an incubator for drying. After drying the immunoglobulins, protein bands appeared clearly. These slides were photographed and scanned for determining the concentration of different protein bands of antibodies.

Results and Discussion

The accurate interpretation of avian plasma proteins is a very important diagnostic tool in host. Protein electrophoresis is a practical and useful test for diagnostic features of the host. Dramatic changes in protein fraction are important event in several diseases and may help in procuring a diagnosis. Gel electrophoresis is valuable in monitoring the response to therapy as well. Avian total proteins consist of albumin and globulin. Serum obtained from the control and infected groups were analyzed for the serum total protein profile concentration of antibodies after 25 and 50 days respectively.

Analysis of serum protein profile

The serum obtained from the control and infected male WLH chicks were analyzed for the serum protein profile by Agarose gel electrophoresis for performing the electrophoretic studies on serum of control and experimentally infected group of male WLH chicks.

Serum obtained from the control and infected groups were analyzed for the serum total protein profile concentration of antibodies after 25 and 50 days respectively

Albumin

Group I: Control Group-In control group of male WLH chicks, the concentration of albumin was found to be 38.55 percent and 39.06 percent after 25 and 50 days respectively (Table-1, Fig-1 A, B).

Group II: Low dose of 500 embryonated A. galli eggs infected group - In this group the male WLH chicks, the albumin concentration was observed to be 34.69 percent and 33.41 percent after 25 and 50 days of post infection (Pi) respectively. The concentration of albumin was found to be significantly ($p<0.005$) decreased in comparison to control group (Table-1; Fig-1A, B).

Group III: High dose 1000 embryonated A. galli infected group - In the above group of male WLH chicks the concentration of albumin was measured to be 30.12 percent and 29.27 percent after 25 and 50 days of Pi respectively. The albumin concentration was found to be highly significantly ($p<0.005$) decreased in comparison to control group (Table-1; Fig-1 A, B).

Alpha-1 Globulin

Group-1 : Control Group -In control group of male WLH chicks the concentration of alpha-1 globulins depicting IgA1 antibodies were found to be 10.78 percent and 9.84 percent after 25 and 50 days respectively (Table-1, Fig-2 A, B).

Group-II : Low dose of 500 infective embryonated eggs of A. galli - In infected group of male WLH chicks the concentration of alpha-1 globulins depicting IgA1 antibodies were observed to be 7.42 percent and 7.46 percent after 25 and 50 days of Pi respectively. The IgA1 antibodies were found to be significantly ($p<0.005$) decreased as compared to control group (Table-1, Fig-2 A, B).

Group-III : High dose of 1000 infective embryonated eggs of A. galli - In infected group of male WLH chicks the concentration of alpha-1 globulins depicting IgA1 antibodies were found to be 30.12 percent and 29.27 percent after 25 and 50 days of Pi respectively. The statistical analysis revealed highly significant ($p<0.005$) fall in IgA1 antibodies as compared to the control group (Table-1, Fig-2 A, B).

Alpha-2 Globulin

Group-I : Control Group-In control group of male WLH chicks the concentration of alpha-2 globulins depicting IgA2 antibodies were found to be 9.44 percent and 9.75 percent after 25 and 50 days respectively (Table-1, Fig-3 A, B).

Group-II: Low dose of 500 infective embryonated eggs of A. galli -In infected group of male WLH chicks the concentration of alpha-2 globulins depicting IgA2 antibodies were measured to be 19.51 percent and 20.50 percent after 25 and 50 days of Pi respectively. The IgA2 antibodies were found to be significantly ($p<0.005$) elevated as compared to control group (Table-1, Fig-3 A, B).

Group-III : High dose of 1000 infective embryonated eggs of A. galli -In infected group of male WLH chicks the concentration of alpha-2 globulins depicting IgA2 antibodies were observed to be 23.30 percent and 18.35 percent after 25 and 50 days of Pi respectively. The IgA2 antibodies were found to be highly significantly ($p<0.005$) increased as compared to control group, depicting increased humoral immune response. (Table-1, Fig-3 A, B).

Beta Globulin

Group-I: Control Group-In control group of male WLH chicks the concentration of beta globulins were recorded to be 15.06 percent and 14.69 percent after 25 and 50 days respectively (Table-1; Fig-4 A, B).

Group-II: Low dose of 500 infective embryonated eggs of A. galli - In infected group of male WLH chicks the concentration of beta globulins were found to be 10.60 percent and 9.12 percent after 25 and 50 days of Pi respectively. The concentration of beta globulins were observed to be significantly ($p<0.005$) decreased as compared to control group (Table-1; Fig-4 A, B).

Group-III: High dose of 1000 infective embryonated egg of A. galli - In infected group of male WLH chicks the concentration of beta globulins were measured to be 8.12 percent and 6.09 percent after 25 and 50 days of Pi respectively. The concentration of beta globulins

were observed to be significantly ($p<0.005$) decreased as compared to control group. (Table-1; Fig-4 A, B).

Gamma Globulin

Group-I: Control Group-In control group of male WLH chicks the concentration of gamma globulins depicting IgG antibodies were found to be 25.44 percent and 26.91 percent after 25 and 50 days respectively (Table-1; Fig-5 A, B).

Group-II: Low dose of 500 infective embryonated egg of A. galli - In infected group of male WLH chicks the concentration of gamma globulins depicting IgG antibodies were recorded to be 28.57 percent and 28.90 percent after 25 and 50 days of PI respectively. The IgG antibodies showed a significantly ($p<0.005$) increase in humoral immune response as compared to control group (Table-1; Fig-5 A, B).

Group-II: High dose of 1000 infective embryonated egg of A. galli - In infected group of male WLH chicks the concentration of gamma globulins depicting IgG antibodies were recorded to be 31.57 percent and 31.91 percent after 25 and 50 days of Pi respectively. The IgG antibodies showed a highly significant ($p<0.005$) elevation in humoral immunity as compared to control group (Table-1; Fig-5 A, B).

Analysis of serum protein profile

The present attempts have been made to ascertain the immunogenecity of low and high doses of *A. galli* and experimental ascaridiasis in White Leg Horn chicks. This was performed by the analysis of serum protein profile by agarose gel electrophoresis. The five major bands observed in male WLH chicks were the albumin, alpha-1 globulin, alpha-2 globulin, beta globulin and gamma globulin. Serum protein includes all plasma proteins except the coaglulation proteins, principally fibrinogen, which is eliminated by clot separation. Protein electrophoresis has been demonstrated to be a very effective diagnostic tool in avian medical science. The immune response against intestinal nematode has been extensively studied in avian and human (Cox 1992; Sher and Coffman 1992; Margaret and Wissman 2001). Albumin is the largest single fraction in healthy bird and it serves as the major reservoir of protein and is synthesized in liver. Increase in albumin concentration is associated with dehydration. Decrease occurs with decreased synthesis (chronic liver disease, diatery protein deficiency or chronic inflammation, renal disease and gastrointestinal disease). Decrease can also occur with blood loss, severe infection and chronic infection (Margaret and Wissman 2001). The total serum protein was found to be higher in male chicks (Meluzzi *et al.* 1992). The intensity of albumin was more in control then in highly infected birds while the gamma globulins in the control birds was very much less. This experiment revealed that the albumin fraction and the gamma globulin fraction were inversely proportional to each other (Rao *et al.* 1983). Leutskaya (1964) reported decreased albumin level and A/G ratio with increased gamma globulin in chicks immunized with homogenized parasitic suspension. Lowered values of albumin with increased level of globulins were also demonstrated by passively immunized chickens.The albumin level decreases in chicks infected with *Ascaridia galli* but albumin level gradually elevated after treatment in comparison to infected group (Raot *et al.* 1991). Albumins have an important property to bind with lipids, hormones, bilirubin and many drugs. *A. galli* could be attributed to increase the host metabolism. The toxins produced by egg or larval stages affect the liver to inhibit the process of albumin synthesis. Vasoactive amines and other toxic substances released during antigen-antibody interaction also inhibit the albumin fraction (Rao and Cohly, 1953).

During the present investigations the electrophoretic evaluation of sera revealed significant decrease of alpha-1 globulin, depicting IgA^1 antibody in all groups, high decrease of alpha-1 was found in infected groups. Whereas alpha-2 globulin, depiciting IgA^2 antibody was found highly increased in experimental infected group in comparison to control group. The present findings are supported by the findings of Hurwitz *et al.* (1972) observed the reduction in *alpha-1* globulin because of infection with *A. galli,* which may be due to reduction in the activity of enterokinase, trypsin, chemotrypsin and amylase in the intestinal tract of infected chicken.The alpha globulins are linked with mucoproteins and glycoproteins of plasma.Joshi and Johri (1978) observed increase in the alpha-2 globulin on the 31st day in chickens experimentally infected with *Ascaridia galli*. They also reported no appreciable changes in the albumin and alpha-1 globulin.

Another reason for the fall in the beta globulin is that beta lipoproteins and possibly glycoproteins are produced in response to tissue damage. Joshi and Johri (1978) also reported a fall in the beta globulin concentration in fowl infected with *Ascaridia galli*. The note worthy features of their experiment was an apparent fall in the level of beta globulin in the infected group as compared to the controls.

Haiba (1966) observed that *A. galli* infected egyptian chicks also revealed a non significant fall in beta globulin. The probable cause for the depletion of the beta globulin component is probably the iron binding protein transferin, which is predominantly present in the beta fraction and hepatoglobulin in alpha-2 globulin. Thus the serum iron level also tends to bring

an over all reduction in beta component (Tizzard 1984).

In present study the higher value of gamma globulin was observed in all groups of WLH male chicks as compared with control group. A high rise was noticed in infected group. Deutsch *et al.* (1982) also found increased gamma globulin levels in chickens treated with human gamma 2 globulin and reported that a direct correlation existed between the gamma globulin level and antibody activity and their utilization at the site of parasite invasion. The rise in gamma globulin percentage directly reflects the response of reticulo-endothelial system to foreign antigen. Increase in gamma globulin level in normal and *Ascaridia galli* infected chicks was also reported by Leutskaya (1964), Haiba (1966)

An increased gamma globulin level in serum of animals against vaccination is one of the most important criteria for antibody response and good immunity (Williams and Chase 1967). Thus the rise of gamma globulin percentage directly reflects the response of reticuloendothelial system to foreign antigen. IgA and IgG antibodies contribute to the development of immunity by neutralizing or inactivating vital metabolic enzymes of *Haemonchus contortus* (Gill *et al.* 2000). Befus *et al.* (1982) reported the specific IgA and IgG are found in the intestinal secretion of the respective host. Almond *et al.* (1986) found that serum IgA and IgG antibodies against parasite surface antigens are prominent in the host response against *Trichinella spiralis.* IgM antibodies must have appeared in the first week of challenge infection and after treatment with antigen it disappeared and only IgA antibodies were identified. Some modification or modulation might have occurred in the virulence of the *Ascaris* antigens or some modulation could also be there in the antigenic determination of *A. lumbricoides*.

Conclusion

The serum obtained from the control and infected male WLH chicks were analyzed for the serum protein profile by Agarose gel electrophoresis for performing the electrophoretic studies on serum of control and experimentally infected group of male WLH chicks. The five major bands were clearly visible on amido black electrophotograms. These bands were albumin, alpha-1 globulin depicting IgA^1 antibody, alpha-2 globulin depicting IgA^2 antibody, beta globulin and gamma globulin. Marked reduction in albumin level was observed in experimentally infected chicks. The elctrophoretic evaluation revealed significant decrease in alpha-1 depicting IgA^1 antibody in all groups. In experimentally infected group, highly significant fall was observed. The male WLH chicks with experimental ascaridiasis showed highly significant elevation in alpha-2 concentration. In present study the beta globulin decreased in infected group of male WLH chicks. In the present experiment higher value of gamma globulin percentage pointed towards antibody production in all groups. In conclusion, the study on the alteration in Immune Responses by analysis of serum protein profiles in White Leg Horn Chicks with Experimental different doses of infection of Ascaridia galli and the data obtained would be extremely helpful in designing the ant parasitic agents against nematodes infection in chicks. This investigation would be extremely helpful in the development of vaccine against ascaridiasis in chicks.

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Table 1: Serum protein profiles in male WLH chicks infected with 500 and 1000 *A. galli* eggs

Parameters	Day	Control	Low dose infection	High dose infection
Albumin	25	38.55 ± 1.2365	34.69 ± 0.3564	30.12 ± 1.2354
	50	39.06 ± 0.6598	33.41 ± 1.5984	29.27 ± 1.6746
Alpha-1 globulin	25	10.78 ± 1.6565	7.42 ± 1.6565	4.81 ± 1.3654
	50	9.84 ± 1.6989	7.46 ± 1.6598	5.20 ± 1.9989
Alpha-2 globulin	25	9.44 ± 0.6598	19.51 ± 0.6986	23.30 ± 0.3697
	50	9.75 ± 0.2365	20.50 ± 0.3265	18.35 ± 0.3265
Beta globulin	25	15.06 ± 0.5864	10.60 ± 0.6598	8.12 ± 0.2356
	50	14.69 ± 0.8564	9.12 ± 0.3698	6.09 ± 1.6584
Gamma globulin	25	25.44 ± 0.3654	28.57 ± 0.1254	31.57 ± 0.4251
	50	26.91 ± 0.3695	28.90 ± 0.2525	31.91 ± 0.2000

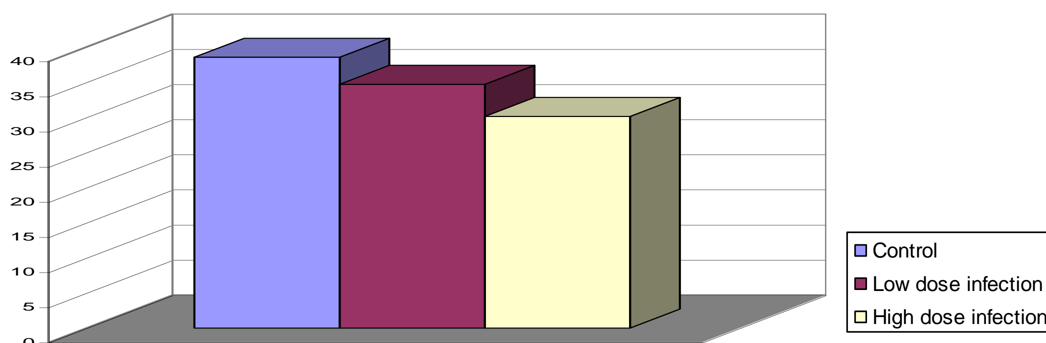

Fig.: 1 A

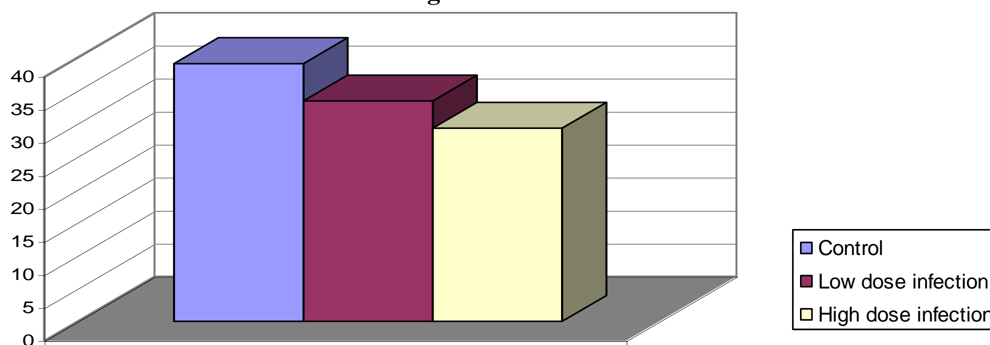

Fig.: 1 B

Fig. 1A & 1B: Values of albumin in male WLH chicks in control and with low and high doses of *A. galli* eggs

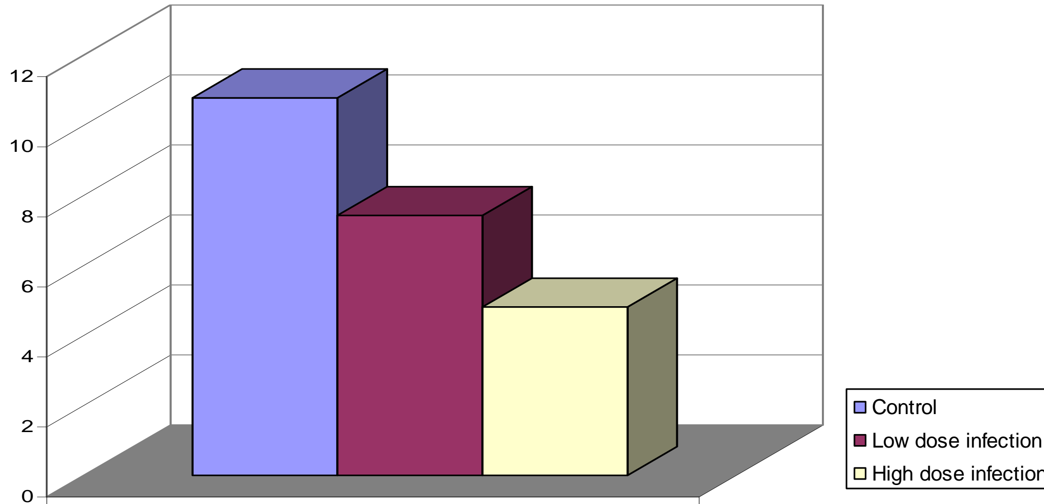

Fig.: 2 A

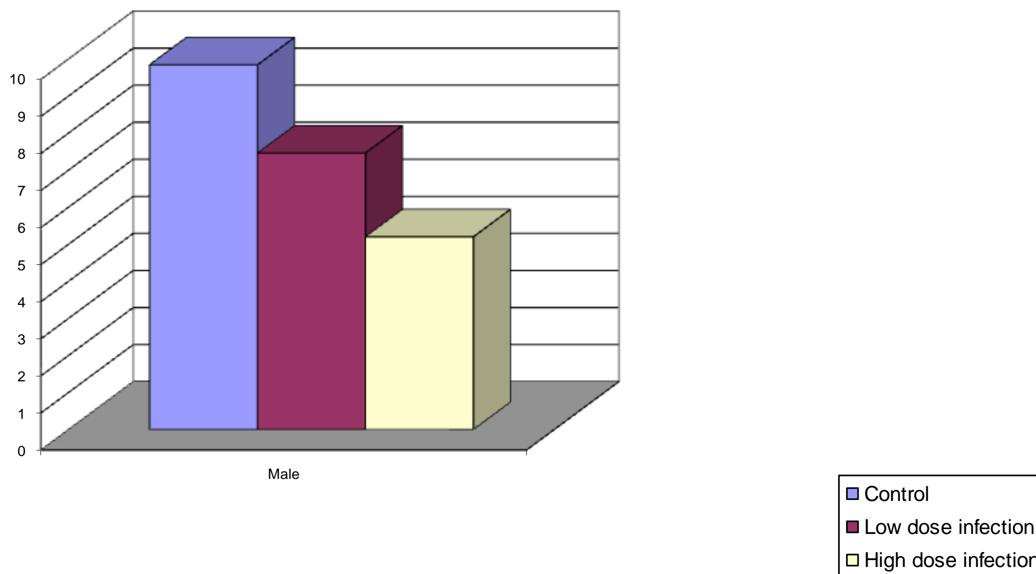

Fig.: 2 B

Fig. 2 A & 2 B: Values of alpha-1 globulin in male WLH chicks in control and infected with low and high doses of *A. galli* eggs

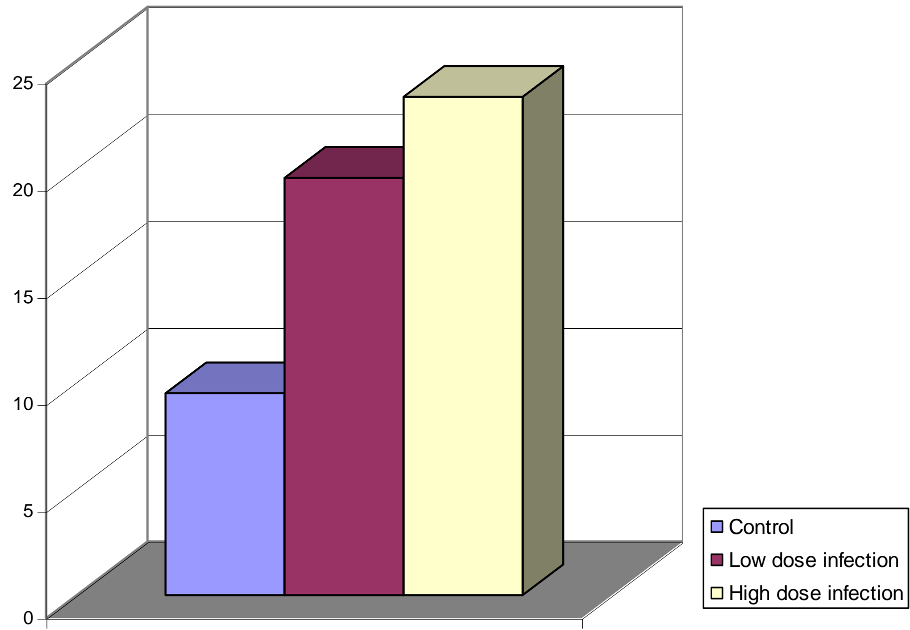

Fig.: 3 A

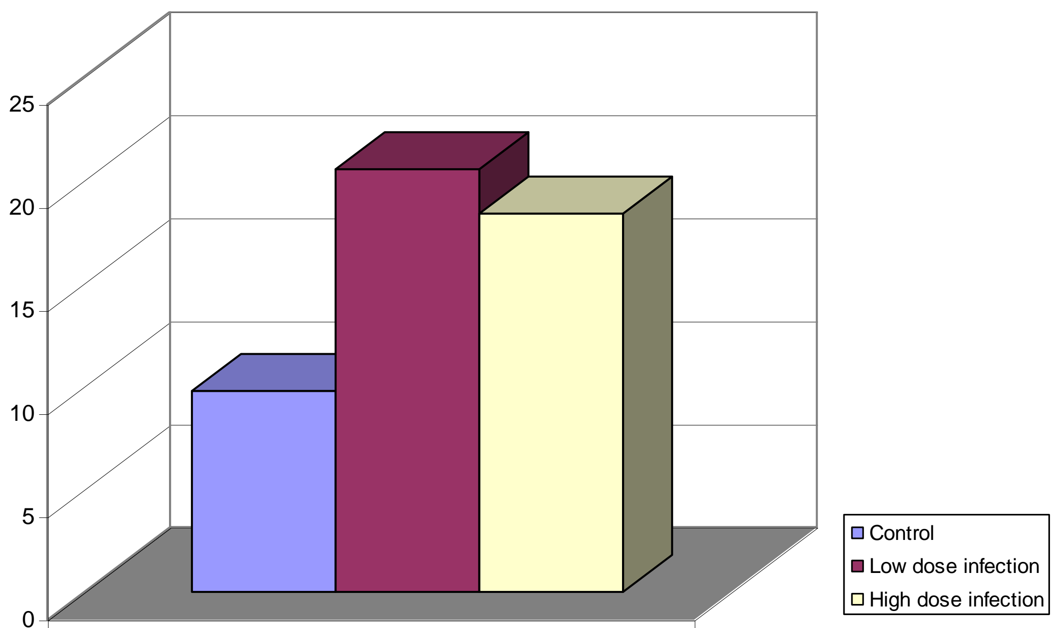

Fig.: 3 B

Fig. 3 A & 3 B: Values of alpha-2 globulin in male WLH chicks in control and infected with low and high doses of *A. galli* eggs

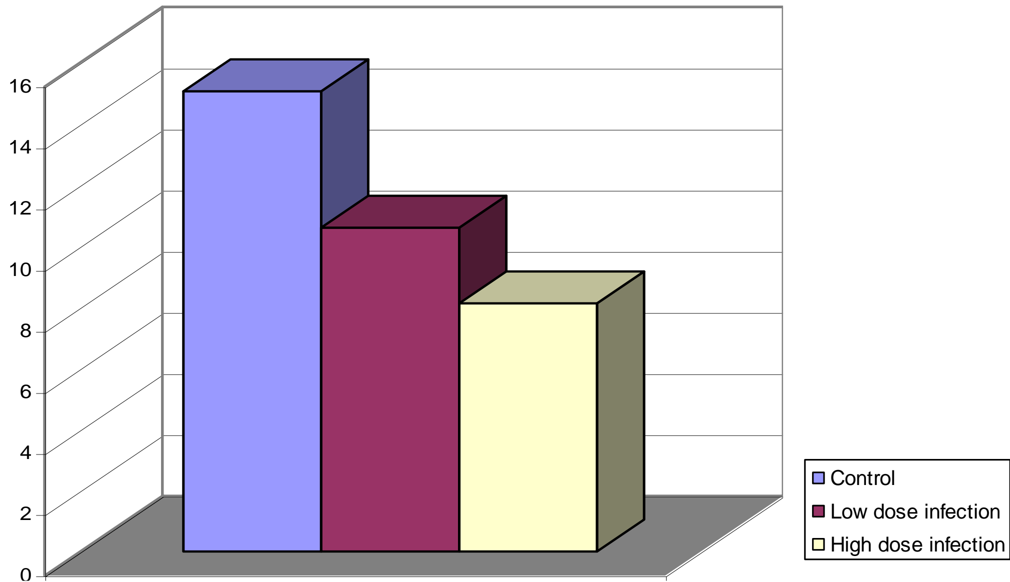

Fig.: 4 A

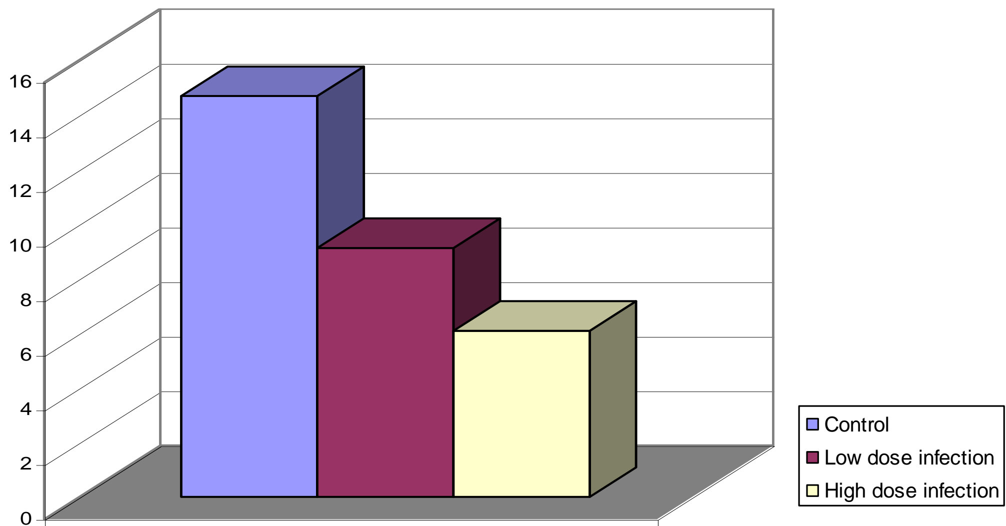

Fig.: 4 B

Fig. 4 A & 4 B: Values of beta globulin in male WLH chicks in control and infected with low and high doses of *A. galli* eggs

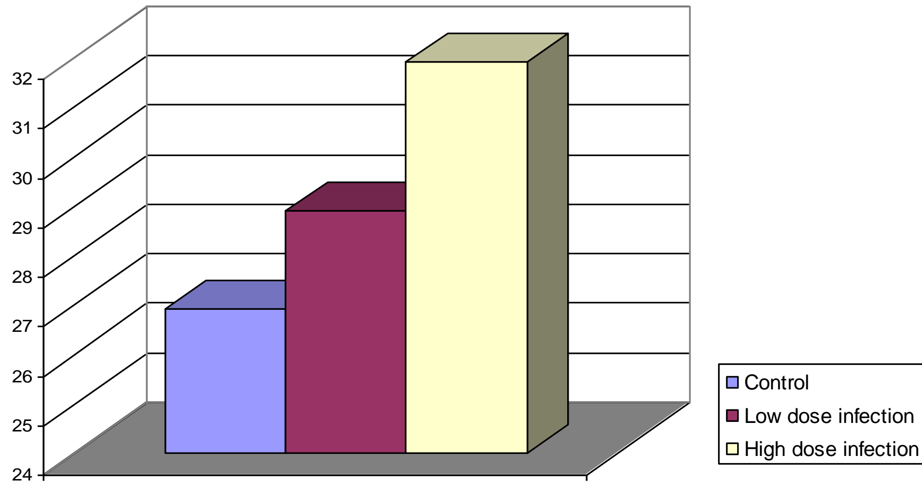

Fig.: 5 A

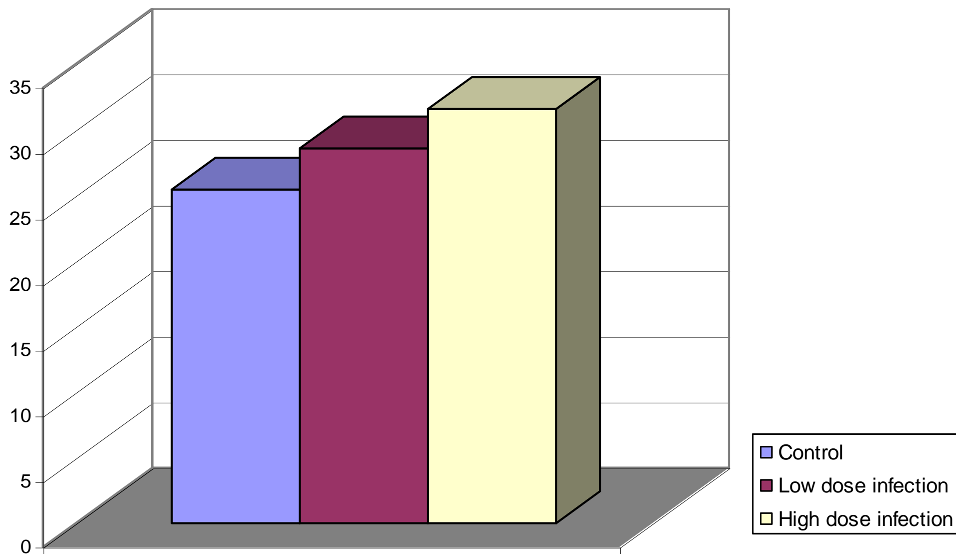

Fig.: 5 B

Fig. 5A & 5 B: Values of gamma globulin in male WLH chicks in control and infected with low and high doses of *A. galli* eggs

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