

Effect of vaccination on some biochemical parameters in broiler chickens

I. M. Kudair and N. A. J. Al-Hussary*

*Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq, E-mail: ahmed_jargees@yahoo.com

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Abstract

The present study was conducted to evaluate the effect of vaccination against Newcastle disease, infectious bronchitis and infectious bursal disease on some biochemical parameters in serum of broiler chickens. Two hundred and fifty Ross 1-day old unsexed broiler chicks were divided into two groups (unvaccinated and vaccinated), blood samples were obtained from each group weekly until 8 weeks old. The results revealed significant differences in values of some biochemical parameters according to various ages of chickens within either unvaccinated or vaccinated groups, the effect of different ages on parameters was more clearly observed in vaccinated group. It was also revealed from this study that vaccination had no effect on levels of glucose, total protein, total lipids cholesterol, triglycerides, high density lipoprotein-cholesterol (HDL-c) and low density lipoprotein-cholesterol (LDL-c) and the activity of alanine aminotransferase (ALT). Vaccinated chickens showed a significant reduction in the level of albumin, albumin/globulin ratio and alkaline phosphatase (ALP) activity accompanied with an elevation in the level of globulin and activities of aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) compared with their values in non vaccinated chickens. These data suggest the interference of age and vaccination in broiler chickens on some biochemical parameters.

Keywords: Vaccination; Broiler chickens; Biochemical parameters.

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تأثير التحصين على بعض المتغيرات الكيميائية الحياتية في فروج اللحم

إسراء محمد خضير و نبيل أحمد جرجيس الحصري*

*فرع الفلسفة والكيمياء الحياتية والأدوية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

تضمنت الدراسة الحالية تقييم تأثير التحصين ضد مرض النيوكاسل ومرض التهاب القصبات الخمجي ومرض التهاب غدة فابريشيا المعدي في بعض المتغيرات الكيميائية الحياتية في مصل الدم لفروج اللحم. تم استخدام ٢٥٠ فرخ فروج لحم من نوع Ross الانكليزية الأصل بعمر يوم واحد غير منفصلة الأجناس، وتم تقسيمها عشوائياً إلى مجموعتين غير محصنة وأخرى محصنة. تم جمع عينات الدم من كل مجموعة أسبوعياً حتى الأسبوع الثامن من عمر الأفراخ. أشارت النتائج الإحصائية إلى وجود فروقات معنوية في قيم بعض المعايير الكيميائية الحياتية عند المقارنة بين الأعمار في كل من المجموعة غير المحصنة والمجموعة المحصنة. وكان التأثير أكثر وضوحاً في المجموعة المحصنة. لم يلاحظ من النتائج تأثير للتحصين على مستويات الكلوكون، البروتينات الكلية، الكولسترول، الكليسيريدات الثلاثية، الشحوم الكلية، كولسترول الشحوم البروتينية المرتفعة الكثافة وكولسترول الشحوم البروتينية الواطئة الكثافة وكذلك فعالية أنزيم ناقلة أمين الألبومين. بينما أظهرت المجموعة المحصنة انخفاض معنوي في مستوى الألبومين ونسبة الألبومين/الكلوبولين وفي فعالية أنزيم الفوسفاتيز القاعدي مع وجود ارتفاع معنوي في مستوى الكلوبولين وفي فعالية أنزيم ناقلة أمين الأسبارتيت ونازعة هيدروجين اللاكتيت عند مقارنتها مع المجموعة غير المحصنة. يستنتج من الدراسة الحالية أن التحصين قد أدى إلى إحداث تغيرات معنوية في بعض القيم الكيميائية الحياتية في مصل دم فروج اللحم. كما لوحظت التغيرات في بعض المعايير باختلاف الأعمار وكان التأثير أكثر وضوحاً في المجموعة المحصنة.

Introduction

The primary purpose of all poultry vaccination is to induce protective immune response that could prevent or reduce the economic lost caused by viral infection and diseases (1). Serum biochemical parameters may provide valuable information for evaluation of health status of birds and reflect many metabolic alterations of organs and tissues (2). Several factors may have an influence on biochemical parameters in the chicken's serum including feed additives (3), drugs(4), environmental temperature (5) and poultry diseases (6). Newcastle disease (ND), infectious bronchitis (IB) and infectious bursal disease (IBD) remains the major viral poultry diseases affecting domestic and wild birds with high mortality rate induction (7,8), a little attention has been given to the role of vaccination on biochemical changes in the serum of chickens. A significant changes in the serum levels of total protein, triglycerides and albumin following vaccination was recently reported (9).

Our trial was conducted to evaluate the effect of vaccination against ND, IB and IBD on some serum biochemical parameters in broiler chickens including changes in the activities of several enzymes as alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) in addition to the levels of glucose, total lipids, cholesterol, triglycerides, HDL-c, LDL-c, VLDL-c, total proteins, albumin and globulin.

Materials and methods

Chickens

A total of 250 ROSS 1- day -old broiler chicks were randomly divided into two groups (control and vaccinated). These chicks were reared in cages and housed in a separate rooms of the animal house research division in the College of Veterinary Medicine, University of Mosul. The birds were allowed *ad libitum* access to food and water and maintained with recommended ambient temperature and other environmental conditions to 8 weeks of age.

Vaccine and vaccination

The chickens of the second group were vaccinated according to Jordan *et al* (10), vaccination program was designed as day 1 with spray IB vaccine (H120), day 7 with drinking IBD vaccine (Gumbol), day 10 with drinking ND vaccine (B1), day 13 with drinking IBD vaccine (IBDL), day 15 with drinking IB vaccine (H120), day 22 with drinking ND vaccine (B1), day 35 with spray ND vaccine (Lasota).

Blood sampling and serum collection

On day 7 and at weekly intervals until 56 day old, labeled blood samples (number of birds and date) were

taken from jugular vein using 1 ml syringes, kept in room temperature until clotted, and were centrifuged at 1,000 g for 15 minutes, Serum specimens obtained were frozen and stored at -20 C° until the time of analysis.

Estimation of biochemical parameters

Serum LDH, total cholesterol and triglycerides levels were estimated by the colorimetric method using diagnostic kit (Biolabo, France). AST and ALP activities and albumin level were measured by the colorimetric kit (Biomerieux, France). ALT activity was measured using colorimetric kit (Spinorect, Spain). Glucose level was measured using colorimetric kit (Biocan, Diagnostic, GmbH, Burbach, Germany). HDL-c concentration was estimated colorimetrically using diagnostic kit (Biomaghreb, Tunisia). Total protein and total lipids levels were measured according to (11) and (12), respectively.

Statistical analysis

Data were expressed as mean \pm SE, the data were analysed by (ANOVA). Significant treatment differences were assessed using Duncan's multiple range test. Differences in the means between the two groups were examined with students' t test. $P < 0.05$ was considered as statistically significant (13).

Results

The results obtained (Table 1) revealed that the activities of AST, ALT and LDH were increased at the fifth week old in vaccinated chickens. A significant increase in AST activity at seventh week old was only seen in non vaccinated chickens. Activity of ALP in the serum tend to be decreased with age from the third week in both vaccinated and non vaccinated chickens (Table 1). Vaccination produced an elevation of LDH and a reduction of ALP activities from the first week till the end of the experiment, while activity of AST in vaccinated group was increased at fifth week in vaccinated group compared with value obtained from non vaccinated group. Table (2) revealed that the total cholesterol was increased at the eighth week old only in vaccinated chickens. Serum levels of triglycerides was decreased at the sixth and eighth week old in both vaccinated and non vaccinated chickens, respectively. This table also showed an increase in serum level of glucose at the eighth week old in both vaccinated and non vaccinated chickens. Comparison of levels of cholesterol, total lipids, triglycerides and glucose for vaccinated chickens with their levels of corresponding parameters for non vaccinated chickens were not significantly differed. Results of Table (3) showed that values of HDL-c, LDL-c and VLDL-c were not affected by age and vaccination. From Table (4) it was observed that

levels of total protein and albumin in the serum tend to be increased gradually by age from the second week in both vaccinated and non vaccinated chickens. An elevation in the level of globulin accompanied with a significant

reduction in the level of albumin and albumin/globulin ratio were observed in vaccinated chickens in comparison with their levels in the non vaccinated chickens (Table 4).

Table (1): Effect of vaccination on serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) in broiler chickens.

Age in weeks	AST (U/L)		ALT (U/L)		LDH (U/L)		ALP (U/L)	
	Non vaccinated	vaccinated	Non vaccinated	vaccinated	Non vaccinated	vaccinated	Non vaccinated	vaccinated
1 st	17.86	16.58	42.90	43.94	750.10	*2661.31	109.506	*82.672
	±1.42	±1.50	±0.10	±5.20	±26.63	±241.51	±6.45	±1.38
	a	a	a	a	a	a	a	a
2 nd	19.84	18.54	45.60	47.17	751.95	*2665.79	105.570	*80.573
	±1.17	±1.73	±1.82	±2.93	±84.53	±135.06	±5.18	±1.47
	a	a	a	a	a	a	a	a
3 rd	19.67	22.87	47.75	47.81	751.10	*2669.91	81.076	*60.572
	±1.23	±0.43	±2.32	±3.39	±31.58	±100.18	±0.91	±2.02
	a	a	a	a	a	a	b	b
4 th	20.53	22.57	48.68	48.68	754.16	*2769.94	81.065	*57.376
	±0.97	±1.10	±4.08	±4.08	±41.07	±210.66	±1.50	±0.88
	a	a	a	a	a	b	b	b
5 th	21.55	*33.69	49.60	59.17	754.83	*2878.16	76.591	*33.052
	±0.85	±0.78	±4.46	±6.55	±34.88	±225.64	±0.51	±1.25
	a	b	a	b	a	c	b	c
6 th	21.39	*35.97	50.35	59.47	756.67	*2879.67	75.157	*33.847
	±0.60	±1.38	±6.20	±4.57	±32.01	±186.25	±0.96	±1.80
	a	b	a	b	a	c	b	c
7 th	37.72	*49.30	50.64	60.20	756.66	*3092.80	56.121	*30.590
	±1.06	±3.07	±3.70	±3.32	±27.33	±220.31	±1.92	±1.22
	b	c	a	b	a	d	c	c
8 th	37.28	*49.88	51.82	60.29	759.46	*3125.46	50.334	*30.100
	±2.32	±2.06	±5.70	±1.01	±27.69	±184.63	±1.82	±1.01
	b	c	a	b	a	d	c	c

Values are expressed as mean±SE, Different letters in the same column refers to the significant differences at level P≤0.05, *Significant differences from non vaccinated data at P<0.05.

Discussion

A little work only had been done for influence of vaccination on blood analysis and utilization of biochemical profile in avian medicine.

Data obtained from this study revealed that serum ALP activity in non vaccinated and vaccinated chickens was extremely high in the first week then decreased gradually by age. These findings come in agreement with those reported by (14). These changes correspond with the growth and may be attributed to differences in bone formation or to physical role in general metabolic activity (15). The main cause for serum ALP reduction is damage of intestine (16). The predominant ALP isoenzyme in plasma originates in the gut (17). Appearance of abnormal amounts of certain enzymes of intercellular origin in the blood reflect damage

to an organ or tissue (18). Liver is rich in some enzymes as ALT, AST and LDH and it's damage often results in releasing these enzymes to the blood (19). In the present study, serum ALT activity was not affected by age in both vaccinated and non vaccinated chickens. Vaccination also showed no significant change in serum ALT activity. This observation was in contrast to previous study (14) who demonstrated that the activity of serum ALT was higher in vaccinated chickens at the first two weeks of age. Vaccination was accompanied with a rise in the AST activity. This result was in agreement with the finding of (14) who found that serum AST activity was increased following vaccination with ND vaccine, this indicated some damage to an organ or tissue, or may be due to heart infarction and acute kidney disease (20). Serum LDH activity was unchanged in chickens of non vaccinated

group by age. This observation was in contrast to previous study (21) who indicated that serum LDH activity in chickens was decreased by age. The elevation of serum LDH activity postvaccination seems to be agree with that reported by (14) who demonstrated that this finding may be due to damage effect of the vaccine on tissues or organs which lead to the rise in it's level in the serum. This increasing may be also result from brain and nervous system damage resulting in paralysis and muscular atrophy and consequence metabolic changes (22). According to the age husbandry periods the elevation of both cholesterol and glucose levels and the reduction in level of triglycerides at the eighth week of age was in agreement with previous reports (23-25). Vaccination in the present work did not cause any significant changes in the levels of total lipids, cholesterol and glucose. These findings come in agreement with those reported by (26) for total lipids and (25) for cholesterol and (9) for glucose. Unchange in the level of cholesterol in the vaccinated chickens of the present work may reflect the intact liver function as it was postulated from the unalteration in the activity of ALT in the present study. Unchange in level of triglycerides was not confirmed

by other study (9) who found a significant increase in serum triglycerides in chickens postvaccination. Levels of both glucose and triglycerides may be correlated. It has been found that elevation of glucose concentration could induce 50% reduction in oxidation of fatty acids and this followed by an increase in serum level of triglycerides (27). Intact liver functions may be the cause of unalteration in the concentrations of HDL-c, LDL-c and VLDL-c in the present work. Liver damage thought to be the result in alteration of above parameters (28). This work has also shown that the level of albumin was significantly reduced in vaccinated chickens when compared with the non vaccinated group. This finding was in agreement to (14). Reduction of serum albumin may be due to traumatic injury to kidney and urinary system (29), or may be due to tissue damage of kidney causing loss of large amount of albumin (18). This view may be supported by (30) who reported that vaccination with IB vaccine caused traumatic injury to kidney. Data also reported that serum globulin was increased post vaccination. Similar observation was recorded by (6) using IBV vaccine. This effect may be due to stimulation of the immune system in the birds (31).

Table (2): Effect of vaccination on serum levels of total lipids, cholesterol, triglycerides and glucose in broiler chickens.

Age in weeks	Total lipids (mg/ 100 ml)		Cholesterol (mg/ 100 ml)		Triglycerides (mg/ 100 ml)		Glucose (mg/ 100 ml)	
	Non vaccinated	vaccinated	Non vaccinated	vaccinated	Non vaccinated	vaccinated	Non vaccinated	vaccinated
1 st	267.80	265.10	136.26	140.93	80.41	78.83	199.64	184.04
	±1.42	±1.86	±6.78	±6.60	±2.62	±2.49	±3.86	±4.37
	A	a	a	a	a	a	a	a
2 nd	268.60	266.00	135.47	143.04	76.96	76.83	194.22	184.02
	±1.72	±1.63	±1.62	±6.86	±2.27	±2.81	±4.28	±7.72
	A	a	a	a	a	a	a	a
3 rd	268.90	268.70	137.97	143.40	76.26	77.50	195.73	187.89
	±2.57	±2.25	±0.92	±7.31	±2.12	±2.16	±4.37	±7.74
	A	a	a	a	a	a	a	a
4 th	270.50	270.20	137.07	145.39	74.30	74.55	195.74	188.70
	±2.66	±1.88	±7.10	±1.39	±2.64	±2.84	±5.10	±3.62
	A	a	a	a	a	a	a	a
5 th	270.60	273.66	138.12	145.46	74.42	70.38	197.56	188.65
	±3.83	±1.52	±1.16	±0.99	±2.50	±2.51	±5.41	±4.08
	A	a	a	a	ab	a	a	a
6 th	273.39	272.98	141.93	145.76	68.07	59.04	206.29	196.27
	±2.57	±2.93	±1.78	±1.12	±2.26	±2.15	±6.99	±7.81
	A	a	a	a	ab	b	ab	ab
7 th	272.90	274.43	146.39	153.21	65.46	59.15	204.60	196.30
	±2.03	±2.14	±1.12	±1.10	±2.85	±2.91	±5.36	±6.38
	A	a	a	ab	ab	b	ab	ab
8 th	276.41	275.90	146.60	156.68	55.84	50.33	210.56	200.50
	±2.37	±1.20	±1.20	±7.93	±2.18	±2.11	±6.60	±7.11
	A	a	a	b	b	b	b	b

Values are expressed as mean±SE, Different letters in the same column refers to the significant differences at level P<0.05.

Table (3): Effect of vaccination on serum concentrations of high density lipoprotein- cholesterol (HDL-c), low density lipoprotein-cholesterol (LDL-c) and very low density lipoprotein- cholesterol (VLDL-c) in broiler chickens.

Age in weeks	HDL-c (mg/ 100 ml)		LDL-c (mg/ 100 ml)		VLDL-c (mg/ 100 ml)	
	Non vaccinated	vaccinated	Non vaccinated	vaccinated	Non vaccinated	vaccinated
1 st	68.43±1.61	71.29±3.32	52.75±1.50	54.66±2.30	16.08±1.52	15.57±1.60
2 nd	67.62±3.08	72.00±2.80	52.60±2.79	54.68±1.56	15.25±1.83	15.36±1.10
3 rd	68.29±1.57	73.00±3.04	51.29±0.92	54.90±1.29	15.39±1.44	15.50±0.68
4 th	67.71±2.93	73.71±2.13	54.50±2.51	56.77±1.42	14.86±0.71	14.91±0.93
5 th	70.14±2.93	73.71±1.52	53.50±2.75	55.75±2.33	14.48±0.80	14.00±1.18
6 th	71.29±1.30	76.38±2.28	56.23±1.72	59.14±0.53	13.61±1.20	11.80±0.95
7 th	71.29±1.98	77.84±2.03	62.01±2.82	62.04±1.60	13.61±0.57	11.80±1.38
8 th	73.33±3.73	77.10±1.07	61.14±2.04	62.50±0.92	11.16±1.28	11.66±1.75

Values are expressed as mean ±SE.

Table (4): Effect of vaccination on serum concentrations of total protein, albumin, globulin and albumin/ globulin ratio in broiler chickens.

Age in weeks	Total protein (g/ 100 ml)		Albumin (g/ 100 ml)		Globulin (g/ 100 ml)		Albumin/Globulin ratio	
	Non vaccinated	vaccinated	Non vaccinated	vaccinated	Non vaccinated	vaccinated	Non vaccinated	vaccinated
1 st	2.50 ±0.22 a	2.53 ±0.21 a	1.50 ±0.08 a	*0.90 ±0.07 a	1.00 ±0.04 a	*1.63 ±0.05 a	1.50 ±0.11 a	*0.55 ±0.03 a
2 nd	3.14 ±0.17 b	3.13 ±0.12 b	2.10 ±0.06 b	*1.50 ±0.10 b	1.01 ±0.01 a	*1.74 ±0.01 a	1.90 ±0.04 a	*0.86 ±0.03 a
3 rd	3.29 ±0.14 b	3.27 ±0.30 b	2.10 ±0.1 b	*1.52 ±0.14 b	1.19 ±0.16 a	*1.79 ±0.17 a	1.76 ±0.02 a	*0.82 ±0.04 a
4 th	3.33 ±0.23 b	3.27 ±0.10 b	2.14 ±0.11 b	*1.51 ±0.05 b	1.19 ±0.02 a	*1.81 ±0.01 a	1.79 ±0.02 a	*0.80 ±0.04 a
5 th	3.33 ±0.10 b	3.35 ±0.23 b	2.15 ±0.11 b	*1.54 ±0.05 b	1.18 ±0.13 a	*1.81 ±0.03 a	1.82 ±0.24 a	*0.85 ±0.07 a
6 th	3.38 ±0.17 b	3.46 ±0.09 b	2.20 ±0.13 b	*1.59 ±0.10 b	1.18 ±0.14 a	*1.87 ±0.02 a	1.86 ±0.01 a	*0.85 ±0.03 a
7 th	3.38 ±0.18 b	3.50 ±0.20 b	2.20 ±0.10 b	*1.58 ±0.11 b	1.18 ±0.05 a	*1.92 ±0.04 a	1.86 ±0.02 a	*0.82 ±0.09 a
8 th	3.49 ±0.37 b	3.50 ±0.25 b	2.22 ±0.11 b	*1.58 ±0.13 b	1.19 ±0.05 a	*1.92 ±0.07 a	1.86 ±0.02 a	*0.82 ±0.01 a

Values are expressed as mean±SE, Different letters in the same column refers to the significant differences at level P<0.05, *Significant differences from non vaccinated data at P<0.05.

It is concluded from this study, that vaccination have an effect on some biochemical parameters in serum of broiler chickens. Significant age related differences have also been found for these parameters in both non vaccinated and vaccinated groups.

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References

1. Tripathy DN. The impact of vaccine and the future of genetically modified poxvirus vaccines for poultry. *Anim Health Res Rev* 2004;5:263–266.
2. Rivetz B, Begin E, Hornstein K, Nerdinger M. Biochemical changes in fowl serum during infection with strains of Newcastle disease virus of different virulence. Changes in serum proteins, uni acid, lipids and electrolytes. *Res Vet Sci.*1977;22:285–291.
3. Cetin M, Deniz G, Polat V, Yalcin A, Tarih G. The effects of inorganic and organic selenium supplementation on biochemical blood parameters in broiler.Uludg University, *J Vet Med.* 2002; 21:59-63.
4. Zaman Q, Khan MZ, Lslam N, Muhammed G. Experimental furazolidone toxicosis in broiler chicks: effect of dosage, duration and age upon clinical signs and some blood parameters. *Acta Vet. Hungaria*,1995;43:359-367.
5. Vecerek V, Strakova E, Suchy P, Voslarova E. Influence of high environmental and biochemical indexesbin broiler chickens, *Zek, Anim Sci* 2002;47:176-182.
6. Panigrahy B, Rowe LD, Corrier DE. Haematological values and changes in blood chemistry in chickens with infectious bursal disease. *Res Vet Sci.*1986;40:86-88.
7. Sharma JM, Kim LJ, Rautenschlein S, Yen HY. Infection bursal disease virus of chickens pathogenesis and immuno- suppression. *Dev Comp Immunol.* 2000;24:223-235.
8. Alexander DJ. Newcastle Disease, Other Avian Paramyxo- viruses, and Pneumovirus Infection. In: Saif YM, Barnes HJ, Glisson IR, Fadly AM, McDougald JR. and Swayne DE. (Ed.), *Disease of Poultry.* 11th ed. Iowa State University Press, Ames.2003;pp.63- 92.
9. Talebi A. Biochemical parameters in broiler chickens vaccinated against ND, IB, and IBD. *Int J Poult Sci.* 2006;5(12):1151- 1155.
10. Jordan F, Pattison M, Alexanders D, Faragher T. *Disease of poultry.* W.B. Saunders. London. 2002;2:99-100.
11. Plumer DT. *An introduction to Practical Biochemistry.* 2nd ed., MCGRAW Hill Book Company UK.1987; pp.143–144.
12. Toro G, Ackermann PG. *Practical clinical chemistry* Boston: Little Brown and Company.1975; pp. 354.
13. SAS SAS /STAT Users Guide. Ver 6. SAS Institute Inc. Cary, NC. 1989.
14. EL-Toukhy N, Aly SA, Soliman MK. Physiological studies on the level of some electrolytes and enzymes in normal and Newcastle vaccinated chicks. *Assiut Vet Med.*1989;21(42)7-15.
15. Rizvi F, Anjum AD, Khan A, Mohsan M, Shaszad M. Pathological and serum biochemical effects of salinomycin. *Pakistan Vet J.*2008; 28(2):71-75.
16. Rivetz B, Bogin E, Hornstein K, Merdinger M. Biochemical change in chicken serum during infection with strains of Newcastle disease virus of differing virulence. 1. Enzyme study. *Avian Pathology.*1975; 4:189-197.
17. Bide RW.(1970). Plasma alkaline phosphatases in the fowl: Differentiation of the tissue isoenzymes by urea. *Technicon International congress, Chicago, 1969, Vol 3, p. 169, New York, Medical Inc.*
18. Wilson DD. *Manual of laboratory and diagnostic tests.* McGraw-Hills New York, Chicago, Sanfrancisco, Lisbone.2008.
19. Kaplan LA, Pesce AJ, Kazmierczak SC. *Liver Function.* In: Sherwin, JE. *Clinical Chemistry.* 4th ed. Mosby. An affiliate of Elsevier Science. St. Lais, Toronto.2003.
20. Mc Daniels LS, Chate HL. Enzyme activity levels in chickens plasma. *Am J Vet Res.*1961;22:99-103.
21. Whitby LG, Smith AF, Beckett GJ. *Clinical chemistry.* 4th ed. Blackwell Scientific Publication, Oxford, London,1988; pp:223-226.
22. Rivetz B, Bogin E. Enzymatic changes in serum and tissues in fowl infected with a neurotropic–mesogenic strain of Newcastle disease virus. *Avian Pathology.*1982;11:407-425.
23. Almeida JG, Vieira SL, Gallo BB, Conde ORA, Olmas AR. Period of incubation and posthatching holding time influence on broiler performance. *Brazilian Journal of Poultry Science,*2006; 8(3): 153-158.
24. Safameher A. Effects of clinoptilolite on performance,biochemical parameters and hepatic lesions in broiler chickens during aflatoxosis. *J Anim Vet Adv.*2008;7(4):381-388.
25. Silva PRL, Freitas NOC, Laurentiz AC, Junqueira OM, Fagliari JJ. Blood serum components and serum proteins test of hybro-PG broilers of different stages. *Rev Bras Cienc Avic.*2007;9(4):1-8.
26. Prudnikov AV, Maksimovich VV. Influence of a vaccine against as infectious bronchitis and Newcastle disease on morphological and biochemical parameters of chickens blood. *Repository Search Results.* (Abstract).2007.
27. Sidossis LS, Mittendorfor B, Walser E, Chinkes D, Wolfe RR. Hyperglycemia – induced inhibition of splanchnic fatty acid oxidation increases triacylglycerol secretion. *AM. J. Physiol. Endocrinol. Met.*,1998;275(5):798-805.
28. Blocks VW, Plosch T, Goor HV, Roelofsen H, Baller J, Havinga R, Verkade HJ, Tol AV, Jansen PL, Kuipers F. Hyperlipidemia and atherosclerosis associated with liver disease in ferrochelata-se-deficient mice. *J Lip Res.* 2000;2:41-50.
29. Bunch SE. Hepatobiliary and Exocrine Pancreatic Disorder. In: Nelson, R. W. and Couto, C. G. (Editors) *Small Animal.* 3rd ed. Inter Med Mosby,2003; pp. 486.
30. Bowes VA, Julian RJ, Stirlzinger T. Comparison of serum biochemical profiles of male broilers with female broilers and white leghorn chickens. *Can J Vet Res.*1989;53:7-11.
31. Glick B. *Immunophysiology.* In: Sruke, P.D. *Avian Physiology.* 5th ed. Springer-Verlage-New York.1998; pp.657-666.