INFLUENCE OF LECTIN ON SOME HEMATOLOGICAL, BIOCHEMICAL, IMMUNOLOGICAL PARAMETERS AND PERFORMANCE IN BROILERS EXPERIMENTALLY INFECTED WITH ECOLI

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Abstract

This study aimed to investigate the effect of mannose-binding lectin (MBL) on infections with Escherichia coli in chickens. .One hundred and twenty five birds divided into five equal groups. The first group was kept as negative control.The second group was given Lectin 1cm/L orally in drinking water at 18day of age for 3successive days .The third group was infected with inculum containing Ecoli O78 (1×106 cfu)intra crop at 21 days of age. The fourth group was given Lectin 1cm/L orally in drinking water at 18 day of age for 3successive days then infected with inoculum containing Ecoli O78(1×106 cfu) intra crop .The fifth group was infected with inoculum containing Ecoli O78 (1×106 cfu) intra crop at as group 3 and treated with Lectin 1cm /L orally in drinking water for 3successive days. On the days 28,35 and 42 of age ,blood samples were collected from all groups for haematological and immunological examinations .The results of this experiment reveled non significant changes in Total leucocytic count in 1st week and 3rd week but there is significant decrease ($P<0.05$) in Total leucocytic count in 3rd group (infected with E coli)in the 2nd week only .Heuterophil percent showed non significant changes during 1st week and 3rd week but there was sig. decrease ($P<0.05$) in group 4in the 2nd week only. Eosinophils showed sig .increase($P<0.05$) in group 2 only in 1st week but showed sig .decrease($P<0.05$) in group 5 and in group 3 in 2nd and 3rd week respectively .Basophil reveled sig. increase in all groups in compared with control in 1stweek only . Lymphocytes percent showed sig. increase($P<0.05$) in group 3 ,4 and 5 in 2nd week but in 3rd week there was sig increase in group 3 only.Total protein and albumin reveled non sig. changes in all groups except group 3 showed sig. decrease($P<0.05$) in albumin only but in the 2nd week there were sig. decrease ($P<0.05$)in globulin in group 3 and sig. increase ($P<0.05$)in group 2 beside these results in the 3rd week there were sig. decrease($P<0.05$) in globulin level in groups 3 and 5 .Total immunoglobulin showed sig. increase($P<0.05$) in group 2 only but other groups reveled slight sig. decrease($P<0.05$) at the end of experiment . In conclusion, it has been found that the use of MBL seems to be important in protecting chickens against E. coli infections and as a good immunostimulant

Keywords: mannose-bindinglectin (MBL), Ecoli O78, avian immunology.

INTRODUCTION

The word lectin is from the Latin legere which means "to bind" or to "pick and choose." Lectins are abundant in nature. . MBL plays a major role in the first-line innate immune defense against bacteria, viruses, and parasites. Although MBL is an important part of the innate immune defense, the serum levels of MBL in commercial chicken lines are unknown Norupet , et al. 2009 .

When MBL has bound to a microorganism, it promotes killing of the microorganism either by acting directly as an opsonin or via the MBL-associated serine proteases (MASP-1 and MASP-2) by activating the lectin complement pathway. Similar to mammals, chickens produce MBL in the hepatocytes, secrete it into the blood, and upregulate the production during acute stages of virus infections (Laursen and Nielsen, 2000; Juul-Madsen et al., 2003). MBL is regarded as a minor acute phase protein in that it is elevated only 2- to 3-fold in virus-infected chickens compared with uninfected controls (Nielsen et al., 1999; Juul-Madsen et al., 2002,2003). However, Juul-Madsen et al. (2007) showed that propagation of infectious bronchitis virus (IBV) in trachea was impaired in chickens with a high basal serum level of MBL compared with chickens with a low serum level of MBL. This demonstrates that MBL plays an important role in the immunological defense against pathogens. After infecting 2 Vietnamese breeds experimentally with Pasteurellamultocida, chickens developing systemic infections had a significantly lower mean MBL concentration than the rest of the chickens (Schou et al., 2008). Furthermore, disease resistance in humans and pigs has been shown to be highly associated with the level of MBL (Summerfield et al., 1997; Lillie et al., 2007). The experiment was performed in chicken to evaluate the importance of MBL in interaction with E. coli.

MATERIALS AND METHODS

Drug:- Lector 50 is a drug produced by Microbiotechinc (USA) and distributed by Bioline company,Tanta –Egypt .Each iml contain 50000 mg lectin and given orally for chickens by the dose of I ml |L.

Bacterial strain :-Ecoli O78 strain was obtained kindly from microbiology department - faculty of veterinary medicine Zagazeg university .

Experimental design:-

One hundred and twenty five 1-day-old broiler chickens were obtained from commercial breeder farm were kept under strict hygienic and isolation measures and given ration adlibitum

The first group was kept as negative control .The second group was given Lectin 1cm/L orally in drinking water at 18days of age for 3successive days .The third group was infected with inculum containing(1×106cfu) Ecoli O78 intracrop at 21 days of age.The fourth group was given Lectin 1cm/L orally in drinking water at 18day of age for 3successive days then infected with inculum containing (1×106cfu) Ecoli O78 intracrop .The fifth group was infected with inculum containing(1×106 cfu) Ecoli O78 intracrop at as group 3 and treated with Lectin 1cm /L orally in drinking water for 3successive days

Vaccination:-

All groups were vaccinated against Newcastle disease with Hitchiner B1 at 7days and LaSota at 21 days of age. Also all groups were vaccinated against Gumboro disease with IBD at 13 day of age .

Sampling:-

Three blood samples were collected from all groups at the days28,35,42 days of age .The first blood sample was collected on EDTD from wing vein for leukogram (Total leucocytic count ,Differential leucocytic count). The 2nd blood sample was collected by heart puncture under strict aseptic condition on heparin for immunological examination .The 3rd blood sample was collected without anticoagulant for serum separation for measuring Total protein ,albumin and immunoglobulin fractions

Hematological examinations:-

Blood samples were collected from all groups for TLC evaluation using Natt and Herrick solution as special avain diluent according to Coles (1986) . Blood smear were stained with wright'stain for differential leucocytic count and absolute values were calculated according to Schalm(1975) .

Biochemical analysis :-

Serum total protein was evaluated according to Henry (1974).Albumin was determined according to Doumas (1971).

Immunological test:-

Phagocytic assay was estimated according to (Woldenhiwet, 1987 and Woldenhiwet and Rowan 1990).

Statistical analysis :-Dunkan grouping was done according to SAS,(1992).

RESULTS AND DISCUSSION

The results of this experiment reveled non significant changes in Total leucocytic count in 1st week and 3rd week but there is significant decrease in Total

leucocytic count in 3rd group (infected with Ecoli)in the 2nd week only (table 1,2,3) .Heuterophil percent showed non significant changes during 1st week and 3rd week but there was sig. decrease in group 4in the 2nd week only. Eosinophils showed sig . increase in group 2 only in 1st week but showed sig .decrease in group 5and in group 3 in 2nd and 3rd week respectively .Basophil reveled sig. increase in all groups in compared with control in 1stweek only . Lymphocytes percent showed sig. increase in group 3 ,4 and 5 in 2nd week but in 3rd week there was sig increase in group 3 only (table 1,2,3) .Total protein and albumin reveled non sig. changes in all groups except group 3 showed sig. decrease in albumin only but in the 2nd week there were sig. decrease in globulin in 3 and sig. increase in group 2 beside these results in the 3rd week there were sig. decrease in globulin level in groups 3 and 5 (table 4,5,6). These results may be due to the biological function of MBL is to bind to the carbohydrates on the cell wall of foreign microorganism by means of the CRD. This is followed by an interaction with phagocyting cells carrying MBL receptors on their cell surface. Binding of the MBL to the MBL receptor triggers a number of cellular defence mechanisms including phagocytosis, modulation of cytokines and immunoglobulin secretion(table7). MBL has been shown to bind to a variety of bacteria, viruses, fungi, mycobacteria, parasites and protozoa (reviewed in Epstein et al. 1996). In addition, MBL can activate the complement system independently of antibody and C1q by activating the MBL-Associated Serine Proteases (MASP), which is thereby able to drive C4 and C2 into the classic complement activation. This pathway is now called the lectin pathway of the complement activation (Ohta et al., 1990; Matsushita et al., 1992, Thiel et al., 1997). The level of MBL is up regulated during an infection, and the MBL is therefore a typical positive acute phase protein (Turner 1996). MBL's possibility of alternative complement activation emphasises the importance of the protein in the acute

Table(1): Effect of Ecoli infection and Lectin on blood picture parameters during 1st week of experiment .

	control	Lectin only	E.coli inf.	Lect + infect	Infection + lect
Total WBCs (x103/mm3)	23.7±1.2ab	25.68±4.04a	24.4±3.12ab	21.56±1.33b	21.60±3.81ab
Heutrophils(%)	47.9±1.43a	53.83±1.98a	51.81±2.13a	47.84±3.01a	49.65±2.09a
Eosinophils(%)	4.66±1.3b	7.28±0.93a	5.89±0.78b	8.01±0.73a	5.70±0.67b
Basophil(%)	1.82±0.05b	4.17±0.87a	4.02±0.67a	4.15±1.07a	3.24±1.01a
Lymphocytes(%)	44.2±0.5a	39.69±2.02a	38.75±1.45b	38.11±2.98a	39.7±2.34a
Monocyts(%)	1.44±0.19a	2.97±0.25a	2.26±0.34a	1.98±0.41a	1.99±0.24a

Values are means + standard error, Means with different letters at the same raw differ significantly at ($p<0.05$)

Table (2): Effect of Ecoli infection and Lectin on blood picture parameters during 2nd week of experiment.

	control	Lectin only	E.coli inf.	Lect + infect	Infection + lect
Total WBCs (x103/mm3)	23.75±2.78a	23.52±3.77a	19.47±3.22b	21.05±1.13ab	22.45±2.31ab
Heutrophils (%)	53.96±2.79a	51.94±2.61ab	49.45±2.92ab	46.77±1.87b	51.14±2.69ab
Eosinophils (%)	8.54±1.70a	7.95±1.17a	6.85±0.90ab	8.97±0.82a	5.59±0.88bc
Basophil(%)	4.09±1.1a	4.28±1.16a	4.02±0.95a	4.47±0.48a	3.23±0.75a
Lymphocytes (%)	30.15±1.15b	32.43±1.9b	39.95±2.02a	38.04±1.53a	38.62±1.76a
Monocyts (%)	3.27±0.45a	3.53±0.89a	2.46±0.01a	1.80±0.26b	1.79±0.45a

Values are means + standard error, Means with different letters at the same raw differ significantly at ($p<0.05$)

Table (3): Effect of ofEcoli infection and Lectin on blood picture parameters during 3rd week of experiment.

	control	Lectin only	E.coli inf.	Lect + infect	Infection + lect
Total WBCs (x103/mm3)	24.8±2.15a	25.84±5.54a	23.36±3.32a	24.6±4.2a	27.8±2.39a
Heutrophils (%)	55.72±3.01a	52.45±2.41a	50.91±2.78a	51.96±2.75a	51.53±2.54a
Eosinophils (%)	8.12±1.09a	7.19±1.10ac	6.03±0.98bc	6.3±0.95bc	7.64±1.10ac
Basophil(%)	3.06±0.98ac	4.18±0.96a	2.13±0.78bc	2.59±0.78bc	2.95±0.92bc
Lymphocytes (%)	30.89±2.45bc	34.6±2.18ac	38.79±2.08a	36.31±2.22ac	35.92±2.09ac
Monocyts(%)	2.22±0.23a	1.56±0.28a	2.13±0.31a	1.45±0.35a	1.96±0.21a

Values are means + standard error, Means with different letters at the same raw differ significantly at ($p<0.05$)

Table (4): Effect of Ecoli infection and Lectin on phagocytic activity and index at 1st ,2nd and 3rd week of experiment.

		control	Lectin only	E.coli inf.	Lect + Infect	Infection + lect
Phagocytic activity	1st week	19.92±1.22ab	21.72±1.96a	18.44±0.83b	18.38±1.46b	19.39±2.38ab
Phagocytic index		2.05±0.37a	2.43±0.83a	1.97±0.12a	2.10±0.20a	2.22±0.41a
Phagocytic activity	2nd week	26.84±3.67a	23.53±2.83ac	19.47±2.42b	21.25±1.17bc	18.70±1.8b
Phagocytic index		2.87±0.82a	2.65±0.75ac	1.82±0.62bc	2.14±0.37ac	1.81±0.54bc
Phagocytic activity	3rd week	25.91±3.79a	23.79±3.22ac	20.0±1.7bc	21.86±2.47bc	22.81±5.54ac
Phagocytic index		2.41±0.44a	2.67±0.65a	2.22±0.27a	2.44±0.49a	2.61±0.99a

Values are means + standard error, Means with different letters at the same raw differ significantly at ($p<0.05$)

Table(5): Effect of ofEcoli infection and Lectin on some serum parameters during 1st week of experiment.

	Control	Lectin only	E.coli inf.	Lect + infect	Infection + lect
Total protein (g/dl)	6.08±0.76a	6.18±1.03a	6.24±0.97a	6.88±0.33a	6.63±0.61a
Albumin (g/dl)	5.23±0.66b	5.16±0.71bd	5.38±0.55cd	6.23±0.26a	5.49±0.38bc
Globulin (g/dl)	0.85±0.43a	1.02±0.5a	0.86±0.65a	0.64±0.45a	1.14±0.67a
A/G Ratio	6.92±3.34ac	4.22±0.21bc	6.02±2.41bc	9.07±4.2a	4.24±1.8bc

Values are means + standard error, Means with different letters at the same raw differ significantly at ($p<0.05$)

Table (6): Effect of ofEcoli infection and Lectin on some serum parameters during 2nd week of experiment.

	control.	Lectin only	Ecoli infection	Lect + infect	Infection + Lect
Total protein (g/dl)	5.78±0.76a	5.70±0.64a	6.06±0.25a	6.00±0.36a	6.17±0.14a
Albumin (g/dl)	3.77±1.4a	3.91±1.13a	4.78±0.19a	4.49±0.85a	4.74±0.53a
Globulin (g/dl)	2.02±0.7b	3.1±0.65a	1.28±0.20c	1.51±0.45bc	1.43±0.61b
A/G Ratio	1.94±0.52d	1.28±0.46dc	3.76±0.77b	5.32±1.78b	4.04±1.13b

Values are means + standard error, Means with different letters at the same raw differ significantly at ($p<0.05$)

Table (7): Effect of ofEcoli infection and Lectin on some serum parameters during 3rd week of experiment .

	control .	Lectin only	E.coli inf.	Lect + infect	Infection + lect
Total protein (g/dl)	5.58±0.83a	5.96±0.72a	5.46±0.89a	5.77±0.65a	5.23±0.73a
Albumin (g/dl)	4.28±1.12a	4.65±0.73a	4.75±0.37a	4.45±0.98a	4.73±0.51a
Globulin (g/dl)	1.31±0.65a	1.12±0.2ac	0.71±0.33bc	1.32±0.46a	0.51±0.19b
A/G Ratio	3.49±1.70b	4.5±1.90b	6.36±1.56a	3.89±2.03b	9.63±2.53a

Values are means + standard error, Means with different letters at the same raw differ significantly at ($p<0.05$)

Table (8): Effect of ofEcoli infection and Lectin on Total immunoglobulin during 3rd week of experiment .

Groups	Total immunoglobulin
Control	3.22±0.03 b
Lectin only	3.92±0.01 a
E.coliinf	2.88±0.05 d
Infection +lectin	2.94±0.01 ed
Lectin + infect	2.88±0.21 d

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"دراسة تاثير اللاكتين على التغيرات الدموية والبيوكيميائية و المناعية على اداء دجاج التسمين المصابة تجريبياً بميكروب الايشيريشيا كولاى"

سعاد سعد بليح[1] ، أمل فتحى الزعبى[2] ، أمل أحمد محمود رجب[3]

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تهدف هذه التجربة إلى دراسة تأثير اللكتين على عدوى الدجاج بميكروب الإيكولاى ومن أجل هذا الهدف أجريت التجربةعلى عدد ١٢٥ كتكوت قسمت إلى خمس مجموعات متساوية . المجموعة الأولى اعتبرت مجموعة ضابطة . المجموعة الثانية أعطيت اللكتين بجرعة ١سم/لتر ماء شرب عند ١٨ يوم من عمر الطيور لمدة ٣ أيام متتالية . المجموعة الثالثة تم عدواها بميكروب الإيكولاى لكل طائر داخل الحويصلة عند عمر ٢١ يوم . المجموعة الرابعة أعطيت اللكتين عند عمر ١٨ يوم بجرعة ١سم /لتر ماء لمدة ٣ أيام متتالية ثم تم عدواها كما حدث فى المجموعة الثالثة . المجموعة الخامسة تم عدواها كما حدث فى المجموعتين السابقتين ثم علاجها باللكتين بجرعة ١سم /لتر ماء شرنتاب لمدة ٣ أيام متتالية . فى أعمار ٢٨ -٣٥ -٤٢ تم سحب عينات دم وسيرم للفحص الهيماتولوجى – كمياء السيرم والمناعة . وقد أظهرت النتائج مايلى :- عدم وجود أى تغير معنوى فى العد الكلى لكرات الدم البيضاء على مدار الثلاث أسابيع ماعدا إنخفاض معنوى فى المجموعة الثالثة التى تم عدواها بميكروب الإيكولاى فى الأسبوع الثانى فقط (جدول ١,٢,٣) . أما العد النوعى لكرات الدم البيضاء أوضحت النتائج إنخفاض معنوى فى عدد خلايا الهتيروفيل فى المجموعة الرابعة فى الأسبوع الثانى فقط وكذلك زيادة معنوية فى نسبة خلايا الإزينوفيل فى المجموعة الثانية فقط فى الاسبوع الاول ولكن إنخفضت معنويا فى المجموعة الخامسة والثالثة فى الأسبوع الثانى والثالث على الترتيب.

أم الخلايا الليمفاوية أظهرت زيادةمعنوية فى المجموعة الثالثة والرابعة والخامسة فى الأسبوع الثانى ولكن فى الأسبوع الثالث وجدت زيادة معنوية فى المجموعة الثالثة فقط .

الخلايا الليمفاوية أظهرت زيادة معنوية فى المجموعات الثالثة والرابعة والخامسة ولكن فى الأسبوع الثالث وجدت زيادة معنوية فى المجموعة الثالثة فقط .

التحليل الكيميائى للسيرم (البروتين الكلى والألبيومين) لم يظهر بها أى إختلاف فى الأسبوع الأول فى جميع المجموعات ماعدا المجموعة الثالثة فقد أظهرت إنخفاضا معنويا فى الألبيومين فقط . أما فى الأسبوع الثانى فقد وجد إنخفاضا معنويا فى الجلوبيولينفى المجموعة الثالثة وزيادة معنوية فى المجموعة الثانية . أما فى الأسبوع الثالث فقد وجد إنخفاض معنوى فى الجلوبيولين فى المجموعة الثالثة والخامسة .

نشاط الخلايا الآكولة إنخفض معنويا فى الأسبوع الثانى فى المجموعات الثالثة والرابعة والخامسة أما فى الأسبوع الثالث فقد إنخفض نشاط الخلايا الأكولة معنويا فى المجموعات الثالثة والرابعة فقط .

قياس مستوى الإميونوجلوبيولين إنخفض معنويا فى المجموعات الثالثة والرابعة والخامسة أما المجموعة الثانية ظهر بها زيادة معنوية .