Effect of garlic supplementation on the humoral and cellular immunity after immunization of broilers by sonicated oocysts of Eimeria tenella

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Abstract

This study was performed to investigate the effect of garlic with sonicated oocysts immunization of E. tenella on humoral and cellular immune responses in broilers. A total of 150 broiler chicks were divided into 6 equal groups. The 1st and 2nd groups inoculated *in ovo* with sonicated oosycts of E. tenella and repeated by I/M injection at day 14. The 3rd and 4th groups injected I/M with sonicated oosycts at day old and repeated at day 14, the 1st and 3rd feeding a basal diet, while the 2nd, 4th and 5th groups feeding basal diet with garlic. The birds of the 6th group fed basal diet and remains as control. All groups were challenged with 50,000 of sporulated oocysts of E. tenella. The results revealed that the immunized groups and immunized with garlic have a significant difference $P < 0.05$ in the lymphoid organs weight and indices, antibody titers against SRBCs (sheep red blood cells) and Phytohaemagglutinin (PHA) skin test in camper with control and garlic groups specially at day 35. At these instants there was no significance difference between the two procedures of immunization *in ovo* and I/M.

In conclusion that *in ovo* and I/M immuinzation of sonicated oocyst showed some evidence of reducing negative performance impact associated with coccidiosis.

Keywords: garlic, sonicated oocysts, Eimeria tenella, broilers

تأثير اضافة الثوم على المناعة الخلطية والخلوية بعد تمنيع دجاج اللحم بأكياس البيض المكسرة لطفيلي الايميريا تينيلا

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المستخلص

اجريت هذه الدراسة لمعرفة تاثيرالتمنيع بوجود الثوم مع مستضد أكياس البيض المكسرة لطفيلي الايميريا تينيلاعلى المناعة الخلطية والخلوية في دجاج اللحم. تم اخذ 150 فرخا من افراخ اللحم قسمت هذه الأفراخ إلى 6 مجاميع متساوية تحتوي كل مجموعة على 25 فرخاً، حقنت المجموعة الأولى والثانية بالبيضة بعمر 18 يوماً

من عمر الجنين وأعيد حقنها بالعضلة بعمر 14 يوم. المجموعة الثالثة والرابعة حقنت بالعضلة بعمر يوم واحد، وأعيد حقنها بالعضلة بعمر 14 يومً. تم الحقن بمستضد أكياس البيض المكسرة لطفيلي الايميريا تينيلا لهذه المجاميع. المجموعة الأولى والثالثة غذيت على العليقة الأساسية بينما غذيت المجموعة الثانية والرابعة والخامسة على العليقة الأساسية بالاضافة الى مسحوق الثوم بنسبة 4غم/كغم علف. وتركت المجموعة السادسة كمجموعة سيطرة. تم إجراء اختبار التحدي لكل الطيور بإعطائها 50,000كيس بيض متبوغ لطفيلي الايميريا تينيلا بعمر 28 يوماً. اظهرت النتائج ان المجاميع الممنعة والممنعة مع مادة الثوم قد سجلت اختلافا معنوياً عند مستوى احتمال 5% من ناحية وزن ونسبة الاعضاء اللمفاوية،الاجسام المضادة للكريات الدم الحمر للاغنام واختبار الحساسية المتأخربالمقارنة مع مجموعة السيطرة والثوم خاصة في يوم 35. ولم يكن هناك أي فرق معنوي بين طريقتي التمنيع بواسطة الحقن بالبيض أو الحقن بالعضلة. من هذا يمكن الاستنتاج بأن التمنيع بواسطة الحقن بالبيض والحقن بالعضلة بأكياس البيض المكسرة حفزت المناعة ووفرت حماية يمكن ملاحظتها ضد الخمج بالايميريا تينيلا.

Introduction

Garlic (*Allium sativum* L.) has been used for remedies and food for more than a thousand years, it still being utilized and accepted in modern day medicine to prevent and treat of a variety of diseases ranging from infections to heart diseases [(1)]. The antioxidant properties of garlic are well documented, on the other hand, it has been founded that garlic as a natural feed additive, improved broiler's growth, feed conversion ratio, haematilogical performance and decreased mortality rate [(2, 3)].

Avian coccidiosis, caused by obligate intracellular parasites belonging to various species of the genus Eimeria, is estimated to cost the world-wide poultry industry several billion dollars annually in mortality and bird productivity [(4, 5)]. The disease has been primarily controlled by use of chemicals, but their extensive use has resulted in the development of drug resistance by these parasites [(6, 7)]. Due to food safety concerns and the cost of new drug development, recent emphasis has centered on elicitation of protective immune response to parasite infection by development and effective use of live or inactivated parasite vaccines [(7)].

The immune response to inactivated sonicated vaccine was used in chickens; parental inoculation of dead antigen is although capable of stimulating circulating antibodies against coccidiosis antigen. In spite of these limitations, vaccination remains the most efficient means of preventing disease and reducing economic losses [(8)]. Particularly, there is an urgent need to develop a felid vaccine against avian coccidiosis that is safe and effective against all relevant parasites. The objective of the present work was to determine if sonicated oocysts immunization of Eimeria tenella with G can significantly enhance immune responses in broiler chickens.

Materials and methods

The experiment was done in the Pathology and Poultry Diseases Department, College of Veterinary Medicine, Baghdad University, poultry house, after cleaning and disinfection.

Birds and management

One-day-old, broiler chicks of the "Cobb strain" were purchased from a local hatchery. Upon arrival, the chicks were raised according to routine management practice as outlined by the National Research Council requirements [9]. All nutrients including water were supplied *ad libitum* to meet the 9 (1994).

Water and diet

Ordinary drinking fresh water used in this experiment, and the diet good grounded seeds with supplements vitamins and amino acids without anticoccidial drugs prepared in the Collage of Agriculture, Baghdad University. High quality garlic bulbs were used in this study purchased from local market, garlic powder added to the basal diet as 4g/kg. Fresh garlic bulb, sun-shade dried and then ground to obtain their powder [10].

Sonicated antigen and vaccine preparation

The sonication applied in Biotechnical Center in Baghdad University, the sporulated oocysts collected in potassium dichromate solution were washed 3-4 times by physiological saline solution (pH 7.2) and concentration to 5000-6000 per ml. The washed sporulated oocysts were subjected to ultra sonication by Soniprep150 (SONY Company) for 2 by 30 seconds in jacketed vessel with cool water. The inactivated vaccine was prepared from sonicated suspension by treating with 0.3 percent formalin (33% formaldehyde) for 96 hours at 37°C [8, 11] and stored at 4°C until use.

Phytohaemagglutinin (PHA) skin test

Pluck the feathers from one side of each wing web, clearing an area minimally large enough to accommodate the contacts of the calipers. The bare skin was swabbed with alcohol and 0.1 ml sonicated oocysts antigen (half dose of the prepared vaccine) as mitogen stimulation was injected intra-dermally into the patagium. The subsequent swelling was measured as an assay of *in vivo* T-cell mediated immune responsiveness [12]. Thickness measurement of the wing-web in the right wing was made to the nearest 0.05 mm with a micrometer immediately before and 24 – 48 h after injection.

Titers of antibodies

Preparation of SRBC and immunization of chickens: Titers were expressed as $\log_2$ of the reciprocal of the highest dilution showing visible agglutination or spreading of the RBCs in response to 0.1ml of a 1% suspension of SRBC antigen administered i.v. between 28 to 35 days of age and the plasma tested for SRBC antibody using the microtiter procedure. If the amount of antibody is insufficient to cause agglutination, the RBCs form a small, dense, round "button" at the bottom of the well [13].

Experimental design

A total of 150 newly hatched commercial broiler chicks "Cobb strain" was purchased from a local hatchery. Upon arrival, the chicks were divided randomly into six equal groups of 25 chicks each 1st and 2nd group's chicks were inoculated *in ovo* with 0.2 ml of sonicated oocyst at day 18 embryonated eggs and repeated by I/M injection at 14 days old. The 3rd and 4th group's chicks were receive 0.2 ml of sonicated oocyst by I/M injection at day one and repeated by same route at day 14. The first and third

group's feed basal diet while second, fourth and fifth groups feed by basal diet with garlic (4 g/kg) from day one to the end of experiment. The 6th group chicks remain as control. All groups are challenged by 50,000 viable sporulated oocysts of E. tenella at day 28. The PHA skin test and antibody titers against SRBC were done at 28 and 35 days old for cellar and humoral immunity response.

Results

The weight and somatic index of thymus of chickens during the experiment are shown in Tables 1 an increased in weight and somatic index was seen, those of birds in the non-immunized groups were lower ($P < 0.05$) than that of the immunized groups at day 35.

Table: 1 the thymus weight and index of broilers during the experimental period (mean±SD)

Group	Thymus weight		Thymus index	
	d 28	d 35	d 28	d 35
In ovo	2.794 ± 0.152 a	4.280 ± 0.150 a	0.208 ± 0.014 a	0.234 ± 0.010 a
In ovo +G	2.971 ± 0.187 a	4.440 ± 0.302 a	0.214 ± 0.014 a	0.239 ± 0.012 a
I/M	2.805 ± 0.297 a	4.509 ± 0.277 a	0.206 ± 0.013 a	0.248 ± 0.015 a
I/M +G	2.900 ± 0.253 a	4.564 ± 0.202 a	0.209 ± 0.018 a	0.245 ± 0.010 a
G	2.947 ± 0.151 a	3.694 ± 0.204 b	0.214 ± 0.010 a	0.215 ± 0.018 b
Control	2.800 ± 0.120 a	3.144 ± 0.242 c	0.203 ± 0.0.10 a	0.206 ± 0.012 b

a, b, c Values bearing similar superscript between column do not differ at ($P < 0.05$)

Similarly, those of the immunized groups were always the highest ($P < 0.05$) bursal weight and indices than those of non-immunized groups at day 35 (Table 2).

Table: 2 the bursa weight and index of broilers during the experimental period (mean±SD)

Group	Bursa weight		Bursa index	
	d 28	d 35	d 28	d 35
In ovo	1.656 ± 0.107 [a]	3.066±0.053[ab]	0.123 ± 0.010 [a]	0.167 ± 0.003 [a]
In ovo +G	1.669 ± 0.094 [a]	3.142 ± 0.109 [a]	0.120 ± 0.007 [a]	0.169 ± 0.006 [a]
I/M	1.502 ± 0.106 [a]	3.027 ± 0.126[ab]	0.111 ± 0.013 [a]	0.166 ± 0.006 [a]
I/M +G	1.574 ± 0.099 [a]	3.118 ± 0.141[ab]	0.113 ± 0.007 [a]	0.167 ± 0.008 [a]
G	1.573 ± 0.148 [a]	2.866 ± 0.100[bc]	0.114 ± 0.013 [a]	0.163 ± 0.008 [a]
Control	1.533 ± 0.100 [a]	2.450 ± 0.212[d]	0.111 ± 0.008 [a]	0.141 ± 0.011 [b]

[a, b, c, d] Values bearing similar superscript between column do not differ at ($P < 0.05$).

Likewise, those of the immunized groups were always the highest ($P < 0.05$) spleen weight and indices than those of non-immunized groups at day 35 (Table 3).

Table: 3 the spleen weight and index of broilers during the experimental period (mean±SD)

Group	Spleen weight		Spleen index	
	d 28	d 35	d 28	d 35
In ovo	1.310 ± 0.074 [a]	2.549 ± 0.141 [ab]	0.098 ± 0.007 [a]	0.139 ± 0.009 [a]
In ovo +G	1.296 ± 0.042 [a]	2.588 ± 0.187 [a]	0.093 ± 0.003 [a]	0.139 ± 0.010 [a]
I/M	1.276 ± 0.100 [a]	2.465 ± 0.101 [ab]	0.094 ± 0.011 [a]	0.134 ± 0.006 [ab]
I/M +G	1.298 ± 0.061 [a]	2.592 ± 0.223 [a]	0.093 ± 0.005 [a]	0.139 ± 0.011 [a]
G	1.258 ± 0.050 [a]	2.250 ± 0.178 [bc]	0.091 ± 0.005 [a]	0.128 ± 0.012 [abc]
Control	1.276 ± 0.060 [a]	1.998 ± 0.079 [c]	0.092 ± 0.003 [a]	0.115 ± 0.005 [c]

[a, b, c] Values bearing similar superscript between column do not differ at ($P < 0.05$) .

Table 4 shows the antibody titers of birds throughout the experimental period. In general, there is an increasing trend of antibody titers in all groups as time advances. At day 35 significantly higher ($P < 0.05$) titer were seen in the *in ovo* +G and I/M +G in compared with other groups.

Table: 4 the antibody titers (lg_2) of responders 7 days after inoculation with SRBC of broilers during the experimental period (mean±SD)

parameter	Groups	Before challenge	After challenge
		d 28	d 35
Ab to SRBCs	*In ovo*	3.20 ± 1.095 ^{ab}	7.90 ± 1.19^{b}
	In ovo +G	5.60 ± 2.190 ^{a}	10.60 ± 1.57^{a}
	I/M	3.80 ± 1.085 ^{ab}	7.80 ± 1.40^{b}
	I/M +G	5.60 ± 2.190 ^{a}	11.20 ± 1.95^{a}
	G	3.00 ± 1.195 ^{ab}	5.20 ± 1.68^{c}
	Control	2.40 ± 0.894 ^{b}	2.80 ± 1.95^{d}

$^{a, b, c, d}$ Values bearing similar superscript between column do not differ at (P<0.05)

Non-immunized groups have resulted in less responsiveness of chickens to PHA skin test at days 28 and 35 (Table 5, 6). No significant changes were seen in the PHA skin test during the pre-injection period. At post injection, highest (P < 0.05) change was seen in the immunized groups. Marked suppression of PHA response was in the control and G groups which were the lowest (P < 0.05) at day 35.

Table: 5 the Phytohaemagglutinin (PHA) skin test at 28 day of broilers during the experimental period (mean±SD)

Groups	Pre 24 h	d 28		d 28	
		Post 24 h	Dif 24 h	Post 48 h	Dif 48 h
In ovo	0.884± 0.026 ^{a}	1.734 ± 0.118 ^{ab}	0.850±0.097 ^{ab}	1.690±0.095 ^{ab}	0.806 ± 0.096 ^{ab}
In ovo +G	0.922 ± 0.069 ^{a}	1.916 ± 0.086 ^{a}	0.994±0.072 ^{a}	1.858±0.081 ^{a}	0.936 ± 0.087 ^{a}
I/M	0.932 ± 0.058 ^{a}	1.846 ± 0.122 ^{ab}	0.914±0.068 ^{ab}	1.774±0.091 ^{ab}	0.842 ± 0.035 ^{ab}
I/M +G	0.906 ± 0.049 ^{a}	1.876 ± 0.113 ^{ab}	0.970±0.088 ^{ab}	1.786±0.104 ^{ab}	0.880 ± 0.083 ^{ab}
G	0.886 ± 0.047 ^{a}	1.732 ± 0.175 ^{ab}	0.846±0.208 ^{ab}	1.704±0.176 ^{ab}	0.818 ± 0.217 ^{ab}
Control	0.906 ± 0.090 ^{a}	1.676 ± 0.079 ^{b}	0.770±0.078 ^{b}	1.606±0.071^{b}	0.700 ± 0.073 ^{b}

$^{a, b}$ Values bearing similar superscript between column do not differ at (P<0.05).

Table: 6 the Phytohaemagglutinin (PHA) skin test at 35 day of broilers during the experimental period (mean±SD)

Groups	Pre 24 h	d 35		d 35	
		Post 24 h	Dif 24 h	Post 48 h	Dif 48 h
In ovo	0.940± 0.032 a	1.772 ± 0.043 ab	0.832 ± 0.040 ab	1.718 ± 0.029 ab	0.778 ± 0.035 ab
In ovo +G	0.976± 0.041 a	1.888 ± 0.062 a	0.912 ± 0.090 a	1.800 ± 0.047 a	0.824 ± 0.076 a
I/M	0.968± 0.083 a	1.812 ± 0.077 ab	0.844 ± 0.024 a	1.746 ± 0.086 a	0.778 ± 0.037 ab
I/M +G	0.970± 0.081 a	1.872 ± 0.059 a	0.902 ± 0.120 a	1.722 ± 0.110 a	0.752 ± 0.170 ab
G	0.884± 0.026 a	1.696 ± 0.087 bc	0.812 ± 0.106 abc	1.686 ± 0.093 bc	0.802 ± 0.110 ab
Control	0.984± 0.087 a	1.626 ± 0.057 c	0.642 ± 0.077 c	1.624 ± 0.125 c	0.640 ± 0.103 ab

a, b, c Values bearing similar superscript between column do not differ at ($P<0.05$).

Discussion

Eimeria tenella is one of the most common pathogenic coccidia causing a severe problem for the poultry industry [4]. E. tenella controlled mostly on the use of chemo-prophylaxis or conventional vaccines [6].

In this study the sonicated sporulated oocysts used as antigen to stimulating the immune response against E. tenella in broiler chicks and this gave a successful result to protect the broilers against felid coccidiosis strain due to stimulate superior immune response (8). Over years, there has been many researches concern about the immune response to coccidiosis in poultry. Chickens infected with Eimeria species produce parasite-specific antibodies that are present in the systemic circulation and mucosal secretions [4, 14].

The *in ovo* inoculation in 18 days old embryos typically and perfectly to stimulate the immunity against coccidiosis and used in other viral diseases like Marek's disease, ND and Gumboro disease [15]. Also, the injection in 1 day old chicks proved their ability to stimulating the immune system [16]. The effectiveness of vaccination in the generation of immunity and protection against subsequent Eimeria infection has been well documented [17]. Our results revealed that there were no statistically differences were detected in the means of parameters and immune response between immunized and non-immunized groups at d 28 (before challenge), this proved that the dose of antigen not effected on the chicks parameters. After challenge at d 35, the statistical analysis showed that (tables 1, 2, and 3) the immunized groups especially the immunized plus garlic have high lymphoid organ weight and their indices. The lymphoid organs are useful indicators of immunological status [18]. The lower lymphoid organs

weight and its associated lesions in the non-immunized groups signified low protection [(19)] invoked by the instilled E. tenella [(20)].

Birds in immunized groups (Table 4) revealed high protection rate against E. tenella was correlated with high detectable levels of antibody titers against SRBC during the challenge experiment. That means immunized groups with whole antigens (sonicated oocyst), may develop a significant humoral immune response. Overall, immunization groups especially the immunized plus garlic conferred higher level of protection compared to non-immunized groups. Coccidian infestation significantly and negatively affected the capacity of the birds to respond against the SRBC injection [(21)]. Also, it has been reported earlier that antibodies do play a role in protective immunity against Eimeria [(14)].

However, it is believed that cell-mediated immunity has a far greater role in protection against coccidial parasites than humoral immunity, but both parts are important for birds to acquire complete protective immunity [(22)]. The *in ovo* inoculation or intramuscular injection of the sonicated sporulated oocysts of E. tenella results in massive infiltration of macrophages, granulocytes and lymphocytes, macrophages and lymphocytes are the source of cytokine production in the intestine during Eimeria infection and thereby modulate the immune response [(4, 23)]. Another important finding is that the slower spatial peak PHA skin response in the non-immunzed groups (Table 5, 6) are possibly due low immune response in compared with immunized groups which have highest ($P < 0.05$) change in PHA skin response. Cell-mediated responses are an integral part of protective immunity as shown in experiments where T-cell depleted animals were unable to resist primary or secondary challenge infections [(4, 24)]. Garlic has several beneficial effects on both human and animals having antimicrobial, antioxidant as well as antiparasitic properties [(1)].

In conclusion immunization broilers chicks with a sonicated oocyst vaccine is an effective tool for the generation of protective immunity against field strain of E. tenella following challenge and garlic supplement in the diet of poultry this antigen increases this protection and eliminates the disease in the poultry.

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