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Effects of γ-Irradiation from Cobalt-60 on Immunogenicity of *Eimeria tenella*

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Cobalt-60 γ-선 조사가 *Eimeria tenella*의 면역원성에 미치는 영향

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초록 : 우리나라 육계산업을 저해하는 중요한 질병중의 하나가 닭의 맹장콕시듐증이다. 그 병원체는 *Eimeria tenella*로서 병아리에 감염하면 증체저하와 폐사율이 높기 때문에 양계농가에 커다란 경제적 손실을 준다. 닭의 맹장콕시듐증을 효과적으로 관리하기 위하여 몇가지 예방백신의 개발을 시도하여 왔으며 근래에는 재조합 DNA기법을 이용, *Escherichia coli*를 매체로 제조한 항원으로 닭에 방어력을 부여시키고자 하는 방법과 조기발육형원충의 작출법에 의하여 생산된 약독원충으로 면역을 부여하는 방법 등에 관한 연구를 진행하고 있으나 아직 만족할만한 성과를 이루지 못하였다. 본 시험에서는 약독원충을 이용하여 닭 맹장콕시듐증에 대한 저항성을 부여하고자 하였다. 콕시듐증의 병원체인 *E tenella*에 Cobalt-60 감마선을 조사한 원충 또는 그 원충을 SPF병아리에 계대증식시켜 회수한 오오시스트로 병아리에 접종하고 체내발육상태와 병원성을 조사하였으며, 면역접종한 후 병원성이 강한 원충의 공격접종에 대한 방어효과를 관찰한 성적은 다음과 같다. 원충감염 닭의 생잔율, 증체량, 혈변도 및 병변도 등에 대한 효과에서 감마선 100 Gy로 조사한 *E tenella*를 접종한 것이 가장 좋았으며, 접종량은 1대 계대한 오오시스트 약 10,000개를 접종한 것이 좋았다. 면역접종한 후 공격접종한 결과 계대원충접종군은 혈변도 및 병변도가 낮게 나타났으며, 최대 오오시스트 배설수(OPG)는 103,900으로서 공격접종대조군의 1,658,900에 비하여 현저하게 낮았으나 적혈구용적율은 계대원충접종군이 가장 높았다. 공격접종한 닭의 1주 및 2주 후의 증체량은 계대원충접종군이 2.6g 및 155.4g으로서 공격접종대조군의 −85.8g 및 63.6g 보다 좋았으며, 사료요구율도 계대원충접종군이 3.28로서 공격접종대조군의 5.60 보다 낮았다. 생잔율은 계대원충접종군이 100%로서 공격접종대조군의 87.0%와 비교하면 방어효과가 있었다. 계대원충접종군의 항콕시듐 지수는 70.5로서 공격접종대조군의 −81.9에 비하면 현저하게 높았다. 그러므로 맹장콕시듐 원충은 감마선 조사가 병원성을 저하시키고 계대수가 증가할수록 병원성은 약간 증가하지만 공격접종에 대한 방어효과가 높아지는 것을 볼 수 있었다. 이는 병원성이 있는 원충이 면역원성도 좋으며 앞으로 면역원성이 유지되며 병원성이 없는 원충의 선발이 필요할 것으로 사료된다.

Key words : *Eimeria tenella*, Cobalt-60, γ-irradiation, the 1st and the 3rd progeny, immunogenicity, anticoccidial index.

Introduction

For the control of avian coccidiosis, various anticoccidial drugs were developed and used. Among the anticoccidial drugs, polyether ionphorous antibiotics are mainly used.[10] Although those durgs are very effective for the protection of avian coccidiosis, some problems are recognized in the use of the anticoccidial drugs. One of those is emergency of drug resistant strains according to the continuous use or misuse of the drugs. Then the development of new drugs and rotation programs in the use of the drugs were demanded. In other hand, as the anticoccidial feed additives are ascended expenditure of poultry products, the drugs or antibiotics would be retained as residue in poultry product, and these residual drugs may have a demerit influence to the final consumer the human being. Therefore, the enhancement of the regulations for the anticoccidial drugs should be strengthen gradually. For the safety and economic control of avian coccidiosis, we are concerned about the development of vaccine.[24] Dickinson et. al. carried out the initial study of coccidial immunity with *E tenella, E acervulina, E maxima, E necatrix* and *E praecox*[5] Rose also reported about immunity and the prospects for prophylactic immunization[18] and Rose and Hesketh studied the immunity to coccidiosis[19]. Long and Millard and Long et al. studied the immunization of young chicken in farms.[12,14]. The possibility of vaccination to the avian coccidiosis was also studied by other scientists.[1~3,16,21] In recent, the development of avian coccidial vaccine is accompanied two methods ; the method of genetic engineering technology and avirulent coccidial oocysts. Danforth and Augustine carried out the method of genetic engineering technology with recombinant DNA technique by *Escherichia coli*, but this immunogen was estimated to have non-effect. The other was the use of avirulent coccidial oocysts with the precocious line and the application of γ-irradiation methodology. Many scientists studied on the precocious line to immunogen of avian coccidiosis.[8,9,11,15,20~22,25,27,28] This method is required of time and labor to make and preserve the passaged oocysts and possibility of interspecific contamination. Fitzerald initiated the method of use of γ-irradiated oocysts with bovine coccidia, such as *E bovis*.[7] Singh and Gill also reported the effect of γ-irradiation to *E necatrix*.[23] Recently, Djajusman et al. reported on the implementation of coccidia radiovaccine (*Eimeria tenella*) in east-Jawa, south Sulawesi and north of Sumatera.[6] To investigate the pathogenicity and immunogenicity of coccidia, they only used with γ-irradiated oocysts. The method used γ-irradiated oocysts was not satisfied because of the problems to preserve of immunogenicity and mass production. In these experiments, for making avirulent coccidia, we treated *E tenella* by γ-irradiation from ^{60}Co and passaged them in SPF chicken. To make use of passaged oocyst to coccidial immunogen, their immunogenicity in chickens challenged with virulent *E tenella after* immunization was investigated.

Materials and Methods

Eimeria tenella. A reference stock of *E tenella* was provided from the Protozoology Laboratory of the USDA and the oocysts propagated in the specific pathogen free(SPF) chickens were used in this experiment on the immunogenicity. The oocysts were preserved in 2% potassium dichromate solution to be sporulated and preserved in a refreezerator(2~5℃)until used. It was used to investigate γ-irradiated dose from ^{60}Co, numbers of immunization, their immunogenicity by comparison of the survival rate, body weight gains, feed efficacy, blood in feces, lesion score or anticoccidial index and to challenge.

Experimental animals : Three hundred and fifty SPF chickens of the same numbers of sex of white colored layer Lohmann were used as the experimental animals. They were reared 10 chickens per group on cage. One hundred and fifty 9 week-old chickens are used to determine proper dose of ^{60}Co γ-ray and immune dose. Two hundred 6 week-old chickens were used to investigate the immunogenicity of γ-irradiated *E tenella* and its progeny.

Experimental feed : Experimental feed is manufactured for early broiler without anticoccidial feed additives at Kun Kuk feed manufacture company in Kun Kuk University and its composition followed the commercial chicken production manual.[17] Feed and water were administered at liberty.

Dose of gamma irradiation from ^{60}Co to oocysts of *E tenella* and determination of irradiated dose : Sporulated oocysts of *E tenella* were irradiated with γ-ray from ^{60}Co

according to the method suggested by the previous report[29]. Ten 9 week-old birds per each group were challenged with 1×10^5 oocysts of virulent *E tenella* after immunization with 1×10^5 oocysts by 100, 200, 300 and 600 Gy γ-irradiated from ^{60}Co. Two groups of 10 birds were control and challenged control groups. All birds of each group were slaughtered to examine lesion score at 1 week after challenge. Body weight gain of each group was investigated at the 1st week after challenge. Blood in feces, lesion score and excreted oocysts per gram in feces were investigated at the 1st week after challenge.

Determination of immune dose : Ten 9 week-old birds per each group were challenged with 1×10^5 oocysts of virulent *E tenella* after immunization with 1×10^2, 1×10^3, 1×10^4, and 1×10^5 oocysts of the 1st and the 3rd passaged *E tenella* irradiated with 100 Gy γ-ray from ^{60}Co, respectively. Two groups of 10 birds were control and challenged control groups, respectively. All birds of each group were slaughtered to examine lesion score at the 1st week after challenge. The body weight gain of each group was investigated at the 1st week after challenge. Blood in feces, lesion score and excreted oocysts per gram in feces were investigated as the same as previous methods.

Degrees of the immunogenicity of experimental *Eimeria tenella* :Ten 6 week-old birds per each group were challenged with 1×10^5 oocysts of virulent *E tenella* after immunization with 1×10^4 oocysts of non-passaged, the 1st and the 3rd passaged *E tenella* irradiated with 100 Gy γ-ray from ^{60}Co, respectively. Two groups of 10 birds were control and challenged control groups. The items of investigation were as follows ; blood in feces, lesion score, packed cell volume(PCV) of erythrocysts, number of excreted oocysts in feces, body weight gains, feed conversion ratios, and anticoccidial index. All items were investigated according to the method suggested by the previous report.[29]

Statistical analysis : Results of body weight gain, feed conversion ratio, lesion score, PCV of erythrocytes and anticoccidial index were analyzed by Tukey's studentized range test.

Results

Immunogenicity of *E tenella* treated with various doses of γ-ray from ^{60}Co : The immunogenicity of the group challenged with 1×10^5 oocysts of virulent *E tenella* after immunization with 1×10^5 oocysts of 100 Gy γ-irradiated *E tenella* was the highest of any other groups by comparison of survival rate, body weight gains, blood in feces, lesion score, and excreted oocysts in feces(Table 1).

Immunogenicity of the 1st progeny of *E tenella* irradiated with 100 Gy γ-ray from ^{60}Co : The immunogenicity of the group challenged with 1×10^5 oocysts of virulent *E tenella* after immunization with 1×10^4 and 1×10^5 oocysts of the 1st passaged *E tenella* was the highest of any other groups by comparison of survival rates, body weight gains, blood in feces and lesion scores(Table 2).

Immunogenicity of the 3rd progeny of *E tenella* irradiated with 10 Gy γ-ray from ^{60}Co : The immunogenicity of the group challenged with 1×10^5 oocysts of virulent *E tenella* after immunization with 1×10^4 and 1×10^5 oocysts of the 3rd passaged *E tenella* was the highest of any other groups by comparisons of survival rates, body weight gains, blood in feces and lesion scores(Table 3).

Immunogenicity with oocysts of 100 Gy γ-irradiated *E tenella* from ^{60}Co and its progeny : In the preliminary

Table 1. The immunogenicity of *E tenella* treated with various dose of γ-ray from ^{60}Co

Group	Survival rate	Body weight gain	Blood in feces	Lesion score	OPG
	1 week	1 week	1 week	1 week	1 week
I	100.0	140.0	−	0.00	0
II	40.0	-60.4	+−++	4.00	1,658,000
III	100.0	-52.0	−+	2.00	45,400
IV	80.0	-73.5	+++	2.50	126,300
V	60.0	-77.7	+++	3.40	325,000
VI	70.0	-85.0	+++	4.00	428,600

group I : control group without immunization and challenge, group II : control group challenged with 1×10^6 of oocysts, group III, IV, V and VI : experimental groups challenged with 1×10^5 of oocysts after 2 weeks of an initial administration of 1×10^5 of oocysts irradiated with 100, 200, 300 and 600 Gy of γ-ray from ^{60}Co, respectively, OPG : oocysts per gram of feces, * : week(weeks) after the 1st inoculum, ** : week after challenge, − : normal, + : mild, ++:moderate, +++ : severe, ++++ : heavy.

Table 2. The immunogenicity of the 1st progeny of *E tenella* irradiated with 100 Gy γ-ray from ^{60}Co

Group	Number of oocysts (immunized)	Survival rate	Body weight gain	Blood in feces	Lesion score
		1 week	1 week	1 week	1 week
I	–	100.0	85.0	–	0.00
II	–	77.8	-11.0	++++	4.00
III	1×10^2	100.0	23.3	+++	3.25
IV	1×10^3	90.0	22.6	++	2.80
V	1×10^4	100.0	36.0	+	2.33
VI	1×10^5	100.0	46.5	+	2.20

group I : control group without immunization and challenge, group II : control group challenged with 1×10^5 of oocysts, group III, IV, V and VI : experimental groups challenged with 1×10^5 of oocysts after 2 weeks of an initial administration of the 1st progeny of *E tenella* irradiated with 100 Gy of γ-ray from ^{60}Co, respectively, – : nomal, + : mild, ++ : moderate, +++ : severe, ++++ : heavy.

Table 3. The immunogenicity of the 1st 3rd progeny of *E tenella* irradiated with 100 Gy γ-ray from ^{60}Co

Group	Number of oocysts (immunized)	Survival rate	Body weight gain	Blood in feces	Lesion score
		1 week	1 week	1 week	1 week
I	–	100.0	83.0	–	0.00
II	–	77.8	-21.0	++++	4.00
III	1×10^2	100.0	28.5	+++	3.00
IV	1×10^3	90.0	32.5	++	2.50
V	1×10^4	100.0	45.0	+	2.00
VI	1×10^5	100.0	54.0	+	1.50

group I : control group without immunization and challenge, group II : control group challenged with 1×10^5 of oocysts, group III, IV, V and VI : experimental groups challenged with 1×10^5 of oocysts after 2 weeks of an initial administration of the 3rd progeny of *E tenella* irradiated with 100 Gy of γ-ray from ^{60}Co, respectively, – : nomal, + : mild, ++ : moderate, +++ : severs, ++++ : heavy.

Table 4. Survival rate after challenge to the chicken immunized with 1×10^4 oocyststs of *E tenella* irradiated with 100 Gy of γ-ray from ^{60}Co and its progeny

Group	Survival rate(%)
C_0	100.0 ± 0.0^a
C_1	87.0 ± 6.0^b
P_0	95.0 ± 5.8^{ab}
P_1	100.0 ± 0.0^a
P^3	100.0 ± 0.0^a

C_0 : control group without immunization and challenge, C_1 : challenged control group, P_0 : treatment group immunized with γ-irradiated oocysts, P_1 : treatment group immunized with the 1st progeny of γ-irradiated oocysts, P_3 : treatment group immunized with the 3rd progeny of γ-irradiated oocysts

a, and b values with different superscripts differ significantly($p < 0.05$).

trials, the numbers of γ-irradiated *E tenella* and its progeny were estimated 1×10^4 oocysts by comparison of immunogenicity. So we immunized with 1×10^4 oocysts of 100 Gy γ-irradiated *E tenella* and its progeny to each bird and challenged with 1×10^5 oocysts of virulent *E tenella*. The results of challenge trials were as follows ;

Survival rate : The survial rates of the groups challenged with 1×10^5 oocysts of virulent *E tenella* after immunization with 1×10^4 oocysts of the 1st and the 3rd passaged *E tenella* were higher than that of the challenged control group(Table 4).

Blood in feces, lesion score and packed cell volume : The immunogenicity of group challenged with 1×10^5 oocysts of virulent *E tenella* after immunization with 1×10^4 oocysts of the 3rd passeged *E tenella* was the mildest of any other groups by comparison of blood in feces, lesion score and PCV. The immunogenicity of the group challenged with 1×10^5 oocysts of virullnt *E tenella* after immunization with 1×10^4 oocysts of the 1st passaged *E tenella* was milder than those of γ-irradiated *E tenella* and challenged control groups(Table 5).

Shedding of oocysts. : Shedding of oocysts in groups challenged with 1×10^5 oocysts of virulent *E tenella* after immunization with 1×10^4 oocysts of the 3rd passaged *E tenella* was smallest of any other groups. Shedding of oocysts in group challenged with 1×10^5 oocysts of virulent *E tenella* after immunization with 1×10^4 oocysts of the 1st passaged *E tenella* was smaller than those of γ-irradi-

Table 5. The immunogenicity after challenge to the chicken immunized with 1×10^4 oocysts of *E tnenella* irradiated with 100 Gy of γ-ray from ^{60}Co and its progeny

Group	Blood in feces					Lesion score	Packed cell volume
	4	5	6	7	8(days)		
C_0	−	−	−	−	−	0.0 ± 0.0^a	30.2 ± 0.7^a
C_1	+	++++	+++	−	−	4.0 ± 0.0^d	20.7 ± 1.5^c
P_0	+	+++	++	−	−	3.5 ± 0.1^c	25.4 ± 0.9^b
P_1	−	+	+	−	−	2.4 ± 0.1^b	31.2 ± 1.2^a
P_3	−	−	+	−	−	1.6 ± 0.3^b	30.0 ± 0.6^a

C_0 : control group without immunization and challenge, C_1 : challenged control group, P_0 : treatment group immunized with γ-irradiated oocysts, P_1 : treatment group immunized with the 1st progeny of γ-irradiated oocysts, P_3 : treatment group immunized with the 3rd progeny of γ-irradiated oocysts. − : normal, + : mild, ++ : moderats, +++ : severe, ++++ : heavy. a, b, c and d values with different superscribpts differ significantly($p<0.05$).

Table 6. Number of oocysts per gram of feces after challenge to the chicken immunized with 1×10^4 oocysts of *E tenella* irradiated with 100 Gy γ-ray from ^{60}Co and its progeny

Group	Shedding of oocysts(OPG×1000)						
	5	6	7	8	9	10	11(days)
C_1	0.0	45.1^b	416.5^b	1658.0^b	329.4^b	189.6^a	34.8^a
	±0.0	±23.4	±293.0	±1198.4	±238.6	±196.9	±17.3
P_0	0.0	27.6^{ab}	187.0^{ab}	651.4^{ab}	222.4^b	63.3^a	13.1^a
	±0.0	±18.8	±39.9	±521.4	±86.2	±21.7	±7.2
P_1	0.0	7.5^a	103.9^{ab}	13.8^a	13.3^a	8.8^a	12.3^a
	±0.0	±7.2	±37.4	±9.3	±6.9	±3.8	±11.0
P_3	0.0	25.8^{ab}	25.5^a	8.9	24.8^a	3.5^a	4.5^a
	±0.0	±18.1	±6.2	±3.7	±18.4	±4.5	±3.7

C_1 : challenged control group, P_0 : treatment group immunized with γ-irradiated oocysts, P_1 : treatment group immunized with the 1st progeny of γ-irradiated oocysts, P_3 : treatment group immunized with the 3rd progeny of γ-irradiated oocysts.
a and b values with different superscripts differ significantly($p<0.05$)

Table 7. Body weight gain and feed conversion ration after challenge to the chicken immunized with oocysts of *E tenella* irradiated with 100 Gy γ-ray from ^{60}Co and its progeny

Group	Body weight gain(g)		Feed conversion ratio
	1 week	2 weeks	2 weeks
C_0	98.7 ± 13.0^a	195.0 ± 20.0^a	2.70 ± 0.13^a
	$(100.0 \pm 0.0)^*$	(100.0 ± 0.0)	
C_1	-85.8 ± 18.0^d	63.6 ± 12.7^c	5.60 ± 1.66^b
	(-89.2 ± 27.1)	(32.6 ± 5.5)	
P_0	-25.3 ± 4.5^c	130.5 ± 4.5^b	3.80 ± 0.05^{ab}
	(-26.2 ± 7.7)	(67.3 ± 6.5)	
P_1	2.6 ± 16.5^{bc}	155.4 ± 7.4^b	3.28 ± 0.17^a
	(4.0 ± 17.6)	(80.3 ± 8.8)	
P^3	11.6 ± 18.3^{bc}	168.9 ± 19.7^b	2.96 ± 0.28^a
	(13.9 ± 22.3)	(87.5 ± 15.7)	

C_0 : control group without immunization and challenge, C_1 : challenged control group, P_0 : treatment group immunized with γ-irradiated oocysts, P_1 : treatment group immunized with the 1st progeny of γ-irradiated oocysts, P_3 : treatment group immunized with the 3rd progeny of γ-irrandiated oocysts. ( )* : rate of relative body weight gain=body weight gain of treatment group/body weight gain of control group.
a, b, c and d values with different superscripts differ significantly($p<0.05$).

ated *E tenella* and challenged control groups(Table 6).

Body weight gain and feed conversion ratio : The body weight gain of the group challenged with 1×10^5 oocysts of virulent *E tenella* after immunization with 1×10^4 oocysts of the 3rd passaged *E tenella* was the greatest of any other groups except control group. The body

Table 8. Anti-coccidial index after challenge with non-irradiated *E tenella* to chickens immunized with 1×10^4 oocysts of *E tenella* irradiated with 100 Gy γ-ray from ^{60}Co and its progeny

Group	Anti-coccidial index(M±SD)
C_0	200.0 ± 0.0^a
C_1	-81.9 ± 32.1^d
P^0	16.3 ± 11.7^c
P^1	70.5 ± 12.4^b
P^3	93.9 ± 25.3^b

C_0 : control group without immunization and challenge, C_1 : challenged control group, P_0 : treatment group immunized with γ-irradiated oocysts, P_1 : treatment group immunized with the 1st progeny of γ-irradiated oocysts, P_3 : treatment group immunized with the 3rd progeny of γ-irradiated oocysts.
a, b, c and d values with different superscripts differ significantly($p<0.05$).

weight gain of the group challenged with 1×10^5 oocysts of virulent *E tenella* after immunization with 1×10^4 oocysts of the 1st passaged *E tenella* was more than those of γ-irradiated *E tenella* and challenged control groups. The feed conversion ratio of the group challenged with 1×10^5 oocysts of virulent *E tenella* after immunization with 1×10^5 oocysts of the 3rd passaged *E tenella* was the lowest of any other groups except control group. The feed conversion ratios of the groups challenged with 1×10^5 oocysts of virulent *E tenella* after immunization with 1×10^4 oocysts of the 1st passaged and γ-irradiated *E tenella* were more than that of challenged control group(Table 7).

Anti-coccidial index : The anticoccidial index of the group challenged with 1×10^5 oocysts of virulent *E tenella* after immunization with 1×10^4 oocysts of the 3rd passaged *E tenella* was the greatest of any other groups except control group. That of the group challenged with 1×10^5 oocysts of virulent *E tenella* after immunization with 1×10^4 oocysts of the 1st passaged *E tenella* was more than those of γ-irradiated *E tenella* and challenged control groups(Table 8).

Discussion

To develop proper immunogen for avian coccidiosis in this experiment, we irradiated γ-ray from ^{60}Co to coccidial oocysts and passaged them to SPF chicken to be weak pathogenicity of *E tenella*. Their immunogenicity after challenge with virulent *E tenella* to the chickens immunized with γ-irradiated *E tenella* and its progeny were investigated by comparison of body weight gains, blood in feces, lesion scores, number of excreted oocysts and PCV of erythrocytes.

In the experiment of effects of ionizing irradiation from ^{60}Co on oocysts of *Eimeria bovis*, Fitzgerald reported that 100 Gy γ-ray from ^{60}Co was the most immune of any other treated groups by comparison of blood in feces and number of excreted oocysts between 100 and 2,000 Gy γ-ray from ^{60}Co.[7] Also, Singh and Gill reported that 100 to 200 Gy γ-ray from Cobalt-60 was more immune than the other irradiation doses.[23] In this experiment, The immunogenicity of 100 Gy γ-irradiated *E tenella* groups was higher than those of the other γ-irradiation dose groups by comparison of survival rate, body weight gains, blood in feces, lesion score, and excreted oocysts. The immunogenicity of groups immunized with 1×10^4 and 1×10^5 oocysts of the 1st and the 3rd passaged *E tenella* was the higher than those of the other inoculum size groups.

Long et al. reported that the groups immunized with 200~500 oocysts of *E tenella* imporved 27.3~49.7% of survival rate, about 0.4 points of feed conversion ratios and 1.2~2.5 points of lesion score in comparison to challenged control groups.[13] McDonald et. al. reported that the groups immunized with 5,000~50,000 oocysts of precocious *E tenella* improved 18~50% of survival rate and 69~117g of body weight gains in comparison to challenged control groups.[15] Long and Johnson reported that the groups immunized with 1,000~5,000 oocysts of precocious *E tenella* improved 0.4~1.4 points of lesion score and 23~103g of body weight gains in comparison to challenged control groups.[11] Djajusma et al. reported that the groups immunized with 1×10^5 oocysts of *E tenella* radiovacine improved 10~50% of survival rate in comparison to challenged control groups.[6] Therefore, they estimated that the immunization of the precocious line of *E tenella* had effect to cecal coccidiosis on condition of improvement of survival rates, body weight gains and lesion scores.

In the preliminary trials, the numbers of γ-irradiated *E tenella* and its progeny were estimated 1×10^4 oocysts by comparison of immunogenicity. So we immunized with 1×10^4 oocysts of γ-irradiated *E tenella* and its progeny to each bird and challegned with 1×10^5 oocysts of virulent *E tenella*. The survival rates of the groups immunized $1\times$

10^4 oocysts of the 1st and the 3rd passaged *E tenella* were higher than that of the challenged control group. The immunogenicity of group immunized with the 3rd passeged *E tenella* was the highest of any other groups by comparison of blood in feces, lesion score, packed cell volume and excreted oocysts in feces. The immunogenicity of group immunized with the 1st passaged *E tenella* was higher than those of γ-irradiated *E tenella* and challenged control groups. The body weight gain and the feed conversion ratio of group immunized with 3rd passaged *E tenella* were the greatest of any other except control group. The body weight gains and the feed conversion ratios of groups immunized with the 1st and the 3rd passaged *E tenella* were more than those of γ-irradiated *E tenella* and challenged control groups.

To compare immunogenicity between γ-irradiated *E tenella* and its progeny, we introduced anticoccidial index to evaluate anticoccidial drugs. The anticoccidial index of group challenged with 1×10^5 oocysts of virulent *E tenella* after immunization with 1×10^4 oocysts of the 3rd passaged *E tenella* was the greatest of any other groups except control group.

We thought that the immunogenicity of γ-irradiated *E tenella* would be increased according to increase the number of generation passaged in chicken. That might be because of increasing the pathogenicity of γ-irradiated *E tenella* according to increase the number of generation passaged in chicken.

Summary

To reveal the immunogenicity of γ-irradiated *E tenella* and its progeny, a series of experiments on the effects of Cobalt-to γ-irradiation was performed. The SPF chickens inoculated with diffenrt doses of inoculum were challenged with 1×10^5 oocysts of virulent *E tenella*. The levels of 100 Gy γ-irradiation from ^{60}Co and of inoculum with 1×10^4 oocysts were recognized as proper as immunogen by comparison of survival rates, body weight gains, blood in feces and lesion scores in the chickens. In these trials of challenge with virulent *E tenella* after inoculation with 1×10^4 oocysts of the γ-irradiated *E tenella* and its progeny, the survival rates of the chickens challenged with the virulent *E tenella* after immunization with the 1st and the 3rd progeny groups of γ-irradiated *E tenella* oocysts were higher(100%) than that(87.0%) of the challenged control group. The signs of blood in feces and the lesion scores were seen markedly lower with the ourput of the smaller number of oocysts, i.e. OPG 103,900 and 25,800 in the groups of the 1st and the 3rd progeny, respectively, than those(OPG 1,658,900) of the challenged control group. The body weight gains of the 1st and the 3rd progeny groups, the 1st week and the 2nd week after challenge, were higher (2.6g and 155.4g, 11.6g and 168.9g respectively) than those(-85.8g and 63.6g, respectively) of the challenged control group, and the feed conversion ratios(FCR 3.28 and 2.96) of the 1st and the 3rd progeny groups were lower than that(FCR 5.60) of the groups challenged control group. The anticoccidial indices(70.5 and 93.9) of the groups challenged with the virulent oocysts of *E tenella* after immunization with the 1st and the 3rd progeny of the γ-irradiated *E tenella* were significantly higher than that (ACI −81.9) of the challenged control group. It was thought that the immunogenicity of γ-irradiated *E tenella* would be increase according to increase the number of generation passaged in chicken. That might be because of increasing the pathogenicity of γ-irradiated *E tenella* according to increase the number of generation passaged in chicken.

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