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## Protective Immunity of *Eimeria acervulina* Oocysts Protein against Intestinal Coccidiosis

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### Abstract

The study was carried out to assesst the protective immune efficacy of *E. acervulina* oocyst protein against homologous challenge in chickens. Immunization was applied on 14th and 18th day of age subcutaneously with that protein at dose of 50 µg per chicken. Immunized chickens were challenged at 32nd day of age, demonstrated that oocyst protein could provide protection around 62% when compared with unimmunized chickens, while parasite development in intestine asses in histological section revealed decreased proliferation. The study results demonstrated that relatively sufficient protection against coccidia by the use of *E. Acervulina* oocyst protein as vaccine in broilers.

**Key words :** *E. acervulina*, oocyst protein, protective immunity

Coccidiosis in poultry is caused by protozoan parasites of the genus *Eimeria*. Coccidiosis is a self limiting infection; primary infections can stimulate solid immunity to homologous challenges (Allen and Fetterer, 2002). An immunological approach is considered more important since a live vaccine containing a virulent or attenuated *Eimeria* strain is available but its use is limited in the poultry industry because of its high cost. Additionally these vaccines consist of several *Eimeria* species makes them labouries as well as higher cost of production. Also, these types of vaccine may revert back to a pathogenic form (Sharman *et al.*, 2010). Therefore, research efforts undertaken in the development of an anticoccidial protein vaccine consisting of antigens as an alternative to live vaccines. One of protein exploration can be

done to oocysts to induce protective immunity. The present study used the oocyst extract as a vaccine to protect broilers from *E. acervulina* parasite.

### Materials and Methods

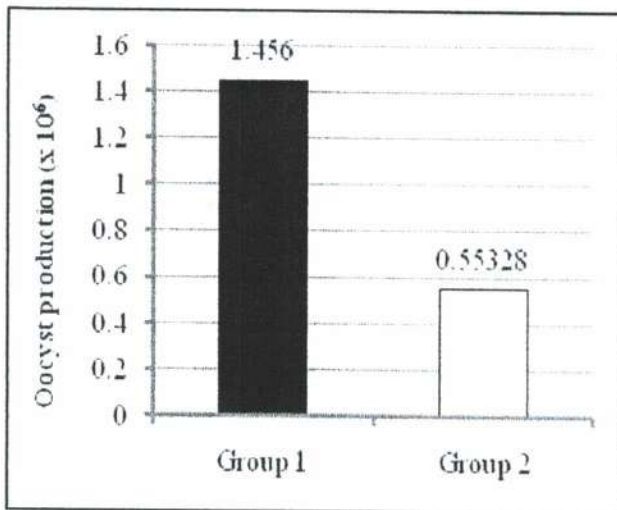
A total number of 12 day old broiler chicks were divided into 2 groups, each group containing six chicks. Group 1 was immunized subcutaneously in the neck with two doses: first dose at 4th day of age with Freund's Complete Adjuvant (FCA) emulsified in PBS and booster dose was given at 18th day of age with Freund's Incomplete Adjuvant (FICA) emulsified in PBS. Group 2 was immunized subcutaneously on the neck with two doses: first dose at 4th day of age with 50 µg antigen (oocyst protein) emulsified in FCA and booster dose was given at 18th day of age with 50 µg antigen emulsified in FICA. After two weeks of last immunization the both groups were challenged orally  $1 \times 10^4$  of virulent *E. acervulina*. The protective efficacy against homologous challenge in chickens evaluated by using protein of *E. acervulina* oocyst was represented through oocyst production and histopathological changes examination (Liu *et al.*, 2018).

### Results and Discussion

Immunized birds were challenged at 32nd day of age, demonstrated that oocyst protein could provide chickens with protection rate around 62%, oocysts number from chickens in the immunized group with oocysts protein significantly decreased than the unimmunized group (Fig. 1). Then few development and proliferation of parasites was seen by histopathological changes (Fig. 2). The immune response to vaccine demonstrated humoral and cellular protection. Li *et al.* [2012] reported specific

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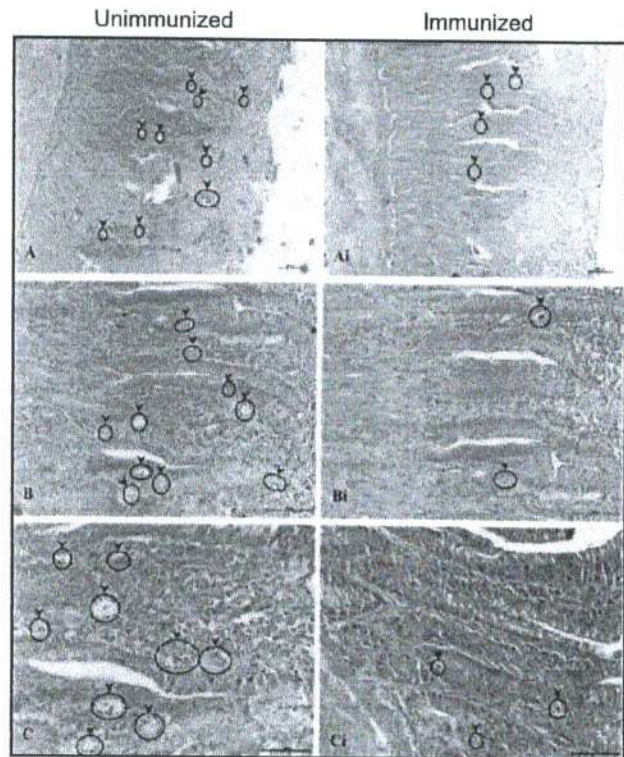


**Fig 1.** The comparison of oocyst production after homologous challenge with *E. acervulina* oocysts which previously non immunized and immunized with *E. acervulina* oocysts protein. Group 1, non immunized; Group 2, immunized.

IgG antibody responses against *E. tenella* was generated in the chickens immunized with recombinant rhomboid like protein expressed in *E. coli* and this protein is capable of eliciting humoral response and activating cell-mediated immunity in birds. Akhtar *et al.* [2001] showed the humoral and challenge responses when the supernatant from sonicated sporulated oocyst was used which induced a strong protection as immune chicks revealed high level of antibodies to resist heavy dose of challenge. Sporozoite that used as protein vaccine gives 66.7 per cent protection (Badawy and Aggour, 2006), while in another studies by Subramanian *et al.* (2008) and Geriletu *et al.* (2011) gave 60% and 77.3%, respectively with the use of recombinant *E. tenella* sporozoite antigen. Finally it was found that in order to get a better protective immunity by using parasite extracts, it requires the inclusion of the correct antigens and exclusion of the irrelevant ones is necessary (Wallach *et al.*, 1994)

### Summary

*E. acervulina* oocysts protein can generate protective immunity against homologous challenge through reduction of proliferation parasite and the presence of parasites disabilities. The further study for investigating efficacy of *E. acervulina* oocysts protein in induction



**Fig 2.** The Comparison of histological section of intestine of each groups non immunized and immunized with *E. acervulina* oocysts protein after challenged with *E. acervulina* oocysts homologous. head arrow, oocysts; (A & Ai, x100; B & Bi, x200; C & Ci, x400; H&E).

of protective immunity against heterologous challenge is required.

### Acknowledgement

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## Effect of Fructose on Fertility in Fowl Semen

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(Received : September, 2019 320/19 Accepted : November, 2019)

### Abstract

The purpose of the study was to determine the fertility of fowl semen after addition of fructose in egg yolk skim milk diluent. The three treatments with added levels of fructose in egg yolk skim milk diluent, respectively at: 5% (T1); 7.5% (T2); and 10% (T3) and then compared with control: egg yolk skim milk diluent without fructose (C). The results showed that the three treatments differ with control on fertility ( $\alpha < 0.05$ ). The conclusion, fructose in egg yolk skim milk diluent to the cock semen helps in maintaining of spermatozoal quality, thus improving their fertility

**Key words:** Fertility, Fowl semen, Fructose

The semen diluent for AI is required addition of fructose for increasing motility of sperm, acrosome action, and fertility (Tsuiji *et al.*, 2006). Fructose is main source of energy for sperm to support sperm quality for increasing of fertility (Akbar *et al.*, 2018). Sperm fertility of fowl semen in Indonesia is very low (Hariadi *et*

*al.*, 2019). Efforts is needed to improve fertility of fowl, through the addition of fructose in egg yolk skim milk diluent during semen preservation.

### Materials and Methods

The semen of cock was added fructose (Rochmi and Sofyan, 2019) as diluent, this research has used egg yolk skim milk, respectively at: 5% (T1); 7.5% (T2); and 10% (T3) and then compared with control: egg yolk skim milk diluent without fructose (C). In this study, semen from healthy cock was collected. The cock was 1 year old and possessed shiny fur, glowing eyes, high libido, and displaying agile movement. They were housed in conventional individual cages under 10 h of daily lighting and fed with a standard commercial feed at a rate of 155 g/day.

Observation In vivo fertilization through artificial insemination was carried out randomly on 24 healthy hens, aged 1 year kept in individual cages under uniform conditions. All of the chicken population were divided into 4 groups (C, T1, T2, and T3) with 6 hens in each

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Telah melaksanakan penelitian dengan judul sebagai berikut :

No.	Judul Karya Ilmiah	Tahun pelaksanaan Penelitian
1.	Valuation of The Antigenicity and Immunogenicity of <i>Eimeria tenella</i> by Reproductive Index and Histopathological Changes of Cecal Coccidiosis Virulent Live Vaccine in Broiler Chickens	2018
2.	<b>Protective Immunity of <i>Eimeriacervulina</i> Oocysts Protein Against Intestinal Coccidiosis</b>	2020
3.	Reproductive characteristic of a precocious line of <i>E. tenella</i> sporozoite as material bioactive in embyonating eggs and the implications of the findings appraised	2014
4.	Vaksinisasi Protein Ekskretori-Sekretori <i>Toxoplasma gondii</i> Hasil Biakan in vivo Membangkitkan Respons Imun Non Protektif	2013
5.	Pengaruh ekstrak sambiloto ( <i>Andrographis paniculata</i> ) terhadap produksi ookista dan skor lesi sekum ayam yang diinfeksi <i>Eimeria tenella</i>	2016





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6.	The conformity of diagnostic test between burrow ink test method with skin scrapping method of scabies in rabbit ( <i>Lepus domesticus</i> )	2018
7.	The Endogenous Development of <i>Eimeria tenella</i> in Chickens Injected Subcutaneously with Oocysts Protein as Initially Study of Development of Cecal Coccidiosis Killed Vaccine.	2020
8.	Potency of Attenuated <i>Eimeria tenella</i> in Protective Immunity Induction on Homologous and Heterologous Challenges	2016
9.	Morphospesies and phylogenetic tree analyses of <i>Leucocytozoon caulleryi</i> from chicken leucocytozoonosis cases in Pasuruan, East Java.	2012

Adapun penelitian tersebut layak dilakukan, meskipun belum ada *Uji Etical Clearence* karena menggunakan hewan coba yang minimal dan menghasilkan output yang sangat baik.

Demikian surat kerangan ini kami buat untuk dapat dipergunakan sebagai persyaratan pengusulan Jabatan Fungsional **Guru Besar**

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