Efficacy of galangal (*Alpinia galanga*) extracts against caecal coccidiosis in broilers

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Abstract

Objective - The present study was conducted on broilers to evaluate the anticoccidial efficacy of galangal (*Alpinia galanga*) ethanolic extracts against caecal coccidiosis caused by *Eimeria tenella* in broilers.

Materials and methods - One hundred 20 days old broilers (Ross 308), mixed sexes, were randomly assigned into 5 treatment groups (n = 20/group) as 1) positive control, 2-4) galangal ethanolic extracts at 25, 50, and 75 mg/kg body weight, respectively, and 5) 25 ppm toltrazuril. Broilers in all groups were orally inoculated with 25,000 sporulated oocysts of *E. tenella* at 20 days old. The body weight gain and caecal lesion scores parameters were recorded.

Results - The results showed that galangal ethanolic extract (AEE) was effective against *E. tenella* infection; especially at concentrations of 50 and 75 mg/kg per day for 3 consecutive days that can significantly ($p<0.05$) increase average daily gain (ADG) when compared to positive control. The lowest average caecal lesion score was observed in chickens treated with Toltrazuril.

Conclusion - The study showed that *Alpinia galanga* extract can improve weight gain and reduce the severity in caecum of broilers infected with *E. tenella*. Exploring the maximum potential of galangal in medicine and pharmaceutical applications for further research is needed.

Keywords: caecal coccidiosis, *Eimeria tenella, Alpinia galangal,* ethanolic extract, broiler

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ผลของสารสกัดข่า (*Alpinia galanga*) ต่อโรคบิดไส้ตัน ที่เกิดจากเชื้ออัยเมอเรีย ทีเนลลา ในไก่เนื้อ

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บทคัดย่อ

วัตถุประสงค์ เป็นการศึกษาประสิทธิภาพของสารสกัดข่า โดยใช้เอทธานอลเป็นสารสกัดในการต้านโรคบิดในไส้ตันของไก่เนื้อ

วัสดุ อุปกรณ์ และวิธีการ ใช้ลูกไก่เนื้อสายพันธุ์ Ross 308 จำนวน 100 ตัว เมื่อไก่อายุ 20 วัน ถูกแบ่งออกเป็น 5 กลุ่มการทดลองๆ ละ 20 ตัว และแต่ละกลุ่มการทดลอง ถูกจัดแบ่งออกเป็น 4 กรงๆ ละ 5 ตัว กลุ่มทดลองทั้ง 5 กลุ่มประกอบด้วย 1) กลุ่มควบคุมผลบวก 2) กลุ่มที่ได้รับสารสกัดข่าโดยใช้เอทธานอลที่ความเข้มข้น 25 มก.ต่อกิโลกรัมน้ำหนักตัว 3) กลุ่มที่ได้รับสารสกัดข่าโดยใช้เอทธานอลที่ความเข้มข้น 50 มก.ต่อกิโลกรัมน้ำหนักตัว 4) กลุ่มที่ได้รับสารสกัดข่าโดยใช้เอทธานอลที่ความเข้มข้น 75 มก.ต่อกิโลกรัมน้ำหนักตัว และ 5) กลุ่มที่ได้รับยาโทลธาซูริลผสมน้ำขนาด 25 พีพีเอ็ม ไก่เนื้อทุกตัวในทุกกลุ่มการทดลองถูกป้อนให้กินด้วยโอโอซิสต์ระยะติดต่อของอัยเมอเรีย ทีเนลลา จำนวน 25,000 โอโอซิสต์ต่อตัว

ผลการทดลอง พบว่าสารสกัดข่าที่ใช้เอทธานอลให้ผลต้านเชื้ออัยเมอเรียได้ โดยที่ระดับความเข้มข้น 50% และ 75% สามารถเพิ่มน้ำหนักตัว และลดรอยโรคที่ไส้ตันของไก่ที่ได้รับเชื้ออัยเมอเรีย ทีเนลลา ได้เมื่อเปรียบเทียบกับกลุ่มควบคุมอย่างมีนัยสำคัญ ($p<0.05$) อย่างไรก็ตามโทลธาซูริลที่ระดับความเข้มข้น 25 พีพีเอ็ม ยังเป็นยาที่มีประสิทธิภาพสูงสุดในการต้านเชื้ออัยเมอเรีย ทีเนลลา

สรุป สารสกัดจากข่าช่วยเพิ่มน้ำหนักตัวของไก่ที่ได้รับเชื้อบิดได้ และมีแนวโน้มลดความรุนแรงที่ไส้ตันที่เกิดจากเชื้อบิดอัยเมอเรีย ทีเนลลา ได้ ทั้งนี้ปริมาณของสารสกัดที่ได้จากข่าที่เหมาะสมและให้ประสิทธิภาพสูงในการใช้เป็นยารักษายังคงต้องมีการศึกษาและพัฒนาเพิ่มเติมต่อไป

คำสำคัญ: โรคบิดในไส้ตัน อัยเมอเรีย ทีเนลลา ข่า เอทธานอล ไก่เนื้อ

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Introduction

Coccidiosis is one of the most important diseases of poultry in the world [18]. It causes major economic losses in poultry production due to mortality and morbidity of infected animals. Epidemiologic studies have shown that the mortality of fowl can range from 5 to 70 % [7, 21]. The disease occurs mostly in young birds depending on their immunity status which normally quickly develops after exposure and gives protection against later outbreaks [27]. An estimated $90 million is spent in the US, and over $3 billion spent worldwide for coccidiosis prevention annually [12]. In China, the estimated expense is approximately $30–60 million for the alternative control strategies against coccidiosis [17]. This is probably the largest expenditure for medication in poultry industry. The most common species of coccidian is *Eimeria tenella*, which causes caecal or bloody coccidiosis, and *E. acervulina* and *E. maxima* cause chronic intestinal coccidiosis [16]. *E. tenella* is a protozoan normally develops in the cells of the caeca which are two blind sacs near the end of the intestine. It is one of the most pathogenic coccidia causing high morbidity and high mortality in infected chickens. [15]. *E. tenella* is highly pathogenic, in spite of advance methodology in immunology, biotechnology and genetic. The control of coccidiosis mainly depends upon prophylactic chemotherapy with anticoccidial drugs [22]. However, the emergence of drug resistance in coccidia is a great problem in most of the drugs and it has been reported against almost all the compounds used in control and treatment of *E. tenella* [3]. According to the toxic effects of chemicals on poultry [26, 2], the development of resistance of parasites [9, 11] and the problem of drug residues in poultry meat [24] it is suggested that herbal remedies may be used as an alternative treatment.

Galangal *(Alpinia galanga)*, called "*kah*" in Thai and known variously as "galangal" and "laos root," is an immensely pungent and fiery rhizome related to the common ginger. Its abundant usage in Thai cooking has earned the title of Siamese or Thai ginger [13]. A constituent of the root contains of volatile oil, resin, galangol, kaempferid, galangin and alpinin, starch, etc. The active principles of galangal are the volatile oil and acrid resin. Galangin is dioxyflavanol, and has been obtained synthetically [10]. It has been reported that *Alpinia galanga* component have therapeutic activities as anti-inflammatory, analgesic, anti-allergic, antifungal, anti-diabetic, antibacterial, antiulcer, antiprotozoal, immunostimulating, anticancer, and antioxidant [6]. The flavonoids from galangal have the potential to antibacterial and synergistic activity against amoxicillin resistant *Escherichia coli* [8]. It has been identified that 1'-

acetoxychavicol acetate from Galangal [30] and galangal extract had the strongest inhibitory effect against *Staphylococcus aureus* [25]. It has been reported that 6% galangal powdered supplements (*A. galanga*) in feed to control coccidiosis in broilers could reduce lesion scores of caeca, and the damage from coccidian was adequately controlled [28]. Investigation of the efficacy of galangal *(A. galanga)* ethanolic extract in treatment of caecal coccidiosis *(E. tenella)* in broilers may provide information towards the development of new anticoccidial drugs.

The objective of this study is to study an effect of galangal *(A. galanga)* ethanolic extract against caecal coccidiosis *(E. tenella)* in broilers.

Materials and Methods

Animals

One hundred 1 day-old broilers (Ross 308), mixed sexes, were purchased from commercial company in Khon Kaen. The broilers were raised and assigned into each experiment at 20 days-old.

Oocyst Propagation

Seed stock of *E. tenella* stored at Faculty of Veterinary Medicine, Khon Kaen University was propagated in 10 chickens, and the harvested sporulated oocysts from these chickens were kept in 3% potassium dichromate as inoculums at 4°C until used. Prior to inoculation, the sporulated oocysts were washed, counted and standardized into suspension containing 25,000 oocysts per milliliter.

Galangal extraction

Alpinia galanga rhizomes purchased from the local market in Khon Kaen were washed and then cut into small pieces with approximately 2-3 millimeter thickness. The galangal was dried with the sunlight for 2 days and then milled with blender into powder, and then was extracted using 50% ethanol (v/v) in water at a solid to solvent ratio of 1:10. The extraction procedure was performed in a shaking water bath at 50°C for 1 hr. The extract was filtered through a no.1 sinter glass funnel and the residue was re-extracted with the same conditions. The extracts was combined and concentrated in a rotary evaporator at 50°C. The concentrated extract was dried using a freeze dryer and kept in a glass vial at - 40°C [19]. The galangal extract (AEE) was followed each treatment group dissolved in 1 ml water and given orally to the chickens 1 day after the inoculation.

Experimental designs

One hundred 20 days-old broilers (Ross 308) were assigned to 5 treatment groups as shown in Table 1.

Table 1. Groups of *E. tenella*-inoculated chicken treated with different concentrations of galangal extract and toltrazuril.

Group	Oocyst number	Galangal extract (mg/kg)	Toltrazuril (ppm)
I	25,000	none	None
II	25,000	25	None
III	25,000	50	None
IV	25,000	75	None
V	25,000	none	25

Each treatment group had 4 subgroups with five broilers in each subgroup. Chicken in each subgroup were raised in a 80 cm x 80 cm x 60 cm wired cage. Broilers of all groups (I, II, III, IV and V) were orally inoculated with 1 ml suspension containing 25,000 *E. tenella* sporulated oocysts at 20 days-old. Clinical signs and dropping features of broilers in all groups were observed and recorded.

Feed and water intake

All broilers were raised and fed *ad libitum* with feed and water without any anticoccidial or growth-promoting antibiotic throughout the experiment. Only broilers in groups V were given with water added toltrazuril at 25 ppm concentration for three days (from day 2 after inoculation) starting on the same day that other broilers were treated with galangal extract.

Data collection

Body weights of all broilers were measured on the day of inoculation and necropsy. All animals were euthanized on day 5 after the inoculation to examine pathological changes of the caecal. Lesions of the caecal were examined and scored from 0-4 (Figure 1) as score +1 is a few scattered petechiae, which are reddish or purple in color, +2 is petechiae, which are apparent on the serosal surface, are somewhat more numerous, +3 is bleeding is more severe, with clotting appearing in the distal end of the pouch and +4 is severe bleeding, a much thickened caecal wall, and eroding of the mucosal surface show up on the fifth day of infection.

Statistics analysis

The data were analyzed using the software SPSS version 20.0 for Windows. The parameters of body weight gains were analyzed by using one-way analysis of variance and mean values were compared using the Duncan's multiple range tests. The results were expressed as mean ± SD. The lesion scores were statistically compared using Kruskal-Wallis H test and the results were expressed as median (inter-quartile range). Statistical difference was accepted at the level of $p<0.05$.

The research protocols were strictly followed the National Research Council manual of experimental animal ethics. The experimental animals were euthanized by injection of 70% alcohol into the atlanto-occipital joint.

Results

The average daily gain (ADG) of 25 days old chickens that received 50 and 75 mg/kg body weight per day of AEE and 25 ppm/day of toltrazuril were significantly ($p<0.05$) higher than that of positive control. Chickens in positive control had the lowest ADG (25.25±9.45 g/day) while those received 25 mg/kg body weight of AEE per day had no difference in ADG (34.65 ±17 g/day). The chickens received 50, 75 mg/kg body weight of AEE per day and 25 ppm of toltrazuril per day had ADG 38.43±14.43, 39.25± 13.05 and 41.9±1.41g/day, respectively. The results indicated that chickens treated with either AEE or toltrazuril had no difference in ADG (Table 2).

Table 2. Average body weight and ADG of broilers in each group.

Groups	Average body weight (g)			
	20 days old	25 days old	Weight gain (g)	ADG (g/day)
Positive	610.75 ± 81.66[a]	737.00 ± 89.10[a]	126.25 ± 47.29[a]	25.25 ±9.45[a]
25 mg/kg AEE	595.25 ± 117.82[a]	768.50 ± 138.53[a]	173.25 ± 85.01[ab]	34.65 ±17[ab]
50 mg/kg AEE	587.50 ± 80.58[a]	779.65 ± 116.9[a]	192.15 ± 72.17[b]	38.43 ±14.43[b]
75 mg/kg AEE	609.00 ± 134.43[a]	805.25 ± 166.1[a]	196.25 ± 53.13[b]	39.25 ±13.05[b]
25 ppm toltrazuril	591.75 ± 109.67[a]	801.25 ± 157.1[a]	209.50 ± 65.27[b]	41.9 ±1.41[b]

Means with different superscripts in each column are significantly different at $p<0.05$

It took 5 days after the inoculation for the animals to show clinical signs of mild depression and brown loose faeces. The caecal lesion scores (Figure 1) ranging 0-2 indicated the low virulence of infectivity in infected animals. Chickens inoculated with *E. tenella* with no any treatment had the highest caecal lesion scores (2). Chickens received 25 and 50 mg/kg body weight of AEE had no difference of caecal lesion scores as 1. AEE at 75 mg/kg body weight yielded significantly decreasing ($p<0.05$) of caecal lesion scores as 0.25 compared to AEE at 25 and 50 mg/kg body weight. Toltrazuril at 25 ppm was the most effective substance to inhibit the virulence of *E. tenella* with no lesion score found in caeca.

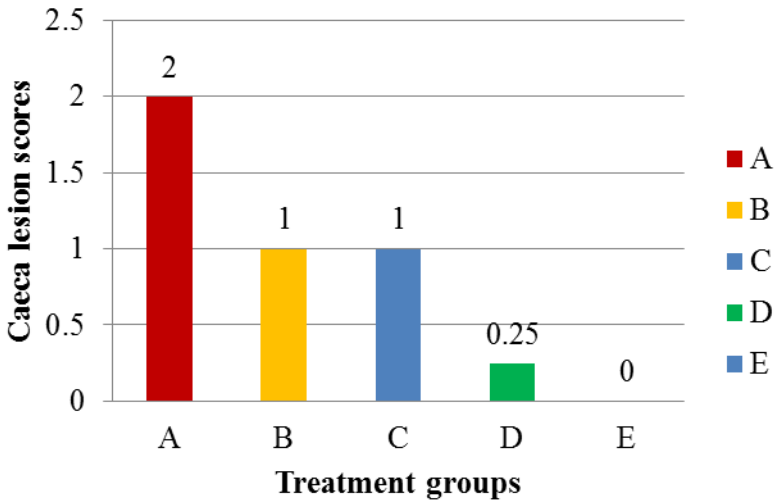

Fig 1. Caecal lesion scores in each treatment groups as Positive control (A), *Alpinia galanga* ethanilic extracts 25 mg/kg (B), 50 mg/kg (C), 75 mg/kg (D) and Toltrazuril 25 ppm (E)

Discussion

The *A. galanga* ethanolic extracts produced an improved weight gain and decreased the virulence in *E. tenella* infected chickens in a similar manner to those in toltrazuril group. However, the caecal lesion score in *A. galanga* treated groups were higher than toltrazuril group. Therefore, *A. galanga* extracts has less efficacy in the treatment of caecal coccidiosis. Many studies used traditional herbs, as *A. galanga* compounds having high antioxidant capacity, containing of high concentration of phenolic compound. Galangin was identified as the principal phenolic component with the highest antioxidant capacity in *A. galanga* [19]. Antioxidant activity of Galangal ethanolic extracts was increased when the concentration was

increased ranging from 0.10 to 1.0 mg/ml [13]. It has been reported that administration of toltrazuril in drinking water yielded a good efficacy after three days of treatment [3]. Moreover, toltrazuril is highly appropriate for the prophylaxis, therapy and intermittent treatment of *E tenella* in infected chickens. Toltrazuril had been shown to be successful as anticoccidial drug when mixed in the drinking water for a control of coccidiosis [21]. However, the cost of coccidiosis treatment and control has been concerned in the chicken production industry [29]. Therefore, an alternative enhancement of immunity in chickens by using herb could play a vital role to minimize the uses of anticoccidial chemo-therapeutic agents in poultry production.

Conclusion

The study demonstrates that *Alpinia galanga* extract can improve weight gain and diminish the severity in caeca of broilers infected with *E. tenella*. Exploring the maximum potential of galangal in medicine and pharmaceutical applications for further research is suggested.

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References

1. Adewole SO. The Efficacy of Drugs in the Treatment of Coccidiosis in Chicken in Selected Poultry. *J Academic Research International*. 2012;2:1.
2. Calo M, Altavilla D, Seminara P, Marini H, Minutoli L, Bitto A, Naccan F, Squadrito F. Inhibition of lipid peroxidation by IRFI 042, a vitamin E analogue, decreases monensin cardiotoxicity in chicks. *J Toxicity and Applied Pharma-col*. 2005;208:137-144.
3. Chapmen HD. Biochemical genetic and applied aspects of drug resistance in *Eimeria* parasites of the fowl. *J Avian. Pathol.* 1997;28:221-244.
4. Chapmen HD. Anticoccidial drugs and their effects upon the development of immunity to *Eimeria* infections in poultry. *J Avian Pathol.* 1999;28: 521-535.
5. Conway DP, McKenzie ME. Poultry coccidiosis: diagnostic and testing procedures. 3rd edition. Oxford, UK: *Blackwell Publishing Ltd*. 2007;pp, 30-32.

6. Chudiwal AK, Jain DP, Somani RS. *Alpinia galangal willd*-An overview on phyto-pharmacological properties. *Indian J on Natural Pro and Res*, 2010; 1(2):143-149.
7. Du A, Hu S. Effects of a herbal complex against *Eimeria tenella* infection in chickens. *J Vet Med B Infect Dis Vet Public Health*. 2004;51(4):194-197.
8. Eumkeb G, Siriwong S, Phitaktim S, Rojtinnakorn N, Sakdarat S. Synergistic activity and mode of action of flavonoids isolated from smaller galangal and amoxicillin combinations against amoxicillin-resistant *Escherichia coli*. *J of Applied Microbiology*. 2011; 1364-5072.
9. Fanatico A. Parasite Management for Natural and Organic Poultry: Coccidiosis, *Sustainable Agriculture Information Service*. 2006; pp:3.
10. Greive M. Modern herb. http://botanical.com/botanical/mgmh/g/galang01.html. 1995; Retrieved 14 December, 2012.
11. Guo FC, Suo X, Zhang GZ. Shen AG. Efficacy of decoquinate against drug sensitive laboratory strains of *Eimeria tenella* and field isolates of *Eimeria spp.* in broiler chickens in China. *J Vet Parasitol*. 2007;147: 239-245.
12. Hix K. Coccidiosis in Broiler. *Published in Diamond V The Trusted Experts in Nutrition and Health.* 2013;pp:3.
13. Juntachote, T. & Berghofer, E. Antioxidative properties and stability of ethanolic extracts of Holy basil and Galangal, *J Food Chem,* 2005;92: 193-202.
14. Kasma L. Galangal Thai food and Travel. *It Rains Fishes & Dancing Shrimp.* 2000;pp: 1.
15. Kennedy MJ. Coccidiosis in Chickens. Agri-facts practical information for Alberta's Agriculture Industry. *Animal Health Division Agdex.* 2001;pp:663-35.
16. Kennedy MJ. Hanson. Alberta Agriculture Coccidiosis in Chickens. *Animal Health Division*. 2001;pp.1.
17. Lai L. Bumstead J. Liu Y. Garnett J. Campanero-Rhodes MA, Blake DP. Palma AS. Chai W. Ferguson DJ. Simpson P. Feizi T. Tomley FM. Matthews S. The role of sialyl glycan recognition in host tissue tropism of the avian parasite *Eimeria tenella*. *J PLoS Pathol.* 2011;7(10):e1002296.
18. Lee SH. Lillehoj HS. Park DW. Jang SI. Morales A. Garcia D. Lucio E. Larios R. Victoria G. Marrufo D. Lillehoj EP. Protective effect of hyper immune egg yolk IGY antibodies against Eimeria maxima infections. *J Veterinary Parasitology*. 2009;163:123-126.

19. Lu M. Yuan B. Zeng M. Chen J. Antioxidant capacity and major phenolic compounds of spices commonly consumed in China, *J Food Research International*. 2011;44:530-536.
20. Mahae N. Chaseri S. Antioxidant activities and antioxidativecomponents in extracts of *Alpiniagalanga*(L.) SW. *J Kasetsart: Natural Science.* 2009;43:358- 369.
21. Mathis GF. Froyman R. Kennedy T. Coccidiosis control by administering toltrazuril in the drinking water for a 2-day period. *J Vet Parasitol,* 2004;121:1-9.
22. McDougald LR. Reid WM. Coccidiosis. Diseases of Poultry. *Calnek, B.W. (Ed.) Affiliated East West Press Privete. Ltd.* 1994; pp:14.
23. Michels MG. Bertolini LC. Esteves AF. Moreira P. Franca SC. Anticoccidial effects of coumestans from Eclipta alba for sustainable control of *Eimeria tenella* parasitosis in poultry production. *J Vet Parasitol.* 2011; 177(1-2):55-60.
24. Ogbe AO. Mgbojikwe LO. Abdu PA. Atawodi SE. Organ and carcass weight variation and histopathological changes in *Eimeria tenella* infected broiler chickens treated with aqueous extract of a wild mushroom (Ganoderma lucidum). *J Electronic of Environmental, Agricultural and Food Chem*. 2008;7(5):2906-2913.
25. Oonmetta-aree J, Suzuki T, Gasaluck P, Eumke G. Antimicrobial properties and action of galangal *(Alpinia galanga Linn.)* on *Staphylococcus aureus. Learn with Technologies- J Food Science and Technology.* 2006;39:1214-1220.
26. Singh T. Gupta RP. Clinico-haematological and mineral studies on experimental maduramycin toxicity in chickens. *J Vet Parasitol,* 2003;116(4):345-353.
27. Vegad JL. A Colour atlas of Poultry Diseases an Aid to Farmers and Poultry Professionals. *International Book Distributing Co. (Publishing Division).* 2007; pp:76.
28. Waramit W. Noppon B. Sanchaisuriya P. Laopaiboon B. Uses of Galanga (*Alpinia galanga* Linn.) on Coccidiosis Controls in Broilers. *The Proceedings of 44th Kasetsart University Annual Conference, Animals, Veterinary Medicine, 30 Jan. -2 Feb.* 2006; pp:533-538.
29. Williams RB. A compartmentalised model for the estimation of the cost of coccidiosis to the world's chicken production industry. *International J. Parasitol,* 1999; 29:1209-1229.
30. Yang X, Eilerman RG. Pungent principal of *Alpinia galangal* (L.) Swartz and Its applications. *J Agri and. Food Chem*. 1999; 47:1657-1662.