ISSN 1819-1878

Asian Journal of Animal Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

Asian Journal of Animal Sciences 1 (1): 26-32, 2007
ISSN 1819-1878

Effect of Leaves Extract of *Carica papaya*, *Vernonia amigdalina* and *Azadiratcha indica* on the Coccidiosis in Free-range Chickens

Z.I.A. AL-Fifi
Department of Biological Sciences, Faculty of Science, King Abdulaziz University, P.O. Box 80302, Jeddah 21589, Saudi Arabia

Abstract: An alternative coccidiosis treatment in response to the side effects of routinely use of conventional anticoccidial drugs was investigated on 600 *E. tenella* experimentally infected day-old Harco chicks in a factorial design at the Benin Agronomic Faculty Research Station. The effect of *Carica papaya*, *Vernonia amygdalina* and *Azadiratcha indica* dry leaf powder in the conventional feed (15% incorporation) was evaluated. *C. papaya*, the most ingested plant reduced the infected untreated control OPG down to 53% and *V. amygdalina*, down to 35%, even though it was less ingested than *C. papaya*. However, this reduction constituted 56 and 37% (*C. papaya* and *V. amygdalina*) of the conventional coccidiostatic effectiveness. The OPG reduction might be ascribed on the hand to the bitterness and on the other to the chemical compound, papaine and Vernoside. *A. indica* was less ingested due to it higher bitterness, repellency and toxicity. Consequently it exalted the disease and produce more oocysts than the control group. The plant OPG reduction mechanism and the accepted leaf powder dose need to be investigated.

Key words: Chick, *Eimeria tenella*, *Carica papaya*, *Vernonia amigdalina*, *Azadiratcha indica*, papaine, venoside

INTRODUCTION

Coccidiosis is one of the most expensive and common diseases of poultry production systems in spite of advances in chemotherapy, management, nutrition and genetics (McDougald *et al*., 1987). It remains a big concern for the commercial chicken production because of the high costs to control the disease. Williams (1999) estimated the total cost of chicken coccidiosis control in UK in 1995 at £38.5million. McDougald (2003) reported that the current expense for preventive medication exceeds $90 million in the United States and more than $300 million worldwide. Coccidiosis may strike any type of poultry in any type of facility (McDougald, 2003). The disease is also present in free-range production systems; in Zimbabwe coccidiosis was reported to be the most important endoparasitic disease and a second direct and indirect cause of loss in Village chicken after Newcastle disease according to Kusina *et al*. (2004).

The discovery of sulfanilamide by Levine (1939) has led to the use of anticoccidial feed additives more recently, polyether ionophorous antibiotics, have also been developed and used (Matsuda *et al*., 1989). The routinely use of those drugs in one hand has led to parasite drug resistant strains (Long, 1982) and on the other hand, prejudicial to consumer health because of drug or antibiotic residues in poultry products (Youn and Noh, 2001). In the recent years, the pharmaceutical industry has shown little interest in developing new products for anti-parasitic use and it is unlikely that we will have replacements for the products already available (McDougald, 2003). The search of alternative tools such as medicinal plants could be consequently envisaged in producing ecological meat, particularly in free-range production systems where the so-called organic product is increasingly demanded by consumers.

In Benin the use of medicinal plants in human malaria treatment is a common practice. Various sour and bitter leave or roots are used for medicinal purposes, but the efficacy is not documented (Personal observation). In many tropical and subtropical countries, numerous traditional medicinal plants have been used for centuries (Dharma, 1985; Perri, 1980). According to Lal *et al*. (1976), preparation of *B. frondosa*, *Carica papaya*, *Momordica charantia* and *Sapidus trifoliatus* have been found effective *in vitro* against poultry *Ascaridia galli*. In Senegal, farmers have traditionally used such plants to treat their chickens against endoparasites, for example, *Capsicum* sp. extracts and the leaves or barks of *Azadirachta indica are* added to drinking water and given to birds. In Cameroon, Agbédé *et al*. (1995) reported good results from the use of plants such as *Kalanchoe crenata* for coccidiosis and papaw (*Carica papaya*) leaves for diarrhea, while the use of human medicines (especially antibiotics, Ampicillin, Tifomycin) achieved no success. Huffman and Wrangham (1994) showed a positive effect of *Vernonia amygdalina* on chimpanzee infected with parasites.

The objective of this study was to try out the effect of *Azadiratcha indica*, *Carica papaya* and *Vernonia amigdalina* on infections with *Eimeria tenella* in chickens.

MATERIALS AND METHODS

Study Design and Duration

The study was designed as a factorial design with anticoccidials and oocysts doses as group factors. The duration was 2 months with 3 replications.

Eimeria tenella

Eimeria tenella infective oocysts (3000 and 30000 oocysts doses in 2% potassium dichromate solution) were provided by Institut de Recherche Agricole in Tours, France and conserved in a fridge at 0-4°C.

Experimental Animals

600 day-old Harco male chicks were provided by the hatchery Poussin du roi and reared in the Department of Biological Sciences, King Abdulaziz University Faculty of Science, Jeddah. Saudi Arabia. The breeders were imported from France by the hatchery. On the day of delivery all chicks were vaccinated against Newcastle disease and Infectious bronchitis.

Interventions

Experimental Infection

Chicks were reared in a chick room before transferring them to the treatment rooms at the age of 21 days. The 200 chicks were placed in 10 different rooms. Chicks in 5 rooms were orally challenged with 3000 *E. tenella* oocysts doses and the other 5, by 30000 *E. tenella* oocysts.

Experimental Feed

The experimental feed was a starter feed with non-anticoccidial additives. The feed was mixed with dry leaves powder at 15% incorporation level. The leaves were *Azadiratcha indica* (AI), *Carica papaya* (CP) or *Venonia amigdalina* (VA). Fresh leaves were collected and dried in rooms. The exposure to the sun was avoided in order to ensure the leaf chemical constituents conservation.

Treatment

The 10 chicken rooms were allocated to 5 groups. Each group comprised 2 rooms: the first was the 3000 oocyst dose infected chicks and the second 30000 oocysts dose infected chicks. Three groups received dry leaves feed additive, the 4th one was treated with a coccidiostatic (Anticox^{R}) in drinking water throughout the experiment. The treatment lasted for 4 weeks after *E. tenella* experimental infection. The last group was the infected untreated control group.

Data Collection

The body weight gain, the cumulative feed intake, the feed conversion ratio, the survival rate, the number of animals with bloody diarrhea and the lesion score were investigated during the 4 weeks following the *E. tenella* experimental infection. Feces were collected from the chick rooms around the water container in the morning to reduce the litter content. The oocyst excretion was daily monitored during the 14 days patent period.

Laboratory Examination

420 feces samples were taken to count the oocysts using the Mc Master technique. Dead birds were examined and the lesion score obtained using the scoring method described by Johnson and Reid (1970).

OPG Calculation

The number of oocysts counted from the McMaster chamber multiply by 200 gave the OPG. This number of oocysts is the mean of the oocysts found in the cells in the Mc-Master Chamber.

Statistical Analysis and Graphs

The descriptive analysis was made using the Proc Univarite Model (SAS version 8.2). The hypothesis was tested using the Proc Mixed Model (SAS, V. 8.02). The survival rate comparisons were tested in a Chi-square model using the GraphPad Prism Program (Version 4.02). The graphs were made using the chart facility of MS Excel 2000.

RESULTS

Parasitological Results

The survivabilities of *V. amigdalina* (64%), *C. papaya* (72%) and *A. indica* (61%) treated chicks were statistically similar; but significantly lower than the coccidiostatic group survivability (84%) and higher than survivability of the control group (59%) (Table 1a and b). The post-mortem of dead birds revealed hemorrhages or thickening whitish mucosa and cores of clotted blood in the caeca which specific for *Eimeria tenella* infection. The number of bloody diarrhea and the lesion score followed

Table 1a : 21 day-old chicks parasitological results during 4 weeks experimental *E. tenella* post infection period (treatment effect)

	Survivability				
Treatment	P (%)	SEM	Blood in feces	Lesion score	OPG
V. amigdalina	64^{ac}	4	3.5 ± 0.8^{a}	2.8 ± 0.4^{a}	35783 ± 12175^{a}
C. papaya	72^{a}	4	3.1 ± 0.7^{a}	2.1 ± 0.4^{b}	25588 ± 8435^{a}
A. indica	61^{ac}	4	3.8 ± 0.9^{ac}	2.8 ± 0.4^{a}	69510 ± 16131^{b}
Conventional coccidiostatic	84^{b}	3	0.0 ± 0.0^{b}	1.0 ± 0.0^{c}	3136 ± 3339^{c}
Control	59^{c}	4	4.6 ± 0.5^{c}	3.1 ± 0.4^{a}	54680 ± 15191^{d}
F- value			38***	33.50***	27.50***

Table 1b : 21 day-old chicks parasitological results during 4 weeks experimental *E. tenella* post infection period (oocyst doses effect)

	Survivability				
Oosyts doses	P (%)	SEM	Blood in feces	Lesion score	OPG
30000	66^{a}	3	2.8 ± 1.7^{a}	2.3 ± 0.8^{a}	34501 ± 22158^{a}
3000	68^{a}	3	3.2 ± 1.7^{a}	2.4 ± 0.9^{a}	40977 ± 29630^{a}
F-value			0.52	0.18	0.46

P: mean proportion, SEM: Standard Error on the Mean, OPG: Oocysts Per Gram, Columns that is not sharing superscript letters are statistically different in Chi-square test (survival rate, Graph pad prism), Blood in feces (genmod procedure, SAS), Lesion score and OPG (proc mixed hypothesis testing, SAS) almost the same trend. The coccidiostatic treated chicks had a higher survival rate (83%) and no bloody diarrhea was observed in their rooms. Significantly lower survival rate (58%), higher number of bloody diarrhea (4.6) and higher lesion score (3.1) were observed in the control group compared to the 4 treated groups Lower mean OPG was observed with coccidiostatic treated chicks (3136) that constituted 6% of the control OPG and higher OPG in *A. indica* treated group constituting 127% of the control OPG. The OPG reduction induced by *C. papaya* leaves treatment was 53% of the OPG of the infected untreated control group, which represented 56% of the conventional coccidiostatic treatment efficacy. Similarly the OPG reduction caused by *V. amigdalina* leaves treatment was 35% of the OPG of the control group, which represented 37% of the conventional coccidiostatic treatment efficacy. No significance OPG difference was observed between the *C. papaya* and *V. amygdalina* treated groups. The peak of OPG (Fig. 1) observed in the 7th day of oocysts excretion in the control group was delayed about 2 days in the *C. papaya* group (on day 9) and one day in the *V. amigdalina* group (8th day). The effect of oocyst doses was not significant on the parasitological results (p>0.05).

Chick Growth Performance

V. amigdalina and *A. indica* infected treated chicks exhibited significantly growth performance (weight gain and feed efficiency compared to *C. papaya*, Coccidiostatic treatment and the control group (Table 2a and b). Higher weight gain (388 g) and daily body gain (10 g) were observed with coccidiostatic treated chicks and lower values (weight: 213 g and DBG: 3 g) with *A. indica* treated chicks. The cumulative feed intake was higher in *C. papaya* (914 g), coccidiostatic treated chicks (905 g) and the control group (896 g) than in *V. amigdalina* (511 g) and *A. indica* treated chicks

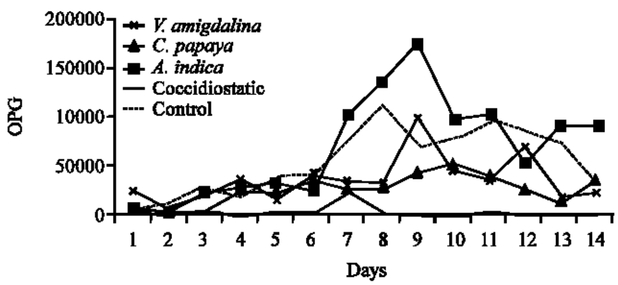

Fig. 1: OPG evolution during 14 days patent period

Table 2a: 21 day-old chicks growth performance during 4 weeks experimental *E. tenella* post-infection period (treatment effect)

Treatments	Weight day 28 PI (g)	DBG mean (g)	CFI (g)	FCR mean (g)
V. amigdalina	224±53^{a}	4±1^{a}	511±157^{a}	5±2^{a-c}
C. papaya	329±58^{b}	8±1^{b}	914±220^{b}	5±1^{a-c}
A. indica	213±49^{a}	3±1^{a}	494±146^{a}	7±2^{a-c}
Anticox	388±65^{b}	10±1^{b}	905±238^{b}	4±1^{a}
Control	345±71^{b}	8±2^{b}	896±278^{b}	4±1^{a}
F-value	10.04**	31.98***	6.39*	4.11*

*, ** and *** are significant at the level of p<0.05, p<0.01 and p<0.005, respectively

Table 2b: 21 day-old chicks growth performance during 4 weeks experimental *E. tenella* post-infection period (oocyst doses effect)

Oocyst doses	Weight day 28 PI (g)	DBG mean (g)	CFI (g)	FCR mean (g)
30000	301±88^{a}	6.5±2.6^{a}	754±307^{a}	4.9±1.5^{a}
3000	298±94^{a}	6.3±3.1^{a}	734±265^{a}	4.9±1.8^{a}
F-value	0.01	0.04	0.04	0.02

PI: Post Infection, DBG: Daily Body Gain, CFIW4: Cumulative Feed Intake at Week 4, FCR: Feed Conversion Ratio
Columns that are not sharing superscript letters are statistically different in proc mixed hypothesis testing (SAS)

(494 g). The feed conversion ratios of almost all experimental groups were statistically similar, with exception of the *A. indica* group, which disposed of a higher feed conversion ratio (7 g feed/g weight gain). The effect of the oocyst doses was not significant on chick growth performance (p>0.05).

DISCUSSION

Carica papaya

C. papaya leaves powder in the feed was the most ingested plant because of less bitterness of the dry leaves and the presence of carotene (vitamin A) that can enhance the feed palatability and consequently impact positively chick growth performance (Eramus *et al*., 1960). However, it did not influence the chicks' growth performance when compared with the coccidiostatic treated chicks and the control. The papaya treatment reduced significantly the OPG down to 53%. This result is in line with previous experiments showing similar activity of papaya latex against infections of Ascaridia galli, in chicken (Mursof and HE, 1991). Satrija *et al*. (1994), by trying out the efficacy of papaya latex against Ascaridia suum in pigs, found a decrease of the eggs produced (by 99%) and the number of adult worms (by 80%). Purwati and He (1991) indicated the effectiveness of papaya latex on the worm organism as well as the egg infectivity. According to these authors, the anthelmintic activity of papaya latex might be ascribed to proteolytic enzymes such as papain, chymopapain and lysozyme present in the latex as well as in the leaves, seeds and roots (Winarmo, 1983). Obviously, worms or parasites in general, being a proteinic organism can be digested by the papaine. The vitamin A supplementing feed showed a great reduction of excreted *E. tenella* oocysts (Eramus *et al*., 1960; Coles *et al*., 1970; Singh and Donovan, 1973). Furthermore, *C. papaya* is known to have an anti-inflammatory properties, certainly due to it riches in Vitamin A, used against tumors, ulcers and can accelerate wound healing (Beuth *et al*., 2001). In the current study, the lesion score and the bloody diarrhea recorded was middle compared to the *V. amygdalina* and *A. indica* treatment and the control. The OPG reduction induced by *C. papaya* dry leaves powder treatment might be ascribed in one hand to the direct *E. tenella* protozoits digestion in the caeca by a synergistic action of pancreas chymotrypsin and papaine. The anti-inflammatory property of the *C. papaya* concentrated vitamin A on the other hand might act in caecal epithelium cell protection, detrimental to the coccidia reproductive activities. Nevertheless, the observed OPG reduction constituted only 56% of the coccidiostatic treatment efficacy and the Survival rate was significantly lesser than the later.

Venonia amygdalina and *Azadiratcha indica*

The feed containing *V. amigdalina* and *A. indica* dry leaf powder was less ingested. Consequently, the treated chicks exhibited a very poor growth performance. This was almost 50% of the growth performance observed in the *C. papaya* group. The drying process did not reduce the bitterness of these 2 plants, as it was the case with *C. papaya*. *A. indica* is reputed for it toxicity and the smoke from the fresh burned leaves is traditionally used to repel mosquitoes (personal observation). Richard *et al*. (2003), by evaluating neem extract for use against the turnip root fly (*Delia floralis fabi*) indicated a repellent effect that reduced the fly oviposition. The toxicity of the neem leaves and *V. amygdalina* leaves in lesser extent (Huffman *et al*., 1996) might repel the chicks from feeding, detrimental to the growth performance. Even though *V. amygdalina* was less consumed it OPG reduction effect revealed to be significant (35%) and constitute 37% of the conventional coccidiostatic efficacy. Huffman and Wranghan (1994) noted that multiple parasitic affected chimpanzees chew *V. amygdalina* pith. *V. amygdalina* bioassay examination indicated an antiparasitic effect. Further analysis done by Ohigashi *et al*. (1994) revealed chemicals categorized as Sesquiterpen lactones and steroid glycosides, which are known for their anti-tumor activity. Specifically vernoside B1 and vernoniol B1 extracted from the pith, suppressed movement and egg-laying activity in a bioassay of

Schistosoma japonicum, a parasitic worm and have proven effectiveness against drug-resistant malarial parasites a protozoan organism. The toxicity of *V. amygdalina* can be directly prejudicial to the *E. tenella* sporocysts. The bitterness of the medicinal feed might enhance the gastrointestinal enzymes (chymotrypsin) production and the digestion of the sporozoits. In addition the vernoside B1 or vernoniole B1 reduction activities against Eimeria is conceivable. However, further studies need to be carried out to elucidate theses suggestions. Contrary, the *A. indica* powder increased the oocysts production of the control group up to 127% and aggravated the disease. The necropsy from the neem leave treated chicks showed enlarged yellowish liver (observation of the author but not quantified), which might be ascribed to the high toxicity of the plant. Such condition can also weaken the chick immune system and cause the organism natural defense reduction.

CONCLUSION

Carica papaya leaves and *venonia amygdalina* have showed a positive effect on OPG reduction compared to the neem leaves treatment OPG, where the value passed largely the control one. Their action might be ascribed to the bitterness or toxicity. In addition it might be in keeping with enzyme activities that can digest and eliminate the sporozoits. The protection of caecal epithelium in the case of *C. papaya* is suggestible. Nevertheless the *E. tenella* OPG reduction observed was almost 50% of the conventional coccidiostatic efficacy. Either ways, some researches need to be carried out to precise the oocyst reduction mechanism in the ceaca as well as the acceptable incorporation level of *A. indica* and *V. amigdalina* dry leaves powder.

REFERENCES

Agbédé, G.B., A. Teguia and Y. Mangeli, 1995. Enquente sur lelevage traditionnel des volailles au Cameroon. Tropicultura, 13: 22-24.

Beuth, J., B. Ost, A. Pakdaman, E. Rethfeldt, P.R. Bock, J. Hanish and B. Schneider, 2001. Impact of complementary oral enzyme application on the postoperative treatment results of breast cancer patients. Results of an epidemiological multicentre retrolective cohort study. Cancer Chemother. Pharmacol., 47: S45-S54.

Coles, B., J. Biely and B.E. March, 1970. Vitamin A deficiency and *Eimeria acervulina* infection in the chick. Poult. Sci., 49: 1295-1301.

Dharma, A.P., 1985. Tanaman obat traditional Indonesia. Jakarta, PN: Balai Pustaka, pp: 291.

Eramus, J., M.L. Scott and P.P. Levine, 1960. Relationship between coccidiosis and vitamin A nutrition in chickens. Poult. Sci., 39: 565-572.

Huffman, M.A. and R.W. Wrangham, 1994. Diversity of Medicinal Plant Use by Chimpanzees in the Wild. In: Chimpanzee Cultures, Wrangham, R.W., W.C. McGrew, F.B.M. de. Waal and P.G. Heltne (Eds.) Harvard Univ. Press, Cambridge, pp: 129-148.

Huffman, M.A., K. Koshimizu and H. Ohigashi, 1996. Ethnobotany and Zoopharmacognosy of *Vernonia amygdalina*, A Medicinal Plant Used by Humans and Chimpanzees in the Wild. In: Compositae: Caligari, P.D.S. and D.J.N. Hind (Eds.), Biology and Utilization Vol 2., Kew, The Royal Botanical Garden, pp: 351-360.

Johnson, J. and M. Reid, 1970. Anticoccidial drug: Lesion scoring techniques in battery and floor-pen experiments with chickens. Exp. Parasitol., 28: 30-36.

Kusina, N., T. Kusina and J. Mhlanga, 2004. A survey on village chicken losses: Causes and solutions as perceived br farmers. Department of Animal Science, University of Zimbabwe, PO BOX MP 167, Mount Pleasant, Harare, Zimbabwe, pp: 149-151.

Lal, J., S. Chandra, V. Raviprakash and M. Sabir, 1976. *In vitro* anthelmintic action of some indigenous medicinal plants on *Ascaridia galli* worms. Ind. J. Physiol. Phamarcol., 20: 64-68.

Levine, P.P., 1939. The effect of sulfanilamide on the course of experimental avian coccidiosis. Cornell. Vet., 29: 309-320.

Long, P.L., 1982. The biology of Coccidia. University Park Press, Baltimore.

Matsuda, K., M. Kimura, K. Komai and M. Hamada, 1989. Nematicidal activities of (-)-N-methylcystisine and (-)-anagyrine from *Sophora flavescens* against pine wood nematodes. Agric. Biol. Chem., 53: 2287-2288.

McDougald, L.R., J.M.L. Da Silva, J. Solis and M. Braga, 1987. A survey of sensitivity to anticoccidial drugs in 60 isolates of coccidian from broiler chickens in Brazil and Argentina. Avian Dis., 31: 287-292.

McDougald, L.R., 2003. Coccidiosis. Diseases of Poultry. 11th Edn. Ames: Iowa State University Press, pp: 974- 976.

Mursof, E.P. and S. He, 1991. A potential role of papaya latex as an anthelmintic against *Ascaridia galli* infection in chicken. Hemera Zoa, 74: 11-20.

Ohigashi, H., M.A. Huffman, D. Izutsu, K. Koshimizu, M. Kawanaka, H. Sugiyama, G.C. Kirby, D.C. Warhurst, D. Allen, C.W. Wright, J.D. Phillipson, P. Timmon-David, F. Delnas, R. Elias and G. Balansard, 1994. Toward the chemical ecology of medicinal plant use in chimpanzees: The case of *Vernonia amygdalina* Del. A plant used by wild chimpanzes possibly for parasiterelated diseases. J. Chem. Ecol., 20: 541-553.

Perri, L.M., 1980. Medicinal plants of east and Southeast Asia. London, The MIT Press, pp: 620.

Purwati, E. and S. He, 1991. Pengaruh getah papaya (*Carica papaya*) terhadap infectivitas telur *Ascaridia galli* pada ayam. Hemera Zoa, 74: 1-5.

Richard, M., S. Randi and B. Per, 2003. The effect of neem extract on the Turnip Root Fly and the Cabbage Morth. Nowegien Crop Research Institute. Proceedings of the 9th Workshop Hohensolms, Germany March. pp: 55-59.

Satrija, F., P. Nansen, H. BjØrn, S. Murtini and S. He, 1994. Effect of papaya latex against *Ascaridia suum* in naturally infected pigs. Danish Center for Experimental Parasitology, Royal Vet. Agric. Univ., pp: 344-345.

Singh, S.P. and G.A. Donovan, 1973. A relationship between coccidiosis and dietary vitamin A levels in chickens. Poult. Sci., 52: 1295-1301.

Williams, R.B., 1999. A compartmentalized model for the estimation of the cost of coccidiosis to the world's chicken production industry. Internat J. Parasitol., 29: 1209-1229.

Winarno, F.G., 1983. Enzim Pangan. PT Gramedia, Jakarta.

Youn, H.J. and J.W. Noh, 2001. Screening of the anticoccidial effects of herb extracts against *Eimeria tenella*. Vet. Parasitol., 96: 257-258.