J. Vet. Malaysia (2000) 12(1): 49-54

CASE REPORT

THE EFFECTS OF COMPETITIVE EXCLUSION, ANTICOCCIDIAL TREATMENT AND *ALPHITABIUS DIAPERINUS* CONTROL IN A MIXED INFECTION OF COCCIDIOSIS AND HYPOGLYCAEMIA-SPIKING SYMDROME ON BROILERS REARED UNDER DEEP LITTER SYSTEM

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SUMMARY

A combined control program of darkling beetle (*Alphitobius diaperinus*) and its larvae, competitive exclusion (Aviguard®) and anticoccidial treatment (Baycox®) were found to be of benefit in mixed infections involving hypoglycaemia-spiking mortality syndrome (HSMS) and *Eimeria tenella* infections in broilers.

Keywords: Anticoccidial agents, Baycox®, Aviguard®, darkling beetle, broilers, deep litter

CASE HISTORY

A deep litter pullet broiler breeder farm, which was left empty for 1.5 years, was converted into broiler houses. In the first grow-out, caecal coccidiosis was diagnosed following clinical and necropsy findings from 18 day-old broilers. Treatment with sulphaquinoxaline, using the 3-2-3 regime, on days 18-20 and 23-26 was not successful. A second treatment on days 30-32 and 35-37 also failed to lower the incidence of caecal coccidiosis while the performance of the flock remained poor. The broilers were also found to exhibit hypoglycaemia-spiking mortality syndrome (HSMS) from 2 weeks of age. The disease, characterised by hypoglycaemia, fine head tremors, blindness and ataxia, had a characteristic high mortality of at least 0.3-0.5% per day for at least 3 days without gross pathological findings. The farm had a history of HSMS since 1995 and a viral aetiology was postulated (Ganapathy *et. al.*, 1998; Haas, *et. al.*, 1999). A high population of darkling beetles *(Alphitobius diaperinus)* and their larvae were observed in the litter. Control of *Alphitobius diaperinus* and their larvae had been shown to be effective in reducing the incidence of HSMS (Haas *et. al.*, 1999).

This report describes the effects of the following actions plans for the broiler grow-out cycle in trying to reduce the disease problem:

a) to observe the efficacy of different anticoccidial drugs (sulphaquinoxaline, amprolium and toltrazuril (Baycox®; Bayer) in controlling *E. tenella*.
b) to assess the benefits of controlling darkling beetles *(Alphitobius diaperinus)* and its larvae in moderating the incidence of HSMS. The beetle have been shown to play a role as vector in the transmission of HSMS (Haas *et al.*, 1999)
c) to observe the effects of competitive exclusion (Aviguard®; Bayer) in moderating the effects of primary infection of coccidiosis. Competitive exclusion with Aviguard® has been observed to moderate the effects of *Clostridium* infections, which plays an important role in the pathogenesis of coccidiosis. It also balances the microflora in the caeca of broilers and assist in the elimination of multi-resistant pathogenic *E. coli* (Day *et al.*, 1997; Hofacre *et al.*, 1998). Competitive exclusion with Aviguard® has been shown to be effective in reducing the prevalence of *Salmonella* Enteritidis (SE) infection in broilers (Zaini *et al.*, 1996). Aviguard® and toltrazuril (Baycox®) have also been shown to moderate SE excretion in mixed SE and *Eimeria tenella* infections in SPF birds (Zaini *et al.*, 1998).

MATERIALS AND METHODS

A total of 110,800 broilers were randomly allotted into 8 houses at a stocking density of 9 birds/m^2. They were divided into 4 treatment groups with two replicates per treatment. The treatments were administered in drinking water according to the manufacturer's recommendations (Table 1).

Table 1. Treatment of broilers

	Treatments I Sulphaquinoxaline	II Amprolium	III Toltrazuril (Baycox®)	IV Baycox® + Aviguard®
Anticoccidia	Days 18 to 20 Days 23 to 25	Days 18 to 23	Days 18 &19	Days 18 & 19
Aviguard				Days 5 & 7 Days 27 & 29*

*a dose of Aviguard® is recommended to be applied at days 2 and 4 after antimicrobial treatment. In this study only ¼ dose was used in each application period.

Broiler houses were washed, cleaned and disinfected with formalin and quarternary ammonium disinfectant followed by a coccidial disinfectant. The interior of the houses were sprayed with a synthetic pyrethroid and the litter in the brooding area was surface-sprayed with a larvicidal insect growth regulator containing triflumuron (Baycidal®; Bayer). The birds in all houses were given similar treatments and vaccinations as follows:

Day 0	ND /IB coarse spray (ND - Newcastle disease B1B1 strain) (IB - Infectious bronchitis H_{120} strain)
Days 1-3	Enrofloxacin - 10 mg/kg for 3 days Glucose - 0.25% solution
Day 7	IBD (drinking water) (IBD- Infectious Bursal Disease)
Day 14	IBD (drinking water)
Day 21	NB B1 Lasota strain (drinking water)
Day 20-23	Colistin (drinking water)

The daily mortality and culling, weekly body weights of 1% of broilers, grow-out feed consumption, body weights at slaughter and percentage depletion, production efficiency factor and coccidial lesion scores, mucosal scrapings and coccidial counts in faeces were monitored. No statistical analysis was carried out.

RESULTS

The growth performance of broilers is shown in Table 2. Birds in the sulphaquinoxaline treated group grew faster than other treatment groups. However, birds in the sulphaquinoxaline and amprolium treated groups were diagnosed to have caecal coccidiosis at 17 to 19 days of age. Same diagnosis was made in the Baycox® and Baycox®-Aviguard® treated groups at 19-20 days of age. Mortality was most severe in the amprolium treated groups at the rate of 13.5% while the Baycox®-Aviguard® treated birds had the lowest mortality rate of 8.4% (Table 3).

The weekly coccidiosis lesion scores of the duodenum, jejunum, ileum, caecum and large intestines in five healthy and sick birds showed that birds in the amprolium treated broilers began to show moderate lesion scores at 14 days of age.

Lesion scores in the sulpahquinoxaline and amprolium treated broilers were more severe from week 4 compared to the Baycox®-Aviguard® treated broilers. The coccidiosis lesion scores, mucosal scraping detection of oocysts and oocysts counts are shown in Table 4.

Table 2. Body weights of the treated birds

	Treatment Group I Sulpha-Quinoxaline	II Amprolium	III Baycox®	IV Baycox® + Aviguard®	Previous flock performance
Day 0	43.1	40.5	43.9	42.3	43.7
Day 7	127.6	99.3	116.2	118.9	118.4
Day 14	308.8	244.0	265.3	265.7	232.5
Day 21	679.7	503.8	584.6	579.3	499.3
Day 28	1066.3	831.2	935.4	979.7	802.2
Day 35	1565.4	1377.8	1430.8	1419.1	1210.1
Day 42	1995.0	1780.0	1793.3	1861.4	1870.0

Table 3. Cumulative culling and mortality

Cumulative Mortality & Culling	Treatment Group I Sulpha-Quinoxaline	II Amprolium	III Baycox®	IV Baycox® + Aviguard®	Previous grow out performance
Day 7	0.74	0.70	1.29	0.90	0.94
Day 14	1.60	1.56	1.92	1.52	1.78
Day 21	3.02	3.76	3.83	2.93	2.97
Day 28	5.27	7.48	6.20	4.90	4.86
Day 35	6.90	10.90	7.98	6.44	7.65
Day 42	8.61	13.48	9.66	8.41	14.63

Table 4. Detection rate of oocysts on mucosa, degree of infection and oocysts counts

Parameter	Treatment Group I Sulphaquinoxaline	II Amprolium	IV Baycox® + Aviguard®
WEEK 1			
- Duodenum	-ve	1/10 mild	-ve
- Mid small intestine	-ve	-ve	-ve
- Lower small intestine	-ve	-ve	-ve
-Caecum	-ve	-ve	-ve
Oocysts/gram	-ve	-ve	-ve
WEEK 2			
- Duodenum	5/10 moderate	4/10 moderate	3/9 mild
- Mid small intestine	1/10 mild	1/10 mild	2/9 mild
- Lower small intestine	1/10 mild	1/10 mild	2/9 mild
-Caecum	-ve mild	1/10 mild	-ve mild
Oocysts/gram	-ve	-ve	-ve
WEEK 3			
- Duodenum	4/10 mild	3/10 mild	3/9 moderate
- Mid small intestine	2/10 mild	1/10 mild	2/9 mild
- Lower small intestine	2/10 mild	1/10 mild	1/9 mild
-Caecum	-ve moderate	-ve moderate	-ve
Oocysts/gram	-ve	18000	-ve
WEEK 4			
- Duodenum	6/10 mild	7/10 mild	4/8 mild
- Mid small intestine	5/10 mild	3/10 mild	4/8 mild
- Lower small intestine	5/10 mild	3/10 mild	4/8 mild
-Caecum	2/10 moderate	2/10 moderate	3/8 mild
Oocysts/gram	23700	6800	9300
WEEK 5			
- Duodenum	3/10 moderate	5/10 moderate	3/10 mild
- Mid small intestine	4/10 mild	1/10 mild	4/10 rnild
- Lower small intestine	4/10 mild	1/10 mild	4/10 mild
-Caecum	2/10 severe	4/10 severe	1/10 moderate
Oocysts/gram	3600	10500	3500
WEEK 6			
- Duodenum	3/10 moderate	4/10 moderate	5/10 mild
- Mid small intestine	10/10 mild	4/10 mild	4/10 mild
- Lower small intestine	10/10 mild	4/10 moderate	4/10 mild
-Caecum	3/10 mild	3/10 moderate	2/10 mild
Oocysts/gram	3000	not available	6000

The grow out performance, percentage depletion and production efficiency of different treatment groups are shown in Table 5. The Baycox®-Aviguard® treated birds showed the best performance amongst the groups whilst amprolium treated groups had the poorest performance.

The cost-benefit effects of medication and increased vector control actions are shown in Table 6. Baycox®-Aviguard® treated birds had an additional 23 to 30 sen/bird advantage over sulphaquinoxaline and amprolium treated birds.

Table 5. Grow out performance, percentage depletion and production efficiency

Parameter	Treatment Group I Sulpha-Quinoxaline	II Amprolium	III Baycox®	IV Baycox® + Aviguard®	Previous grow out performance
DOC intake	27800	27600	27600	27800	32000
Birds sold	24902	22116	24590	24694	26710
Age at depletion (days)	43.0	44.0	44.0	45.5	46.0
% depletion	89.6	80.1	89.1	88.8	83.5
Average weight (kg)	1.86	1.77	1.88	2.04	1.87
Feed/gain (FG)	1.99	1.86	2.00	1.89	2.36
FG/AW.	1.06	1.05	1.06	0.92	1.26
Production Efficiency	177.7	162.8	177.9	201.2	130.7

Production Efficiency = (AW * Birds sold)^2 * 10000/(DOC intake * Feed used * Depletion Age)

Table 6. Cost of medication and cost-benefit

Parameter Cost (sen/bird)	Treatment Group I Sulpha-Quinoxaline	II Amprolium	III Baycox®	IV Baycox® + Aviguard®	Previous grow out performance
Gross Return after feed, DOC depreciation and common programs	46.1	40.7	42.4	78.0	(13.8)
Less: Incremental cost					
Cost of Medication	5.63	6.09	7.96	13.59	2.53
Cost of Vector Control Measures	1.48	1.67	1.50	1.49	
Gross Profit	39.0	32.9	32.9	62.9	(16.3)
Cost-Benefit (in comparison with Baycox®-Aviguard® group	(23.9)	(30.0)	(30.0)		(79.2)

DISCUSSION

Unlike the previous grow-out when the HSMS intensity was severe, mild HSMS signs were observed in about 5% of the birds on day 21 of age in all treatment groups. Following the spraying of internal surfaces of the houses with insecticides, the population of darkling beetle adults and larvae particularly in the litter under bell drinkers and around feeder pans at day 14 of the broiler cycle were low. Since insecticides were not sprayed on the exterior of the houses, the beetles and their larvae could migrate and breed leading to the propagation of HSMS in broilers. The outside periphery of the houses were subsequently sprayed with synthetic pyrethoids on day 15 with the aim of controlling further migration of adult beetles and to kill beetle larvae. When compared with the previous cycle, the efforts in controlling the population of darkling beetles and their larvae appeared to be an effective means to reduce HSMS incidence. An earlier study by Haas *et al.* (1999) revealed that HSMS incidence can be reduced significantly with darkling beetle and larvae control in raised floor open houses reared broilers.

Coccidioisis in broilers is an enigma for broiler producers in the tropics if birds are reared on deep litter systems. This is so despite the inclusion of an in-feed coccidiostat in broiler rations. Coccidiosis control programs employ either a rotation or shuttle in-feed anticoccidial treatment program to overcome acquired resistance build-up and if necessary the use of supplementary anticoccidial treatments when clinical outbreaks occur. Following the different treatments in this study, the incidence and severity of caecal coccidiosis was milder than in the previous cycle. Caecal coccidiosis was observed from days 17-19 in amprolium and sulphaquinoxaline treated broilers and from days 19-20 in Baycox® treated broilers. Baycox® had never been used in this farm and previous studies have shown that it is a superior anticoccidial drug (Rahmat *et al.*, 1996). It is possible that the local isolates of *E. tenella* were resistant to the in-feed coccidiostat used and/or the field challenge was too high that treatment with the three in-feed anticoccidia drugs was ineffective. Thus, farm management had been advised to consider a change of the in-feed coccidiostat for the broilers in following cycle.

The performances of all treatment groups in the second grow-out were superior to the first grow-out. The Baycox® treated group was equivalent in performance with the amprolium and sulphaquinoxaline treated groups in controlling caecal coccidiosis caused by *E. tenella*. The combination therapy of Baycox®-Aviguard® was superior with highest cost-benefit financial return amongst the treatment methods. It is hypothesised that primary caecal coccidiosis can lead to secondary complications if mixed infectious agents such as HSMS virus exist. Previous studies have shown that Baycox®-Aviguard® has a beneficial effect on a mixed infection of *Salmonella Enteritidis* and *E. tenella* infection in SPF broilers (Zaini *et al.*, 1998). Aviguard® alone has been shown to be effective in moderating *Clostridium* infection (Hofacre *et al.*, 1998) and pathogenic and multi-resistant *E. coli* infections in commercial broilers (Day *et al.*, 1997). In this study, only 1/4 dose of Aviguard® was used at strategic times. This dosage has been reported to be effective in broilers reared in Taiwan (Chiang, 1997). The competitive exclusion provided by Aviguard® and its beneficial role in stabilising caecal microflora after caecal coccidiosis and pathogenic enteric bacterial infections needs to be explored in further detail. It would also interesting to observe whether Baycox®-Aviguard® would be cost effective if a suitable in-feed coccidiostat to which the local isolates of coccidia are sensitive to are tested.

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RINGKASAN

KESAN PENYISIHAN BERSAING, RAWATAN ANTIKOKSIDIA DAN KAWALAN ALPHITABUIS DIAPERINUS *DALAM JANGKITAN CAMPURAN KOKSIDIOSIS DAN SINDROM HIPOGLISEMIA-MELONJAK PADA AYAM PEDAGING YANG DIPELIHARA DIBAWAH SISTEM SARAP*

*Manfaat diperolehi melalui program rawatan kawalan kumbang hitam (*Alphitabuis diaperinus*) dan larvanya, penyisihan bersaing (Aviguard®) dan antikoksidia (Baycox®) di dapati bermanafaat dalam mengawal jangkitan campuran melibatkan sindrom hipoglisemia-melonjak (HSMS) dan jangkitan* Eimeria tenella *pada ayam pedaging.*