Kafkas Univ Vet Fak Derg
20 (4): 571-576, 2014
DOI: 10.9775/kvfd.2013.10638

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Effect of Clopidol and Amprolium/Ethopabate on Performance and Intestinal Morphology of Chickens with Experimental Coccidiosis [1]

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[1] *This work had been supported by the funds granted by Vice Chancellor for Research of Shahrekord University*

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Makale Kodu (Article Code): KVFD-2013-10638

Summary

To investigate effects of two anticoccidial drugs on growth performance and intestinal mucosa in experimental coccidiosis, broiler chicks challenged/medicated by oocytes of Eimeria/Clopidol and Amprolium + Ethopabate. There were negative effects on growth performance in chickens infected/medicated by these drugs. Most morphometric parameters of intestine were decreased in infected or medicated groups compared to negative control (P<0.05). Duodenal villus height in infected/unmedicated group and villus surface area in all infected groups were lowest between infected or medicated groups (P<0.05). Duodenal villus width was also decreased in all medicated groups compared to negative control, but this decreasing was only significant in infected / medicated groups. Jejunal villus height, width and surface area in infected/unmedicated group were lowest as compared to other uninfected / medicated groups (P<0.05). Ileal villus height in infected/unmedicated group were lowest as compared to all medicated groups. In infected/medicated groups, ileal villus surface area was significantly lower than uninfected/medicated groups. There were no significant differences between Clopidol- and Amprolium + Ethopabate-medicated groups in intestinal morphometric assessment. It is concluded that Clopidol and Amprolium + Ethopabate have adverse effects on chicken performance and intestinal morphology especially villus dimensions and absorptive surface during control or prevention of coccidiosis.

Keywords: *Intestinal morphology, Broiler chicken, Anticoccidial drug*

Deneysel Koksidiyozlu Tavuklarda Clopidol ve Amprolium/Ethopabate Uygulamasının Performans ve Barsak Morfolojisine Etkisi

Özet

İki antikoksidial ilacın deneysel koksidiyaziste büyüme performansı ve barsak mukozasına etkilerini araştırmak amacıyla broiler civcivlere Eimeria oositleri/Clopidol ve Amprolium + Ethopabate verildi. Bu uygulamaların yapıldığı tavuklarda büyüme performansının negatif olarak etkilendiği belirlendi. Barsağın çoğu morfometrik parametrelerinin uygulama gruplarında negatif kontrole oranla azaldığı tespit edildi (P<0.05). Uygulama gruplarında duodenal villus yüksekliği ve tüm enfekte hayvanlarda villus yüzey alanı uygulama grupları arasında en düşüktü (P<0.05). Duodenal villus genişliği tüm ilaç uygulama gruplarında negatif kontrole oranla azalmıştı. Ancak bu azalma sadece enfekte/ilaç uygulanan gruplarda anlamlı idi. Jejunum villus yüksekliği, genişliği ve yüzey alanı enfekte/ilaç uygulanmayan grupta diğer enfekte olmayan /ilaç uygulanan gruplara oranla en azdı (P<0.05). Enfekte/ilaç uygulanmamış grupta ileum villus yüksekliği tüm ilaç uygulanan gruplara oranla en azdı. Enfekte/ilaç uygulanan gruplarda ileum villus yüzeyi alanı infekte olmayan/ilaç uygulanan gruplara oranla anlamlı derecede daha azdı. Barsak morfometrik değerlendirmede Clopidol- ve Amprolium + Ethopabate-uygulanan gruplar arasında belirgin bir fark yoktu. Kontrol veya koksidiyozu önleme maksatlı Clopidol ve Amprolium + Ethopabate uygulamasının tavuklarda performans ve barsak morfolojisine, özellikle villus boyutları ve sindirici yüzeye, olumsuz etkileri olduğu sonucuna varılmıştır.

Anahtar sözcükler: *Barsak morfolojisi, Broiler tavuk, Antikoksidial ilaç*

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INTRODUCTION

The coccidia as a protozoa are single celled parasites in animals in the phylum *Apicomplexa* and genus *Eimeria*. The coccidia are host-specific. The triggered place of coccidia species is the lining of the intestine or ceca in the chicken [1,2].

In the poultry industry, coccidiosis is a permanent problem; and outbreaks of coccidiosis still occur despite of the improved management conditions in broiler rearing [3,4].

High-intensity systems are leading to dependence on anticoccidial feed additives in broiler rearing to provide prophylactic control against infections due to pathogenetic species of *Eimeria*. In the modern farms, the warm humid environment due to high stocking density, provide suitable condition for *Eimeria* infection. Nine species of coccidia (from genus *Eimeria*) infect poultry [5,6]. The severity of pathogenesis caused by each species of *Eimeria* varies. The most pathogenic *Eimeria* (*E*) in chickens are *E. acervulina, E. brunetti, E. maxima, E. mitis, E. necatrix, E. praecox*, and *E. tenella* which can provide outbreaks of coccidiosis [1,4]. These parasitic agents invade the mucosa, proliferate in the intestinal epithelium and provide different pathological lesions (moderate to severe) in various segments of intestine.

Todays, in the poultry industry, effective control of coccidiosis is still important to obtain profitable production. In this disease, because of oocyst resiliency, chemoprophylaxis by the use of anticoccidial drugs would be necessary. Since the utilization of the sulphonamides as the first chemical anticoccidial drugs, the development and introduction of a continual succession of these and other drugs have been more or less successful in prophylaxis of the disease [7]. At the present study, the effects of two anticoccidial drugs (Clopidol and Amprolium/Ethopabate as chemical drug) were evaluated on the performance and intestinal morphology of healthy and chaleged broiler chicken. The used drugs in this experiment are the usual anticoccidials in the poultry industry, used as prophylactic agents or against clinical and subclinical forms of coccidiosis.

MATERIAL and METHODS

Animals, Management and Treatments

Two hundred and sixteen, one-day-old fast-growing chickens from Ross 308 breed were randomly divided into six equal groups with three replicates per group (36 birds per group). Chicks were reared in floor pens on wood shaving litter at standard condition for six weeks and provided *ad libitum* access to water and a standard basal diet. The basal diets were in mash form and formulated for starter (1-14 d), grower (15-29 d), and finisher (30-42 d) growth periods and the composition is shown in *Table 1* [8]. The drugs were fed to the birds from 21 days of age. Three groups of chickens in this experiment were infected orally at day 21 by a mix of four species of sporulated oocytes, consist of 2.5×10^4 *E. acervulina*, 2×10^4 *E. maxima and* 2.5×10^4 *E. necatrix* and 8×10^4 *E. tenella*. Experimental groups were designed as following:

Group A: As negative control, uninfected/unmedicated control.

Group B: As uninfected/clopidol, uninfected while medicated with 125 ppm clopidol.

Group C: As uninfected/amprolium + ethopabate, uninfected while medicated with 125 ppm Amprolium/8 ppm ethopabate.

Group D: As positive control, infected with 1.5×10^5 mixed oocytes while unmedicated.

Group E: As infected/amprolium + ethopabate, infected with 1.5×10^5 mixed oocytes and medicated with 125 ppm Amprolium/8 ppm ethopabate .

Group F: As infected/clopidol, infected with 1.5×10^5 mixed oocytes and medicated with 125 ppm clopidol.

Feed consumption and body weight were recorded in the each group, feed conversion were calculated at the end of experiment. Excreted oocysts were counted from 5 to 11 days after infection according to Pirali Kheirabadi et al.[9] and offered as OPG (oocytes per gram feces). The mortality rate of chickens in each group was recorded during rearing. The study was approved by the Ethics Committee of Shahrekord University.

Morphometric Analysis of the Intestine

At 42 days of age, 6 chicks from each group were killed by decapitation and their intestinal morphometric variables i.e., villus sizes (height, width, surface area and lamina propria thickness) evaluated according to Zamani Moghaddam et al.[10] in the duodenum, jejunum and ileum. Briefly, midpoint segments of the duodenum, jejunum, and ileum were dissected. The segments were fixed in Clark fixative for 45 min, and then left in ethyl alcohol for longer storage. Each segment was divided into 3 sections along its length. Sections were left in periodic acid Schiff (PAS) reagent for 2-3 min, rows of villi were cut in thickness of the sections, transferred over the glass slides and covered with a cover-slip. These samples were examined by a microscope with eyepiece graticules (10×) and magnification of ×100 [10,11]. The villus height was measured from the top of the villus to the top of the lamina propria. Surface area was calculated using the formula = $(\pi) \times (VW) \times (VL)$ in which VW = villus width and VL = villus length [12]. The lamina propria thickness was measured at the space between the base of the villus and the top of the muscularis mucosa. The muscle layer was measured from the top of the muscularis propria to the serosa [13]. In each bird, three segments of each one of the duodenum, jejunum and ileum was examined.

Statistical Analysis

All data are represented as mean ± SE. Results of OPG, intestinal and performance parameters were compared between groups using one way ANOVA (SPSS Institute Inc.). All data were checked to have a normal distribution and log transformed if necessary. Any data requiring log transformation were back-transformed for presentation of data. *P* values less than 0.05 were significant.

RESULTS

Estimation of Oocyst

The numbers of OPG of feces from the infected/medicated groups of chickens were counted from days 5 to 11 post challenge that are shown in *Fig. 1*. The shedding of oocysts was recorded as early as on the 5th day post challenge in experimentally infected groups (D, E and F) while there was not any oocyst in the feces of uninfected groups (A, B and C) (data not shown). There was significant decreasing of OPG in the groups E and F in days 5, 6, 7, 8 and 9 as compared to other infected group (D, positive control) (P<0.05). There was also significant decreasing of OPG in the group F in days 6, 7 and 8 in compared to group E (P<0.05) *(Fig. 1)*.

Growth Performance in the Experimental Groups

Effects of Clopidol and Amprolium/Ethopabate on the performance of infected and uninfected chickens are presented in *Table 2* and *Table 3*. The parameters of bird performance were presented weekly during rearing. The mean body weight of chickens in the infected or medicated groups was significantly (P<0.05) decreased in weeks 4, 5 and 6 as compared to negative control (Group A). The mean body weight of chickens in group D was lowest in the weeks 4 (P<0.05) and 5 in comparison to other groups *(Table 2)*. Conversely, The FCR of infected or medicated groups was higher than negative control (Group A) in five last weeks of rearing, which this increasing was only significant (P<0.05) in week 4. In this week, chickens in group D had highest FCR (P<0.05) *(Table 3)*.

There were no significant (P>0.05) differences between Clopidol-medicated and Amprolium + Ethopabate medicated groups in growth performance of chickens (*Table 2* & *3*).

The mortality was only observed in group D (Infected/unmedicated) that its rate was about 11.1%.

Intestinal Morphometric Assessment

Intestinal morphometric parameters were compared

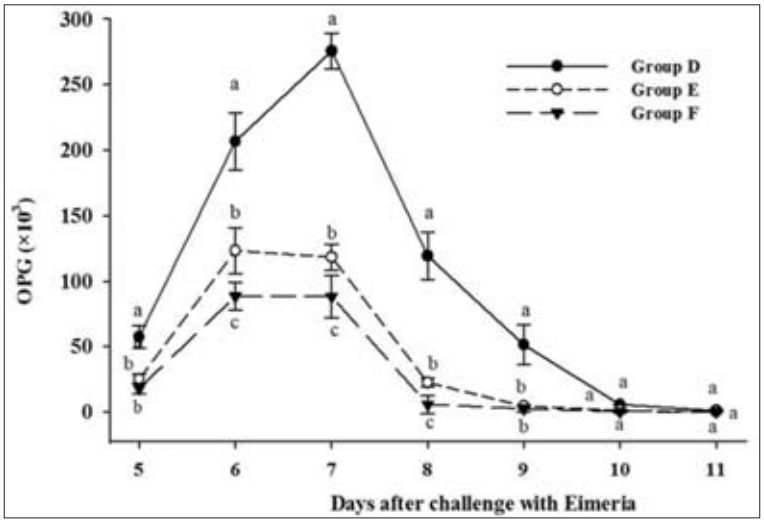

Fig 1. Comparison of number of oocytes per gram feces (OPG) between chicken groups, 5 to 11 days after experimental challenge with *Eimeria,* [a,b,c] Means with the different indices between groups at each day are significantly different for P<0.05. Group D, positive control (infected/unmedicated); Group E, infected/amprolium; Group F, infected/clopidol

Şekil 1. *Eimeria* uygulamasından 5 ile 11 gün sonrasında gruplar arasında her gram dışkıda oosit sayılarının karşılaştırılması, [a,b,c] belirteçleri gruplar arasında her bir gün için anlamlı derecede P<0.05 için farklılığı göstermektedir. Grup D, pozitif kontrol (enfekte/ilaç uygulanmamış); Grup E, enfekte/amprolium; Grup F, enfekte/clopidol

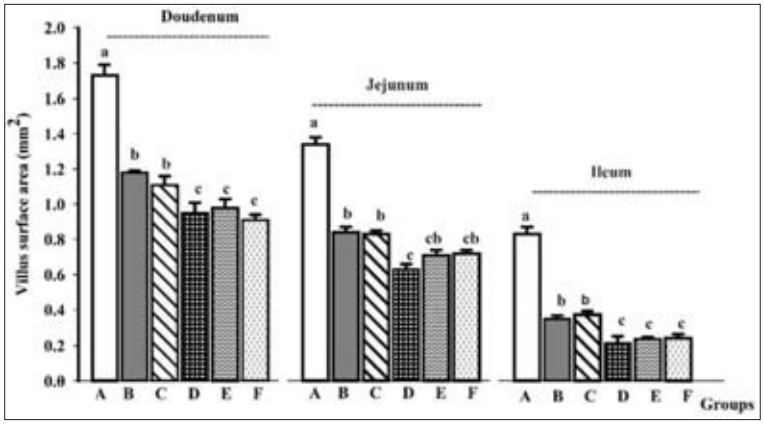

Fig 2. Villus surface area in three intestinal parts in the experimental groups of chickens at 42 days of age, [a,b,c] Means with the different indices between groups are significantly different for *P<0.05*. Group A, negative control (uninfected/unmedicated); Group B, uninfected/Clopidol; Group C, uninfected/amprolium; Group D, positive control (infected/unmedicated); Group E, infected/amprolium; Group F, infected/clopidol

Şekil 2. 42 günlük deneysel gruplardaki tavuklarda üç farklı barsak bölgesinin villus yüzey alanı, [a,b,c] belirteçleri gruplar arasında anlamlı derecede P<0.05 için farklılığı göstermektedir. Grup A, negatif kontrol (enfekte olmamış/ilaç uygulanmamış); Grup B, ilaç uygulanmamış/clopidol; Grup C, enfekte olmamış/amprolium; Group D, pozitif kontrol (enfekte/ilaç uygulanmamış); Grup E, enfekte/amprolium; Group F, enfekte/clopidol

between experimental groups of chickens at 42 days of age that are shown in *Table 4* and *Fig. 2*.

The duodenal villus height, lamina propria thickness and surface area were significantly lower in the infected or medicated groups than negative control (group A) (P<0.05; *Table 4; Fig. 2*). The duodenal villus height in group D (infected/unmedicated) and villus surface area in all infected groups (D, E and F) were lowest between infected or medicated groups (P<0.05). The duodenal width was also decreased in all treated groups compared to negative control (group A), but this decreasing was only significant in groups E and F (P<0.05).

The jejunal villus height, width, lamina propria thickness and surface area were significantly lower in the infected or medicated groups than negative control (group A) (P<0.05; *Table 4; Fig. 2*). The jejunal villus height, width and surface area in group D (infected/unmedicated) were lowest as compared to other uninfected/medicated groups (B, C) (P<0.05).

The ileal villus height, width and surface area were significantly decreased in the infected or medicated groups than negative control (group A) (P<0.05; *Table 4; Fig. 2*).

The ileal villus height in group D (infected/unmedicated) were lowest as compared to groups B, C (P<0.05), E and F. The ileal lamina propria thickness was not different among experimental groups (P>0.05; *Table 4*). In the infected/medicated groups (D, E and F), the ileal villus surface area was significantly lower than uninfected/medicated groups (B and C) (P<0.05; *Fig. 2*).

There were no significant (P>0.05) differences between Clopidol- and Amprolium + Ethopabate-medicated groups in the intestinal morphometric assessment *(Table 4; Fig. 2)*.

Table 1 .*Composition of basal diets*
Tablo 1. *Bazal diyetin kompozisyonu*

Ingredients	Starter (1-14 d)	Grower (15-29 d)	Finisher (30-42 d)
Corn	53.01	58.65	63.95
Soybean meal (44% CP)	39.01	33.51	27.85
Soybean oil	2.89	3.21	3.82
Limestone	1.44	1.35	1.33
Dicalcium phosphate	2.27	2.00	1.83
Vitamin mixture [1]	0.50	0.50	0.50
Mineral mixture [2]	0.30	0.25	0.25
Salt	0.30	0.30	0.30
DL-methionine L- lysine Vitamin E	0.23 0.03 0.02	0.20 0.01 0.02	0.14 0.01 0.02
Calculated Chemical Composition			
ME (Kcal/kg)	2900	3000	3100
Crude protein (%)	22	20	18

[1] Supplied per kg diet: vitamin A, 90.00IU; cholecalciferal, 1.500 IU; vitamin E, 10 IU; vitamin K, 0.5mg; cobalamin, 0.007 mg; thiamin 0.4 mg; riboflavin, 6 mg; folic acid, 1 mg; biotin, 0.15 mg; pantothenic acid 12 mg; niacin, 35 mg; pyridoxine, 4 mg; cholin chloride, 1.000 mg; [2] Supplied per kg diet: Mn, 60 mg; Cu, 5 mg; Zn, 50 mg; I, 0.35 mg; Se, 0.1 mg; iron 40 mg

DISCUSSION

Maintaining bird health, regarding diseases or agents acting on the gastrointestinal tract, is crucial in broiler production, since this is the entry route of nutrients for bird development. The small intestine is responsible for the digestion and absorption of nutrients from food, and the duodenal segment mainly for absorption [14]. Broilers exhibiting shortening of villi have impaired nutrient absorption [14,15]. Cell divisions in the intestine of birds, unlike mammals, are not restricted to crypts, occurring also along the villi [16]. According to Nabuurs [17], the ideal intestinal morphometry in birds are long villi and shallow crypts. That is, length of villi is related to the digestive capacity and intestinal absorptive area [16,18]. However, factors such as dietary supplements, drugs or pathogens can cause changes in the intestinal morphology [19,20].

Table 2. *Comparison of chicken body weights (g) in the experimental groups during rearing*
Tablo 2. *Yetiştirme süresince deneysel gruplarda tavuk vücut ağırlıklarının (g) karşılaştırılması*

Group	n	Weeks					
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Group A	36	107.9	323.1	659.5	1136.6^{a}	1649.2^{a}	2158.2^{a}
Group B	36	104.3	306.9	634.7	986.2^{b}	1431.6^{b}	1948.6^{b}
Group C	36	109.8	299.4	615.5	976.9^{b}	1444.3^{b}	1982.9^{b}
Group D	36	114.3	310.6	668.3	845.9^{c}	1276.4^{c}	1913.9^{b}
Group E	36	106.1	309.2	629.1	946.5^{b}	1389.0^{bc}	1934.6^{b}
Group F	36	107.3	307.6	643.5	930.0^{b}	1360.7^{bc}	1915.6^{b}
Pooled SEM	-	3.7	6.3	20.6	20.7	38.1	38.3

^{a,b,c} Means with the different indices between groups at end of each week are significantly different for P<0.05. Group A, negative control (uninfected/unmedicated); Group B, uninfected/Clopidol; Group C, uninfected/Amprolium; Group D, positive control (infected/unmedicated); Group E, infected/Amprolium; Group F, infected/Clopidol

Table 3. *Comparison of feed conversion rate (FCR) in the experimental groups of chickens during rearing*
Tablo 3. *Yetiştirme süresince deneysel gruplarda yem dönüşüm oranlarının (FCR)karşılaştırılması*

Group	n	Weeks					
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Group A	36	0.98	1.25	1.57	2.04^{a}	2.18	2.33
Group B	36	0.97	1.26	1.65	2.30^{b}	2.38	2.44
Group C	36	0.99	1.24	1.70	2.26^{ab}	2.37	2.34
Group D	36	0.99	1.23	1.59	2.69^{c}	2.49	2.52
Group E	36	1.00	1.24	1.60	2.35^{b}	2.46	2.39
Group F	36	1.01	1.28	1.66	2.40^{b}	2.43	2.50
Pooled SEM	-	0.03	0.01	0.03	0.06	0.07	0.05

^{a,b,c} Means with the different indices between groups at each week are significantly different for P<0.05. Group A, negative control (uninfected/unmedicated); Group B, uninfected/Clopidol; Group C, uninfected/Amprolium; Group D, positive control (infected/unmedicated); Group E, infected/Amprolium; Group F, infected/Clopidol

Table 4 .*Comparison of intestinal morphology between experimental groups of chickens at 42 days of age*
Tablo 4. *42 günlük tavuklarda deneysel grupların barsak morfolojilerini karşılaştırılması*

Intestine	Group	n	Villus Dimensions		
			Height (mm)	Width (mm)	Lamina Propria (mm)
Duodenum	Group A	6	0.79^{a}	0.69^{a}	0.33^{a}
	Group B	6	0.64^{b}	0.58^{ab}	0.23^{b}
	Group C	6	0.62^{b}	0.57^{ab}	0.23^{b}
	Group D	6	0.52^{c}	0.57^{ab}	0.21^{b}
	Group E	6	0.57^{bc}	0.54^{b}	0.21^{b}
	Group F	6	0.57^{bc}	0.51^{b}	0.21^{b}
	Pooled SEM	-	0.01	0.02	0.01
Jejunum	Group A	6	0.61^{a}	0.69^{a}	0.30^{a}
	Group B	6	0.49^{b}	0.54^{b}	0.25^{b}
	Group C	6	0.50^{b}	0.53^{b}	0.25^{b}
	Group D	6	0.45^{c}	0.44^{c}	0.22^{b}
	Group E	6	0.47^{bc}	0.47^{bc}	0.25^{b}
	Group F	6	0.48^{bc}	0.47^{bc}	0.24^{b}
	Pooled SEM	-	0.02	0.01	0.01
Ileum	Group A	6	0.37^{a}	0.72^{a}	0.22
	Group B	6	0.21^{bc}	0.53^{b}	0.22
	Group C	6	0.25^{b}	0.48^{b}	0.20
	Group D	6	0.14^{c}	0.49^{b}	0.22
	Group E	6	0.17^{bc}	0.44^{b}	0.21
	Group F	6	0.18^{bc}	0.43^{b}	0.21
	Pooled SEM	-	0.02	0.02	0.01

^{a,b,c} Means with the different indices between groups at each segment of intestine are significantly different for P<0.05. Group A, negative control (uninfected/unmedicated); Group B, uninfected/Clopidol; Group C, uninfected/Amprolium; Group D, positive control (infected/unmedicated); Group E, infected/Amprolium; Group F, infected/Clopidol; ***n:*** *number of chickens*

Nowadays, it is common that anicoccidials are used prophylactically throughout the entire growing period in chicken to achieve total continual prevention of occurrence or suppression of coccidiosis.

In the present study, we used two anticoccidial drugs, Clopidol and Amprolium + Ethopabate as protection in the diets of healthy chickens for 3 weeks and found their adverse effects on the bird performance i.e. body weight and feed conversion rate. It has also been reported that with no *Eimeria* present in the chicken, each intake of an anticoccidial drug lead to a negative effect on growth or feed conversion of a bird [21]. Anticoccidial drugs have a narrow margin of safety and some of them are toxic [22]. In

fact, in the most experiments, a negative influence is seen. This is different for each drug and depends on the dosage administered. However, as soon as *Eimeria* infections build up in the flocks, the possible growth depressing effect of the drug will be neutralized by effective control of the infection. The compensatory growth seen after withdrawal of the drug from the feed, could also be explained by the growth depression effect of the drug used in their study [23]. Brake *et al.*[24] also reported adverse effects of semduramicin, on broiler breeders. They found that semduramicin causes dose-related decrease in egg production, percentage shell, fertile hatchability and increase in early embryonic mortality.

Hassanpour et al.[21] reported when diclazuril, semduramicin, salinomycin and maduramycin were used as prophylactic drugs against chicken coccidiosis, intestinal morphometric parameters especially villus dimensions and absorptive surface were severely diminished.

To our knowledge, the effects of Clopidol and Amprolium + Ethopabate on intestinal mucosa have not been studied previously in the intact and infected chickens. In this experiment, we found that these drugs decreased villous length, width and surface area, in the duodenum and jejunum and ileum. However these data is probably the evidence of impaired nutrition absorption of intestine and reduced enteric function due to anticoccidial drugs.

In this study, we also reported the reduction of lamina propria thickness in the duodenum and jejunum by anti-coccidials, which apparently show a deminished Lieberkühn's glands in these segments of intestine. Thus, it could be predictable that these drugs may influence intestinal secretion in chickens. Of course, it needs further studies to clarify the effect of anticoccidials on the intestinal secretions in broiler chickens.

The functions of Clopidol and Amprolium + Ethopabate on controlling of *Eimeria* infection, growth performance and intestinal morphology were compared together in the present study. In regarding to reduction of shedding oocysts in the infected chickens, Clopidol had better effect than Amprolium + Ethopabate while adverse effects of these drugs on the body performance and intestine were similar.

It is concluded that Clopidol and Amprolium + Ethopabate have adverse effects on chicken performance and intestinal morphology especially villus dimensions and absorptive surface during control or prevention of coccidiosis. There was also evidence for involvement of these drugs in the intestinal secretion that need more studies to confirm.

REFERENCES

1. Conway DP, McKenzie ME: Poultry Coccidiosis. Diagnostic and Testing Procedures. 3rd ed., 168, Wiley-Blackwell, Iowa, USA, 2007.

2. Sarkar AK: Pathological study of coccidiosis in chick bird. *Res J Anim Vet Sci*, 1, 55-56, 2006.

3. Cox FEG: Control of coccidiosis: Lessons from other sporozoa. *Inter J Parasitol*, 28, 165-179, 1998.

4. Ernik F, Bedrnik P: Controlling coccidiosis in broiler growing. *Poult Inter*, 40, 36-42, 2001.

5. Chapman HD: Evaluation of the efficacy of anticoccidial drugs against *Eimeria* species in the fowl. *Inter J Parasitol*, 28, 1141-1144, 1998.

6. Riviere JE, Papich MG: Veterinary Pharmacology and Therapeutics. 9th ed., 1500, Wiley-Blackwell, Iowa, USA, 2009.

7. Kitandu A, Juranova R: Progress in control measures for chicken coccidiosis. *Acta Vet Brno*, 75, 265-276, 2006.

8. NRC: Nutrient Requirements of Poultry. 9th ed., 176, National Academy of Science Press, Washington D.C., 1994

9. Pirali Kheirabadi KH, Hassanpour H, Nourani H, Farahmand E, Cheraghchi Bashi M, Hossainpour Jaghdani F: Increasing of serum nitric oxide metabolites in chicken *Eimeria* infection. *Iran J Vet Med,* 5, 99-103, 2011.

10. Zamani Moghaddam AK, Hassanpour H, Mokhtari A: Oral supplementation with vitamin C improves intestinal mucosa morphology in the pulmonary hypertensive broiler chicken. *Brit Poult Sci*, 50, 175-180, 2009.

11. Teshfam M, Nodeh H, Hassanzadeh M: Alterations in the intestinal mucosal structure following oral administration of triiodothyronine (T_3) in broiler chickens. *J Appl Anim Res*, 27, 105, 2005.

12. Sakamoto K, Hirose H, Onizuka A, Hayashi M, Futamura N, Kawamura Y, Ezaki T: Quantitative study of changes in intestinal morphology and mucus gel on total parenteral nutrition in rats. *J Surg Res*, 94, 99-106, 2000.

13. Aptekmann KP, Artoni SM, Stefanini MA, Orsi MA: Morphometric analysis of the intestine of domestic quails *(Coturnix coturnix japonica)* treated with different levels of dietary calcium. *Anat Histol Embryol*, 30, 277-280, 2001.

14. Assis RCL, Luns FD, Beletti ME, Assis RL, Nasser NM, Faria ESM, Cury MC: Histomorphometry and macroscopic intestinal lesions in broilers infected with *Eimeria* acervulina. *Vet Parasitol*, 168, 185-189, 2010.

15. Klein BG: Cunningham's Textbook of Veterinary Physiology. 5th ed., 624, Saunders Elsevier, Philadelphia, 2012.

16. Uni Z, Ganot S, Sklan D: Posthatch development of mucosal function in the broiler small intestine. *Poult Sci*, 77, 75-82, 1998.

17. Nabuurs MJA: Microbiological, structural and functional changes of the small intestine of pigs at weaning. *Pig news and Information,* 16, 93-97, 1995.

18. Uni Z, Zaiger G, Gal-Garber O, Pines M, Rozenboim I, Reifen R: Vitamin A deficiency interferes with proliferation and maturation of cells in the chicken small intestine. *Brit Poult Sci*, 41, 410-415, 2000.

19. Pluske JR, Hampson DJ, Williams IH: Factors influencing the structure and function of the small intestine in the weaned pig: A review. *Livest Prod Sci*, 51, 215-236, 1997.

20. Hassanpour H, Moghaddam AK, Khosravi M, Mayahi M: Effects of synbiotic on the intestinal morphology and humoral immune response in broiler chickens. *Livest Sci*, 153, 116-122, 2013.

21. Hassanpour H, Bahadoran S, Koosha S, Askari E, Homai S: Effect of diclazuril, semduramicin, salinomycin and maduramycin as preventive anticoccidial drugs on chicken intestinal morphology. *Global Vet*, 5, 1-5, 2010.

22. Hosseini R, Rajaian H, Hajimohammadi A, Nazifi S, Khaliji E, Asmarian S: ECG alterations and changes in biochemical parameters associated with experimental salinomycin toxicosis in sheep. *Iran J Vet Res*, 14, 120-125, 2013.

23. Tipu MA, Pasha TN, Ali Z: Comparative efficacy of salinomycin sodium and neem fruit *(Azadirachta indica)* as feed additive anticoccidials in broilers. *Inter J Poult Sci*, 1, 91-93, 2002.

24. Brake J, Cummings TS, Pinedo CR, Bafundo KW: Effects of the ionophore anticoccidial semduramicin on broiler breeders. *Poult Sci*, 80, 260, 2001.