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Overdosing of the Ionophore Anticoccidial Semduramicin Induces Unrecoverable Performance Depression Associated with Striated Muscle Lesions

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Abstract: Semduramicin is a potent ionophore anticoccidial provided for prophylaxis against coccidiosis. Here, we provide evidence that overdosing of semduramicin induces unrecoverable suppression of chicken growth accompanied by lesions in the heart and skeletal muscles. Semduramicin at 20 and 60 mg/kg of feed showed high anticoccidial efficacy and improvement of gross and micscopic lesions induced by coccidiosis. Significant compensatory weight gain had occurred after withdrawal of semduramicin at 20 mg/kg. However, in spite of the marked control of coccidiosis, the weight of chicken fed on 60 mg/kg semduramicin was significantly lower than the control infected nontreated group. These finding were associated with degenerative changes and necrosis of heart and skeletal muscles and with a lesser extent focal changes in liver manifested by hepatic cells degeneration, necrosis and aggregation of mononuclear cells. Thus, overdosing of semduramicin has deleterious effects on chicken performance and produces specific affection of striated muscles. There were high recovery from coccidiosis, therefore, these effects might be directly related to the toxic effect of the drug.

Key words: Coccidiosis · Ionophores · Semduramicin · E. tenella

INTRODUCTION

Coccidiosis is caused by Apicomlexan intracellular protozoan parasites belonging to the genus Eimeria remains one of the economically most important diseases in the modern poultry industry [1]. Coccidiosis had impacted the poultry industry and costs the world economy of more than 3 billion US dollars annually [2]. There are seven valid species of Eimeria that parasitise chickens. They are *Eimeria acervulina*, *Eimeria brunetti*, *Eimeria maxima*, *Eimeria mitis*, *Eimeria necatrix*, *Eimeria praecox* and *Eimeria tenella* and they occur throughout the world wherever domesticated fowl are reared. *Eimeria tenella* is the most dangerous species causing dramatic economic losses in poultry farms [3].

Ionophaore antibiotics are very potent anticoccidials, exerting their effect in low concentrations. For more than forty years, ionophores have been used as powerful tools in controlling coccidiosis [4]. Since the introduction of monensin in early 1970s, it took the leading role in most control programs. This raised the interest for developing more potent and less toxic drugs and research studies had lead to the introduction of other ionophores as lasalocid, narasin, salinomycin, maduramicin and semduramicin. Roughly speaking, semduramicin is safe for chicken at recommended doses; however, toxicity issues can rise from overdosing or inappropriate use of the drug [5]. Semduramicin presents a broad spectrum of anticoccidial activity against Eimeria spp. at dietary inclusion levels within the concentration range (20-25mgkg^{-1} complete feeding stuffs) [6]. Overdosing of ionophores anticoccidial had lead to serious toxicity problems. Several studies were adopted to detect the adverse effects associated with overdosing of ionophores as monensin [7,8], lasalocid [9,10], salinomycin [11,12] and maduramicin [13]. In contrast, little is known about the effect of overdosing of semduramicin. Previously three experiments were conducted to assess the effects of contamination of feed with semduramicin on broiler breeders. The data showed that adverse effects of semduramicin require greater than 1 wk of exposure to be evident [14].

Semduramicin is approved for chickens for fattening at a dose range of 20 to 25 mg /kg with a withdrawal time of 5 days. Semduramicin ionophore was added to corn and soybean meal-based broiler diets at the recommended

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level, 25 mg /kg feed for 0, 34, 39, or 42 day for 49 days trial resulted in withdrawal times of 0, 7, 10 and 15 days. Significant differences among experiments were observed but no significant differences due to semduramicin were noted in body weight or feed intake. Feeding semduramicin with a 10-or 15-day withdrawal period resulted in an improvement in feed conversion of about 0.04 units [15].

The use of Semduramicin by the recommended dose is well tolerated by broilers, however with small margin of safety. But breeder hens respond to the recommended dose with a decrease in cumulative egg production and percentage shell and an increase in early embryonic mortality. These adverse effects became evident after 1 week of exposure [14]. In this study, we would like to assess the effect of overdosing of semduramicin on the efficacy against cecal coccidiosis, chicken performance and pathological effects on different organs.

MATERIALS AND METHODS

Facilities and Chicken: Floor-pen studies were carried out to simulate field conditions. Mixed gender one-day old chicks were randomly assigned into five groups (50 chick/group). The first group was kept as non-infected non treated group. The second group was infected and did not receive any treatments. The third and fourth groups were fed on a ration containing with 20 or 60 $mgkg^{-1}$ semduramicin and infected with coccidiosis; S20, S60 inf group respectively. The fifth group (S60 group) was kept noninfected and treated with 60 $mgkg^{-1}$ semduramicin. Standard hygienic measures against infectious diseases were applied. All birds received humane care according to the criteria of the care and use of lab animals.

Isolating a Field E. tenella Strain, Sporulation Oocysts and Experimental Infection: In this study, the chicks were infected with a prepared *E. tenella* field strain. Oocysts were sporulated by pouring the fluid in a measuring cylinder and aerated by using aquarium pump. Identification of *E. tenella* was based on oocyst morphologic characters, predominant caecal lesions and the presence of characteristic schizonts and gametocytes. Oocysts were isolated by using the standard floatation-sedimentation technique. Dose titration was adopted to determine the pathogenesity of isolated strain. Experimental infection was carried out orally by direct inoculation into the crop at adose of 50 oocyst per chich.

Drug Administration: Chicken in groups 1 and 2 received anticoccidial free ration, where, groups 3 and 4 (S20 and S60inf) were fed rations containing semduramicin beginning from the first day of experiment. Experimental infection was carried out at day 14 of age. S60 group were fed on 60 $mgkg^{-1}$ semduramicin and kept free from infection. At day 35 of age, semduramicin was withdrawn from chicken feed and chicken were fed on anticoccidial free ration until the end of experiment at day 42 of age.

Monitored Parameters

Clinical Signs: The intensity of clinical signs of coccidiosis in infected nontreated and treated chicks was one of the most important evaluation methods of coccidiostats efficacy. These symptoms include diarrhea, bloody feces, stop feeding and depression.

Oocyst excretion was determined using ?otation with saturated NaCl solution followed by counting in McMaster chambers. Oocysts shedding started at day 6 post infection and oocysts count was undertaken until day 28 post infection.

Faecal Score: A fecal score (1 to 5) was recorded for each group of birds: a score of 1 indicated normal feces and a score of 5 indicated the presence of severe diarrhoea and/or a profuse amount of blood.

Chicken Performance: performance parameters as body weight and feed conversion rate (FCR) were monitored at day 35 (the end of drug administration) and day 42 (after one week of drug withdrawal).

Histopathological Examination: samples from cecum, liver, heart and skeletal muscle were collected 1 week post infection and at the end of experiment and were fixed in 10% neutral phosphate-buffered formalin solution. Following dehydration in ascending series of ethanol (70, 80, 96, 100%), tissue samples were cleared in xylene and embedded in paraffin and tissue section of 5 μm were stained with haematoxyline and eosin (H and E), for histopathological examination according to [16].

Macroscopic Lesion Scoring: A lesion scoring system was used from 0 to +3. In this scoring system, 0 indicated apparent lack of lesions, +1 little changes as redness or few submucosal hemorrhage, +2 indicated moderate changes as hemorrhagic cecal contents and marked submucosal hemorrhage, while, +3 indicated severe lesions as apparent necrotic changes and severe hemorrhagic lesions and bloody cecal contents.

Microscopic Lesion Scoring: Microscopic lesion scoring system (MLS) was adopted to assess the efficacy of used drug concentrations [17]. MLS depends on the severity of infection and distribution of eimeria stages among villi. The scores were as follows: 0 = no field contained coccidia, 1 = one field contained coccidia, 2 = two fields contained coccidia, 3 = three fields contained coccidia, and 4 = all four fields contained coccidia.

Measurement of Severity Score: Infection severity scoring system (ISS) was adopted to assess the efficacy of used drug concentrations [17]. ISS was based on the percentage of villi in the four fields examined that were parasitized by coccidia. The scores were as follows: 0 = no villi were parasitized, 1 = <25% of villi were parasitized, 2 = 25%-75% of villi were parasitized and 4 = >75% of villi were parasitized.

Statistical Analysis: The test parameters were analyzed by analysis of variance by using "SPSS 10.0 for Windows". The result is recorded as Mean (SD). All signi?cant differences were based upon $P< 0.05$. Coccidia multiply exponentially, not linearly, so pen oocyst counts were log 10 transformed prior to analysis [18].

RESULTS

Anticoccidial Efficacy

Clinical Signs: The infected non treated group showed depression, loss of appetite, anorexia, ruffled feathers and intensive bloody diarrhea one week after infection. S20 group showed no observable alteration in the coccidiosis clinical signs. S20 and S60 groups was free from the clinical coccidiosis, however, chicks were inappetant, depressed, with marked drowsiness even after withdrawal of the drug.

Fecal Scoring: The ceci of the control infected non treated showed the highest lesion score and the highest oocyst count. All treated groups showed highly improved lesion score, nearly +1 and zero, respectively.

Oocysts Count: Semduramicin at 20 $mgkg^{-1}$ showed marked anticoccidial efficacy (Table 1), in which the oocysts shedding was significantly low. Furthermore, S60inf group showed highly significant low oocysts shedding, even it was zero after 9 days post-infection (Table 1).

The Effect on Chicken Performance: At day 35, the body weight in the infected nontreated group was significantly lower than the non-infected group. Chickens given 20 $mgkg^{-1}$ semduramicin showed non-significant improvement in body weight. Furthermore, S60 and S60inf groups showed deteriorated performance. In these groups, chicken lost more than 36% of its body weight compared with the infected non-treated group (Table 2).

At day 42, the body weight in the infected nontreated group was significantly lower than the non-infected group. Chickens given 20 $mgkg^{-1}$ semduramicin showed significant improvement in body weight. Furthermore, S60 and S60inf groups showed deteriorated performance.

Table 1: Oocyst count mean (standard deviation) from the control infected nontreated group or receiving 20 or 60 mg/kg semduramicin

	Oocyst count/g faeces × 10^4		
Days post infection	Control +ve[e]	Semduramicin20 $mgkg^{-1}$	Semduramicin 60 $mgkg^{-1}$
6	3(10)[a]	1.4 (0.3)[a]	0.2 (0.02)[c]
7	180(100)[a]	5(0.5)[b]	0.6 (0.04)[c]
8	950(2.6)[a]	8(1)[b]	1 (0.06)[c]
9	600(0.2)[a]	14 (1.7)[b]	0.6(0.01)[c]
10	200(0.7)[a]	10(1.4)[b]	0[c]
11	50(3.6)[a]	2.5 (0.1)[b]	0[c]
12	40(2)[a]	0.5(0.1)[b]	0[c]
13	10(2)[a]	0[b]	0[b]

[a,b,c] Means within a row with different superscripts are significantly different ($P<0.05$).

Table 2: The effect of different treatments on average body weight and feed conversion rate. Measurements are taken at day 35 and 42 (one week after withdrawal of the drug) of age

Parameter	Control-ve[a]	Control +ve[b]	S20[c]	S60inf[d]	S60[e]
Average body weight (day 35)	1592(35)	1460 (55)	1465(35)	700(66)	688(76)
Average body weight (day 42)	1995(40)	1690(58)	1901(45)	910(100)	900(177)
Feed conversion (day 35)	1.8	2.3	2	2.4	2.4
Feed conversion (day 42)	1.9	2.4	2.2	2.3	2.3

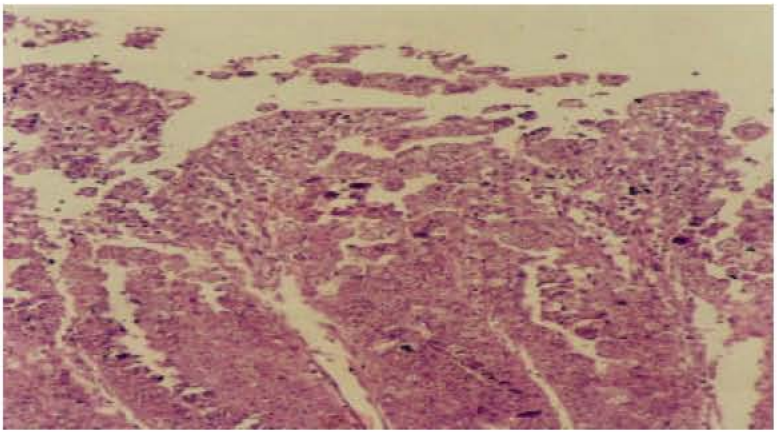

Fig. 1: Section from cecum of the infected nontreated group showing extensive infection of lining epithelium with various parasitic stages of *E. tenella*. The cecal content is overloaded with parasitic stages, inflammatory cells and desquamated epithelium. HE.X20

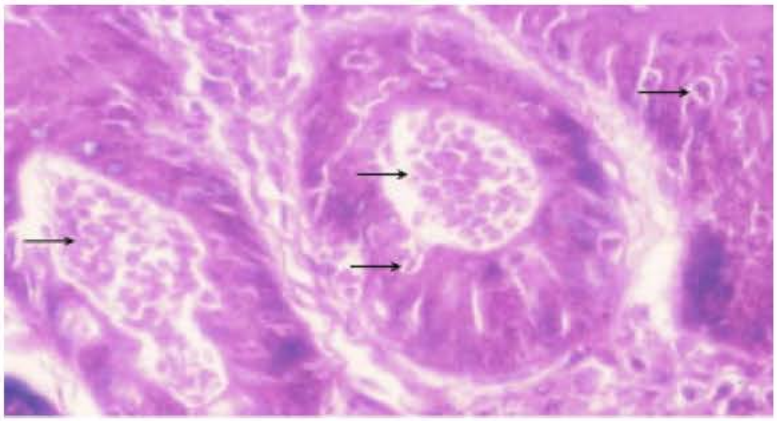

Fig. 2: Section from cecum of the S20 group showing partial desquamation of lining epithelium into the lumen of cecal glands. Few degenerated parasitic stages can be seen in the lining epithelium. HE.X40

In this group, chicken lost more than 35% of its body weight compared with the infected non-treated group. In addition, more than 900 g lighter than the noninfected nontreated group.

The infected non treated group showed a high FCR throughout the experiment. S20 group showed improved feed efficiency, while, S60 and S60inf groups showed higher FCR, which was higher than the infected nontreated group.

Histopathological Examination, Microscopic Lesion Scoring and Infection Severity Scoring: The infected non treated group showed the presence of various stages of coccidia in the cecal glands. Various developmental stages were detectable in the cecal epithelelium, while the cecal content is overloaded with schizonts and oocysts (Fig. 1). In S20 group, few developmental stages of Eimeria were evident in the cecal wall (Fig. 2). In S60inf, group, the lining epithelium showed little reaction of parasitological infection, little developmental stages of coccidia and almostly intact cecal epithelium (Fig. 3). The MLS was 4 for the infected nontreated group, 1.8 for S20 group, 0.2 for S60inf group and zero for S60 group. Other infected-treated groups showed marked reduction in the severity score corresponding to their microscopic lesion scoring system.

The liver of the infected non-treated birds showed hemorrhage, severe congestion and mild degenerative changes in liver. S20 group showed no changes. In S60 and S60inf groups, there were degeneration and necrosis of hepatic cells with mononuclear cell infiltration (Fig. 4).

Noticeable pathological alterations was found in S60 and S60inf group in the form of the myocardial necrosis and mononuclear cell infiltration (Fig. 5,6).

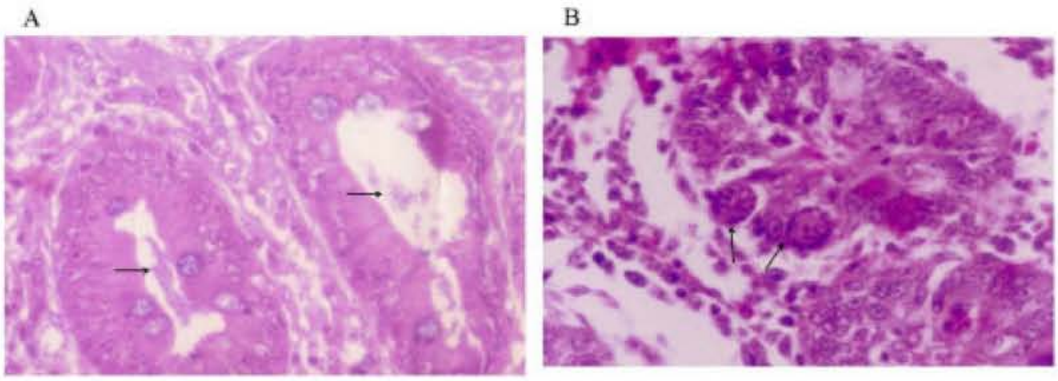

Fig. 3: Section from cecum of the S60inf group showing very little desquamation of lining epithelium into the lumen of cecal glands (3A). Parasitic stages are rarely seen in the lining epithelium. For every 20 examined fields, 1-2 parasitic stages can be detected (3B). HE.X40

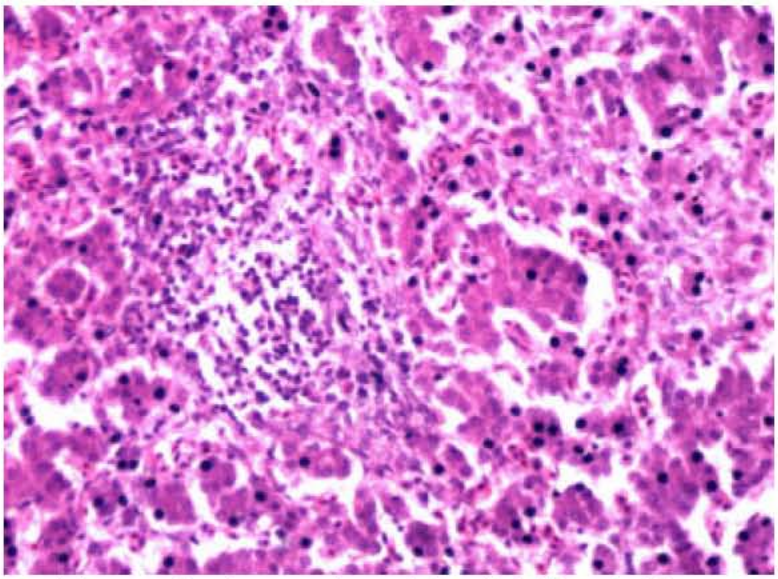

Fig. 4: Liver from S60 or S60inf groups showing area of necrosis heavily infiltrated with momnonuclear cells. HE.X20

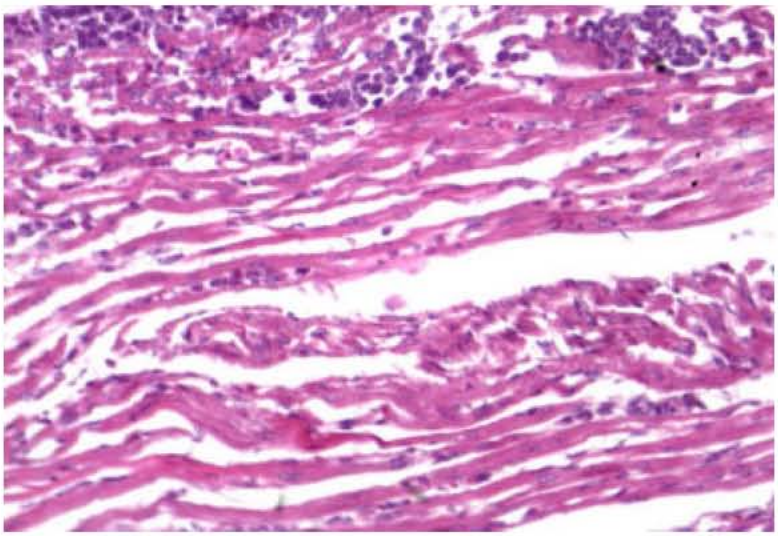

Fig. 5: Heart from S60 or S60inf groups showing infiltration of mononuclear cell in between myocardial muscle. HE.X20

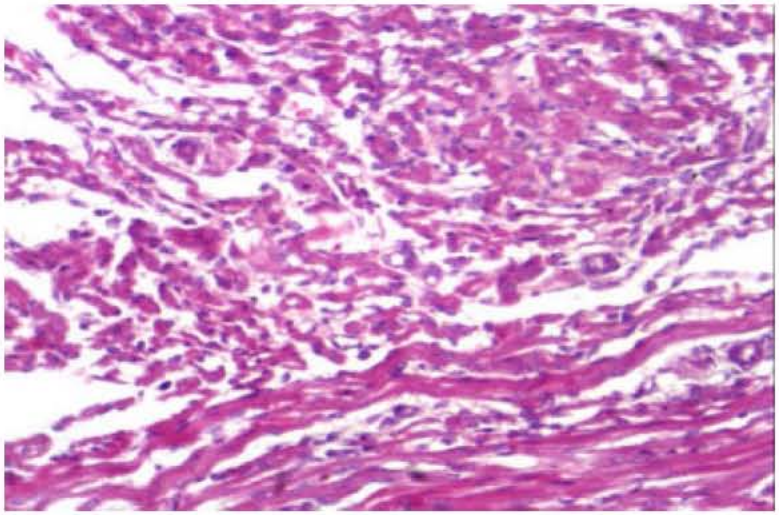

Fig. 6: Heart from S60 or S60inf groups showing degenerative and necrosis myocardial muscle fibers and intermuscular edema. HE.X20

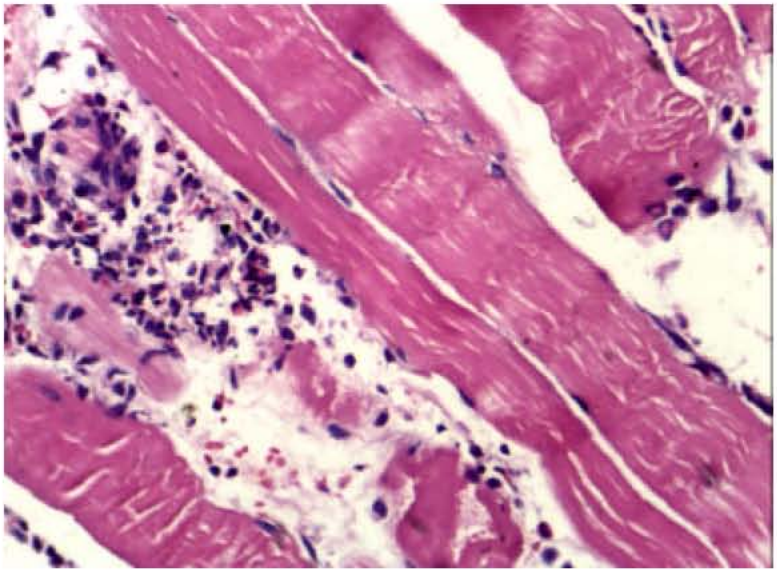

Fig. 7: Skeletal muscle from S60 or S60inf groups showing intermuscular edema, swelling of myofibrils, loss of cross striation, necrotic and fragmented myofibrils and intermuscular mononuclear cells infiltration. HE.X40

Noticeable pathological alterations of the skeletal muscle found in S60 and S60inf group, where intermuscular edema, swelling of myofibrils, loss of cross striation, necrotic and fragmented myofibrils and marked intermuscular mononuclear leucocytic infiltration (Fig. 7).

DISCUSSION

Ionophore anticoccidials are used in poultry diets for control of coccidiosis. The use of these drugs is accompanied with various growth-depressing issues. Sometimes, ionophores can depress the body weight gain when they are added to chicken diet, however, compensatory gain can occur. Hence, withdrawal time is essential for compensatory gain to occur. Accidental overdosing of drugs is very common in food additive mills. For instance, the very low dose level can be unevenly distributed in chicken feed, raising the possibility of toxicity issues for some consumer birds. The hypothesis of this experiment is that semduramicin affect the broiler chicken's response. So that, we tested the effect of the drug in its recommended level (20 $mgkg^{-1}$) and at 3 folds higher doses (60 $mgkg^{-1}$) in the presence or absence of coccidiosis infection.

In the control infected non treated group showed different stages of Eimeria in the cecal glands accompanied with massive esinophilic infilteration. S20 group showed marked drop of macrpscopic and microscopic lesions. S60inf and S60 groups showed progrsseive decrease in developmental stages of coccidia, indicating better control of coccidiosis.

In this study, the withdrawal of semduramicin from the chickens diet lead to marked regain of the lost performance, in this context, the withdrawal of semduramicin was proved to be essential for compensatory gain to occur [15]. Furthermore, several studies approved the lack interaction with various dietary compositions at the recommended level of semduramicin [19-21]. In the view of lack of data about overdosing of semduramicin, we checked the impact of adding 60 mg/kg semduramicin on chicken performance. From this study, we conclude that semduramicin has growth suppressing effect. The degree of growth suppression was more pronounced in chicken given higher dose (60 $mgkg^{-1}$) semduramicin. Interestingly, at the dose of 20 $mgkg^{-1}$, significant compensatory gain occurred after withdrawal of the drug. However, at dose level of 60 $mgkg^{-1}$ there was significant loss of final weight and there was no significant compensatory gain. These results indicated that overdosing of semduramicin produces non-recoverable suppression of performance.

S60inf and S60 groups showed various pathological features, which included cecum, liver, heart and skeletal muscles. Sections from liver showed degenerative changes, focal lymphocytic aggregation and necrotic areas in liver. These changes can be regarded as nonspecific toxic effect of semduramicin. The most predominant features of semduramicin overdosing were the affections of striated muscles. In the heart, there was progressive aggregation of mononuclear cells and degeneration and necrosis of cardiac myofibrils. Furthermore, skeletal muscles showed intermuscular edema, swelling of muscle fibers, degeneration and homogenization of muscle fibers and intermuscular mononuclear cells aggregation. These finding coincides with the toxic effect of semduramicin. The aggregation of mononuclear cells is a regular finding in cases of cellular toxicity [22-24]. The effect of semduramicin on skeletal muscle is proposed due to its ionophoric properties. The binding of semduramicin with monovalent cations may affect the normal function of heart and skeletal muscles. The increased concentration of intracellular cations as Na^+ or K^+ can lead to disturbance of intracellular calcium concentration by exchange with Na^+ or K^+ and hence, stimulate muscle dysfunction and damage.

In conclusion, 60 mg/kg semduramicin had produced efficient control of lesions associated with coccidiosis. However, significant body weight suppression was evident, which was not compensated even after withdrawal of the drug from chicken diets. These findings were associated with degenerative and necrotic changes in liver, heart and skeletal muscles.

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