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PATHOLOGICAL AND SERUM BIOCHEMICAL EFFECTS OF SALINOMYCIN ON LAYER CHICKS

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ABSTRACT

Effects of continuous and over dosage of salinomycin was studied experimentally in layer chicks. Salinomycin was given to layers @ 60, 120 and 180 ppm in feed up to the age of 12 weeks. During the course of trial, the birds of all groups were active and did not show any clinical signs. Body weight started decreasing significantly ($P<0.001$) in chicks receiving 60, 120 and 180 ppm salinomycin compared to the control group from the 11th, 5th and 3rd week onward, respectively. Feed conversion ratio at 12th week was 3.537 in the control compared with 3.701, 4.250 and 4.465 in layer chicks given salinomycin at the rate of 60, 120 and 180 ppm, respectively. Absolute weight of liver at 12th week and absolute weight of kidneys throughout the experiment decreased significantly ($P<0.001$) in chicks receiving 180 ppm salinomycin. Concentrations of AST, ALT, blood urea and creatinine were higher in layers receiving 180 ppm salinomycin, while serum total bilirubin concentration was increased significantly ($P<0.001$) in layers receiving 120 and 180 ppm salinomycin. No gross lesions were observed in liver and kidneys of layers receiving various doses of salinomycin. Microscopically, there was congestion of liver sinusides and vacuolization of hepatocytes in chicks receiving 180 ppm salinomycin. There was hydropic degeneration of tubular epithelium, degeneration and desquamation of most of cells of Bowman's capsule epithelium of kidneys in birds receiving 180 ppm salinomycin. Higher doses of salinomycin seem to alter liver and kidney functions subclinically in layer chicks.

Key words: Pathological and biochemical changes, salinomycin, layer chicks.

INTRODUCTION

Coccidiosis in poultry is widespread in Pakistan and is a problem of economic significance. Various drugs and chemical compounds which have a selective toxicity to coccidia rather than the chicken are used for the prevention of coccidiosis (Albert, 1973). In outbreaks of coccidiosis, poultry farmers usually treat their birds with anticoccidal drugs, including sulfa group and amprolium in the drinking water, as well as anticoccidial feed premixes. This can result in over medication of anticoccidial feed premix for a longer period.

Salinomycin, an ionophoric coccidiostat widely used in chicken feed (Johansen *et al.*, 2007), is a compound that acts by transporting alkali metal ions, resulting in altered ionic gradients and disturbed physiological process in coccidia (Pressman, 1976). Toxicity at a high dietary level probably relates to disturbance of metabolism of ions within the tissues of the host animals or to oxidative damage (Kamashi *et al*., 2004). Continuous use of salinomycin may disturb the physiological process of kidneys and liver. Therefore, a study was conducted to see the effect of salinomycin on the health and function of liver and kidneys in layer chicks.

MATERIALS AND METHODS

Experimental birds and rations

One hundred and twenty day-old layer chicks having almost similar body weight and free from any apparent clinical ailment were procured from a local hatchery. After five days of acclimatization, these chicks were divided randomly into four equal groups i.e., A, B, C and D. The chicks in all groups were fed commercial layer feed (CP 19%, ME 2750 kcal/kg) throughout the study. Chicks in group A served as control, those in groups B, C and D were given salinomycin (Coxistac©) in feed at 60 (recommended preventive dose), 120 and 180 ppm, respectively up to 12 weeks of age. Body weight of 10 birds from each group and feed consumption was calculated weekly. At the 12th week, feed conversion ratio (FCR) was calculated by dividing the total feed consumed per bird by its body weight. Birds were examined twice daily for clinical signs and behavioral alterations. Ten birds from each group were slaughtered on 4th, 8th and 12th week of age. Liver and kidneys were removed for recording their absolute weight.

Serum biochemical and histological examination

Blood samples without anticoagulant were collected from 10 randomly selected birds from each group on 4th, 8th and 12th week of age. Serum was separated and used to determine the concentrations of aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin, blood urea and creatinine spectrophotometrically using commercially available kits (Randox Laboratories Ltd. Crumlin, UK, Cat No. AS 147, AL 146, BI 3338, UR 1068 and CR 510 respectively). On each slaughtering, 10 chicks from each group were slaughtered to observe the gross and histopathological lesions in liver and kidneys (Bancroft and Gamble, 2008).

Statistical analysis

The data thus obtained were subjected to two way analysis of variance. Different group means were compared using Duncan's multiple range test.

RESULTS

During the course of the trial, birds of all groups were active and did not show any clinical signs. Body weight of layer chicks receiving 60 ppm salinomycin decreased significantly (P<0.001) than the control birds from 11th week, whereas body weight in chicks receiving 180 and 120 ppm salinomycin started decreasing significantly (P<0.001) from 3rd and 5th week onward, respectively than the control birds (Table 1).

Values for cumulative feed conversion ratio (FCR) at 12th week were 3.701, 4.250 and 4.465 in layer chicks given salinomycin at the rate of 60, 120 and 180 ppm salinomycin in feed (Fig. 1). A positive correlation between FCR and the dose of salinomycin (r = 0.986, P<0.001) was noted.

Absolute weight of liver was not affected by various doses of salinomycin on 4th and 8th week but was significantly increased on 12th week of experiment at dose of 180 ppm compared to control birds. The absolute weight of kidneys significantly decreased from 4th week of experiment at dose of 180 ppm salinomycin (Table 2).

Differences in concentrations of ALT and AST in layer chicks receiving 60 and 120 ppm salinomycin compared to control chicks were non significant throughout the experiment. In layer chicks receiving 180 ppm salinomycin, AST concentrations on 12th, while ALT on 8th and 12th weeks were significantly (P<0.001) higher than in the control group (Table 3). Non significant difference in serum total bilirubin concentration was observed in layer chicks receiving 60 ppm salinomycin than the control chicks at all experimental weeks. However, it was significantly (P<0.001) increased in chicks receiving 120 ppm on 12th and for 180 ppm salinomycin group on both 8th and 12th week of age than the control group (Table 3).

There was non significant difference throughout the study in concentrations of blood urea and creatinine in layer chicks receiving 60 and 120 ppm salinomycin than the control chicks. However, urea concentration was significantly (P<0.001) higher in layer chicks receiving 180 ppm salinomycin than the control birds 8th and 12th week post-treatment. The concentration of creatinine was also significantly higher in layers receiving 180 ppm slinomycin at 12th week of age (Table 3).

No gross lesions were observed in liver and kidneys of chicks receiving various doses of salinomycin. Microscopically, there was congestion of sinusoidal spaces and degeneration observed as vacuolization of hepatocytes in chicks receiving 180 ppm salinomycin. The same hydropic degenerative changes in tubular epithelium, degeneration and desquamation of most of the epithelial cells of Bowman's capsule in chicks receiving 180 ppm salinomycin were also observed.

Table 1: Effect of different doses of salinomycin on weekly body weight (g) of layer chicks

Age (weeks)	Doses of salinomycin (ppm)			
	Control	60	120	180
1	57.50 ± 1.54	59.70 ± 1.17	55.60 ± 1.20	46.20 ± 1.76
2	98.10 ± 1.70	96.90 ± 2.63	86.80 ± 3.03	80.00 ± 4.64
3	145.00 ± 2.48	141.30 ± 2.55	128.90 ± 2.72	111.70 ± 2.89***
4	249.10 ± 8.34	248.20 ± 6.88	242.10 ± 7.11	206.10 ± 2.56***
5	386.40 ± 13.33	342.50 ± 9.82	301.30 ± 8.90***	291.20 ± 8.80***
6	418.50 ± 2.80	421.10 ± 6.77	397.30 ± 5.33	388.20 ± 7.78***
7	514.00 ± 11.87	513.70 ± 9.83	576.70 ± 19.05***	426.80 ± 5.07***
8	619.60 ± 8.26	633.70 ± 10.80	596.70 ± 13.35***	554.80 ± 14.96***
9	684.90 ± 10.49	686.40 ± 10.8	564.80 ± 13.35***	529.40 ± 13.96***
10	740.00 ± 11.90	731.00 ± 14.81	599.50 ± 10.81***	574.50 ± 14.51***
11	802.00 ± 9.95	757.00 ± 17.24***	633.00 ± 8.57***	579.50 ± 13.25***
12	898.50 ± 18.23	858.50 ± 21.46***	714.00 ± 6.23***	640.50 ± 7.43***

Each figure represents mean (± SEM) of 10 chicks. ***Significant difference (P<0.001) compared with the control.

Table 2: Effect of different doses of salinomycin on absolute weight (g) of liver and kidneys of layer chicks

Age (weeks)	Doses of salinomycin (ppm)			
	Control	60	120	180
Liver				
4	3.09 ± 0.13	3.31 ± 0.14	2.85 ± 0.09	3.25 ± 0.09
8	2.98 ± 0.07	293 ± 0.08	3.07 ± 0.09	3.12 ± 0.09
12	2.40 ± 0.08	2.32 ± 0.10	2.64 ± 0.09	2.68 ± 0.05***
Kidneys				
4	1.18 ± 0.07	1.15 ± 0.07	1.17 ± 0.04	0.86 ± 0.08***
8	0.95 ± 0.03	0.90 ± 0.03	0.88 ± 0.03	0.84 ± 0.04***
12	0.97 ± 0.02	0.95 ± 0.04	10.94 ± 0.04	0.88 ± 0.04***

Each figure represents mean (±SEM) of 10 chicks. ***Significant difference (P<0.001) compared with the control.

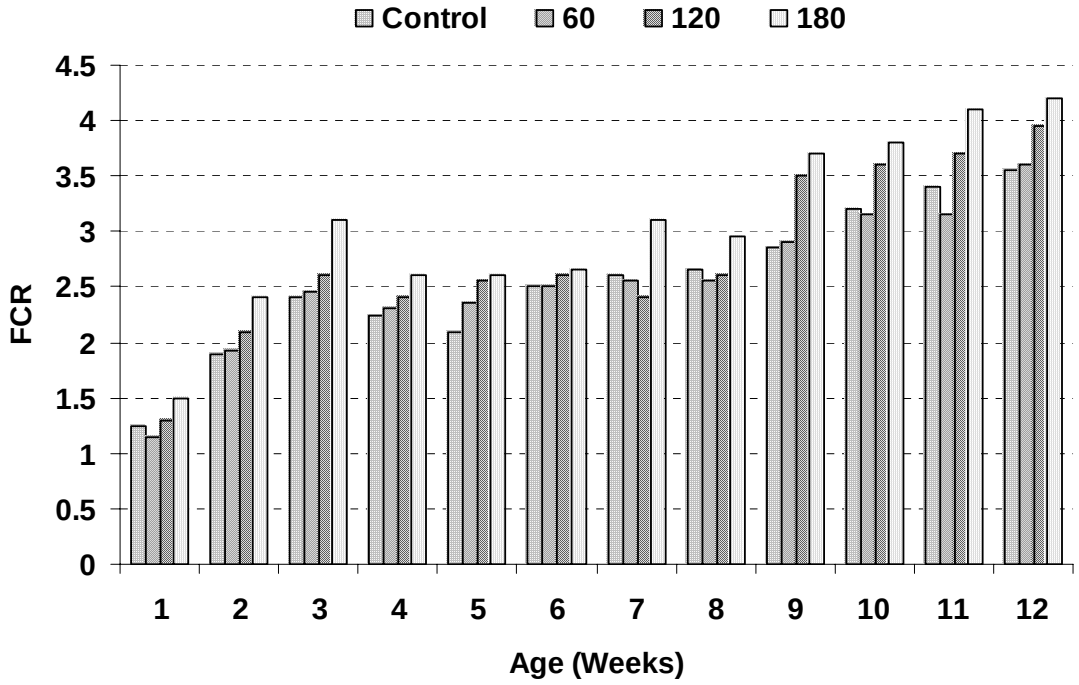

Fig. 1: Effect of salinomycin on FCR of layer chicks at different weeks of age.

DISCUSSION

Salinomycin is not only used extensively in Pakistan for treatment of coccidiosis in poultry but also used worldwide (Kennedy *et al*., 1995). In the present study, no clinical signs were observed in layer chicks at any dose level of salinomycin. However, in turkeys as little as 20-30 ppm salinomycin in feed caused difficult and incoordinated movements, paralysis of leg and neck muscles, dysponea and diarrhea and even 34.5% mortality (Assen, 2006), indicating species variation.

In the present study, 60 ppm salinomycin, the recommended dose, did not affect body weight in female layer chicks up to 10 weeks post treatment, however, thereafter body weight decreased significantly compared to control group ($P<0.001$). It indicates that prolonged use of salinomycin, even at the recommended dose, for prophylaxis of coccidiosis can suppress growth. The growth suppression at 60 ppm in layer chicks is in congruence with findings of various workers (Prohaszka and Rozsnyai, 1990; Thompson *et al*., 2005). In contrast, Pearson *et al*. (1990) observed that 40-80 ppm salinomycin had no effect on body weight in broilers.

At 120 and 180 ppm dose, salinomycin significantly decreased body weight from 5th and 3rd week post treatment in female layer chicks ($P<0.001$). The inverse correlation between dose of salinomycin and weight gain in female layer chicks ($r = -0.979$, $P< 0.01$) further suggests that growth suppression was dose dependent i.e. with the increase in dose of salinomycin, growth was depressed more and earlier than the lower dose. Growth depression observed at higher doses of salinomycin in layer chicks in the present study is unequivocal confirmation of previous studies (Keshavarz and McDougald, 1982; Pearson *et al*., 1990).

This growth suppression in layers receiving higher doses of salinomycin could be due to incompatibility of feed ingredients which vary at various places within a country and around the world. Prohaszka and Rozsanyai (1990) observed that when included in the diet stabilized with TD antioxidant (6,6'ethyliden-bis/2,2;4-trimethyl-1,2-dihydroquinoline), salinomycin caused a strong, monensin a moderate and lasalocid did not cause any growth depression in the broiler chicks. In their study, the growth suppressive effect of salinomycin also increased when given in diet with high saprophyte count. The TD antioxidant is not widely used in poultry feed in Pakistan. However, bacterial count is usually very high under local conditions due to use of fish meal, poultry offal and feather meal, blood meal and bone meal. Saprophytic bacteria, during excessive multiplication in poultry feed, release metabolites of increased bacterial activity which may enhance toxicity of salinomycin and monensin (Prohaszka *et al*., 1987).

Table 3: Effect of different doses of salinomycin on biochemical parameters in layer chicks

Age (weeks)	Doses of salinomycin (ppm)			
	Control	60	120	180
Aspartate transaminase (IU/L)				
4	25.10 ± 3.25	23.20 ± 1.99	30.90 ± 4.57	31.10 ± 1.18
8	33.70 ± 5.12	31.10 ± 8.19	36.80 ± 5.41	47.10 ± 7.18
12	48.50 ± 3.95	45.80 ± 2.79	48.00 ± 4.98	56.00 ± 5.82***
Alanine transaminase (IU/L)				
4	8.90 ± 1.44	7.40 ± 1.14	8.20 ± 1.03	9.20 ± 0.40
8	13.70 ± 1.64	11.20 ± 2.36	17.70 ± 1.33	18.20 ± 2.14***
12	24.50 ± 2.80	24.90 ± 2.23	26.00 ± 2.27	29.10 ± 2.23***
Bilirubin concentration (mmol/L)				
4	0.44 ± 0.01	0.45 ± 0.02	0.52 ± 0.03	0.42 ± 0.03
8	0.88 ± 0.03	0.81 ± 0.02	0.86 ± 0.04	0.92 ± 0.13***
12	1.08 ± 0.05	0.97 ± 0.07	1.23 ± 0.11***	1.33 ± 0.04***
Blood urea (mg/dL)				
4	7.97 ± 1.57	7.85 ± 0.99	10.07 ± 1.78	11.28 ± 1.51
8	14.52 ± 1.52	15.40 ± 0.33	16.46 ± 1.43	18.64 ± 1.35***
12	17.21 ± 0.39	18.85 ± 1.20	18.07 ± 0.43	22.20 ± 1.55***
Creatinine concentration (mg/dL)				
4	0.86 ± 0.15	0.76 ± 0.05	0.84 ± 0.02	0.62 ± 0.05
8	0.95 ± 0.07	0.98 ± 0.05	0.94 ± 0.11	0.76 ± 0.10
12	0.70 ± 0.03	0.76 ± 0.12	1.01 ± 0.17	1.38 ± 0.17***

Each figure represents mean (± SEM) of 10 chicks.
***Significant difference (P<0.001) compared with the control.

Growth depression in layers receiving higher doses of salinomycin could be due to reduced feed intake which was accompanied by poor FCR. There was significant positive correlation between FCR and dose of salinomycin which shows dose related effect of salinomycin. The poor FCR observed in the present study is in line with other workers who observed reduced feed consumption and poor FCR with therapeutic doses (Conway *et al*., 2002), as well as with higher doses of salinomycin in broilers (Keshavarz and McDougald, 1982).

Salinomycin caused damage to the liver at high doses which is also augmented by significant changes in serum chemistry related to liver functions. The AST is a cytoplasmic enzyme and ALT is present both in cytoplasm and mitochondria of hepatic cells. Normal plasma shows low activities of both these enzymes, neither enzyme is specific to liver but ALT occurs in much higher concentration in the liver than elsewhere and consequently increased ALT activity reflects hepatic damage more specifically. These enzymes are liberated into the blood whenever liver cells are damaged and increased plasma activity is a very sensitive index of hepatic damage (Stogdale, 1981). Alteration in serum enzymes and bilirubin reflect damage to the hepatocytes.

In the present study, AST activity was not affected significantly at 60 and 120 ppm salinomycin doses but at 180 ppm it increased significantly (P<0.001). Total serum bilirubin concentration was normal at 60 ppm salinomycin but increased significantly in chicks (P<0.001) receiving 120 and 180 ppm salinomycin. Increased AST activity with salinomycin at 50 ppm in turkeys (Griffiths *et al*., 1989) and 120 mg/kg feed in broilers (Kamashi *et al*., 2004) has been reported. Neufeld (1992) also reported increased AST activity in a flock of turkeys given salinomycin at dose of 15.5 g/Kg feed by mistake. The increase in the concentration of these enzymes in the present study though not substantial, indicates a biochemical change which could be related to mild degenerative changes recorded in hepatocytes.

Salinomycin selectively facilitates transmembrane exchange of sodium and potassium. As a result of this uncontrolled movement, ion gradient and concentration across the cell membrane is altered and physiological process of the coccidia is disturbed (Brander *et al*., 1993). It may be possible that at higher doses salinomycin disturbs the physiological process of host cells, leading to activation of proteolytic enzymes, which affect the cellular and intracellular membrane integrity and cause pathological changes. In the present study, the hepatic biochemical changes could have resulted from any of these mechanisms. However, the exact mechanism of damage needs further investigation.

In the present study, blood urea concentration was not affected at 60 and 120 ppm salinomycin but it was significantly (P<0.001) increased in chicks receiving 180 ppm salinomycin. Urea is the end product of protein metabolism which is excreted through kidneys. In broilers, 120 ppm salinomycin significantly increased urea concentration in serum (Kamashi *et al*., 2004). Serum creatinine concentration was not affected at 60 ppm salinomycin but its concentration was significantly increased in layer chicks receiving higher doses (120 ppm and 180 ppm) of salinomycin at 12th week post-treatment (P<0.001). Increase in blood urea and serum creatinine concentration in broilers with salinomycin treatment have been reported (Kamashi *et al*., 2004) and indicate renal damage (Benjamin, 1978).

It was concluded that salinomycin at the recommended dose of 60 ppm did not affect the liver and kidney functions. However, higher doses of salinomycin seem to alter liver and kidney functions subclinically. Therefore, prolonged use of salinomycin at higher doses should be avoided in layers.

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