Salinomycin Toxicity in Chickens: Biochemical Changes and Treatment with Hypertonic Dextrose

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Abstract: Salinomycin is a monocarboxylic polyether antibiotic with antimicrobial properties. It is used as a coccidiostat in chickens and a growth promoter in ruminants. Although it has proved to be safe at therapeutic doses, a toxic effect can result from overdosage or misuse. Thus, changes in biochemical parameters (AST, ALT and CK) were examined and the effect of hypertonic (50%) dextrose against experimental toxicosis with salinomycin was evaluated in broiler chickens (male and female) and laying hens in this study. Male and female broilers and laying hens each were divided into 6 groups (n=9). Except for group 1, all birds were intoxicated with oral administration of salinomycin (85 mg kg⁻¹). Group 2 received intravenous saline (1 ml kg⁻¹) 1 hour after poisoning. Hypertonic dextrose (1 or 2 ml kg⁻¹, either single or double doses) was similarly injected into the wing vein of the chickens in groups 3 to 6. The number of deaths after salinomycin poisoning was recorded for each group. Blood samples were collected from the wing vein on days 0, 3, 7 and 14. Results indicate that mortalities decreased in those groups receiving dextrose solution, particularly in group 6, compared to the mortality in group 2. In male and female broilers, hypertonic dextrose decreased the mortalities up to 22%, but about 44% in laying hens. AST, ALT and CK levels in serum increased in those groups intoxicated with salinomycin, however, after the administration of dextrose the situation was reversed and the levels of enzymes in the serum mostly decreased in various groups. It is generally concluded that the administration of hypertonic dextrose is partially useful in the treatment of salinomycin toxicosis in the chicken. The mechanism by which dextrose exerts this effect should be investigated in future studies.

Key words: Salinomycin toxicity, biochemical parameters, hypertonic dextrose, chickens

INTRODUCTION

Carboxylic ionophores are a group of polyether antibiotics comprised of many different compounds (Callaway et al., 2003; Butaye et al., 2003). Monensin, lasalosid, narasin, maduramicin and salinomycin are some members of this group (Maas et al., 2001). These antibiotics are characterized by multiple tetrahydrofuran and tetrahydropyran rings connected by aliphatic bridges. Other important features also contributing to their mode of action include a free carboxyl function (EFSA, 2008).

Ionophores are widely used as an anticoccidial drug for poultry and as a growth promoter for ruminants (Anderson et al., 1984; Nagaraja et al., 1996; Kinashi et al., 1973; Wilson, 1980). Generally, ionophores have been shown to be safe and effective in target animals receiving recommended dosage concentrations. However, overdosage or misuse situations can lead to toxic syndromes (Wilson, 1980; Galitzer et al., 1982; Schweitzer et al., 1984; Novilla, 1992; Rajaian et al., 2008).

Salinomycin is a member of ionophores produced by the fermentation of the fungal stereptomyces species which is active against some Gram positive bacteria, coccidia, neospora and toxoplasma (Mckellar and Lawrence, 1980). It has a narrow therapeutic index and is toxic to turkey and mammals at relatively low dose (Todd et al., 1984).

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Chickens are relatively resistant to ionophore poisoning compared to other species (Gregory, 1997). One reason could be the extent of the metabolism of ionophores occurring in this species (Nebbia et al., 2001). The mechanism of action of ionophores at the cellular level is to selective binding, with certain ions creating intra and extra cellular biochemical disturbance. Salinomycin preferentially binds with potassium, interfering with potassium transport across mitochondrial membranes and resulting in low intra cellular energy production.

So far, no antidote or proven treatment regimen has been introduced for ionophore toxicosis (Jones, 2001). The use of emetics (in small animal only) and activated charcoal or mineral oil in combination with saline cathartics, may decrease the absorption of ionophore drugs (Haward, 1993). Compensation for the energy depletion may be useful to alleviate the toxic effects of salinomycin. Therefore, the effectiveness of hypertonic (50%) dextrose (HD) against salinomycin toxicosis in male and female broilers and laying hens was examined in the present study. In addition, changes in several biochemical parameters (AST, ALT and CK) were also evaluated.

MATERIALS AND METHODS

Male and female broiler chickens (6 week old Hi-line breed, weighing 1500-2250 g) and laying hens (44 week old Ross breed, weighing 1350-2050 g) were used in accordance with the international guiding principles involving animals for scientific researches as well as the guidelines of animal welfare. Layer hens and broiler chickens were each randomly divided into six groups (n=9) as follows:

Group 1: not intoxicated with salinomycin and received no treatment.
Group 2: intoxicated with salinomycin and received isotonic saline.
Group 3: intoxicated with salinomycin and received a single dose (1ml kg\(^{-1}\)) of HD.
Group 4: intoxicated with salinomycin and received a double dose (1ml kg\(^{-1}\)) of HD.
Group 5: intoxicated with salinomycin and received a single dose (2ml kg\(^{-1}\)) of HD.
Group 6: intoxicated with salinomycin and received a double dose (2ml kg\(^{-1}\)) of HD.

The first dose was injected one hour after the administration of salinomycin and the second dose was administered 24 hours after salinomycin poisoning. A proper dose of salinomycin was selected on the basis of a series of experiments leading to the determination of LD\(_{50}\) of the ionophore in various birds using up and down dosing (Neuschl et al., 2001; Vaczi et al., 2006; Rajaian et al., 2009). The effect of treatment with HD on the death rate was then examined following the oral administration of salinomycin (85 mg kg\(^{-1}\)). Mortalities in the various groups were statistically compared using the Qui-Square test.

Blood samples were collected from the wing veins of the chickens at different time intervals (0, 3, 7 and 14 days after the administration of salinomycin) and several biochemical parameters (AST, ALT and CK) were measured using a standard autoanalyser with veterinary software (Bayer, Model 560, Germany). Data were analyzed by univariant analysis of variance and Tukey HSD.

RESULTS AND DISCUSSION

The LD\(_{50}\) of salinomycin in broiler chickens and laying hens was determined to be around 106 and 104 (mg kg\(^{-1}\)), respectively. The effects of the treatment with HD on the rate of mortality, following the oral administration of salinomycin (85 mg kg\(^{-1}\)), are shown in Table 1.

A lower death rate was observed in broilers (both sexes) and laying hens compared to the group not receiving HD therapy. Birds in the control group (not intoxicated with salinomycin, group 1) showed no mortality. A similar situation was noticed in group 6 in broilers (both sexes). In male broiler chickens the percentages of death in groups 2, 3 and 5 were around 33, 11 and 11, respectively, but no death has occurred in group 4. In female broiler chickens, the approximate percentages of mortalities in groups 2, 3, 4, and 5 were 44, 33, 33 and 22, respectively. In laying hens, however, the percentages of death in groups 2, 3, 4, 5 and 6 were around 44, 11, 22, 11 and 11, respectively.

Generally, there was no mortality in the control group not intoxicated with salinomycin. The rate of mortality in the intoxicated group not receiving HD therapy was around 40%. This is in accordance with our expectation, as a dose smaller than LD\(_{50}\) has been administered to chickens. On the other hand, the rate of mortality was decreased to at least 4% in groups treated with HD (Table 1).
Values of serum AST, ALT and CK in different groups of broiler chickens and laying hens are depicted in Tables 2, 3 and 4. The enzyme activities of AST and ALT significantly (P<0.05) increased following the administration of salinomycin in the chickens (Tables 2 and 3). Although apparently significant differences exist between the groups in several cases, generally there were no major changes in the serum AST level on day zero. However, from day 3, the enzyme level in the serum increased 2 to 5 fold in most groups compared to the control group (group 1) and did not return to the normal level, even 2 weeks after salinomycin intoxication (Table 2). The situation for the serum ALT level was more or less similar to that for the serum AST. The discrepancies in the data obtained for ALT seem to be higher than those shown for AST (Tables 3 and 2).

The activity of CK was elevated more than ten-fold compared to the control group on day 3 and then started to decrease in the following days (Table 4). In contrast to the gradual increase in the serum AST and ALT levels, the ionophore exerted a sharp increase in the serum CK levels in almost all groups. In addition, the CK levels in those groups treated with HD declined relative to the non-treated control group (Table 4).

### Table 1: Number of mortalities of chickens after oral administration of salinomycin (85 mg kg⁻¹) in various groups of birds

<table>
<thead>
<tr>
<th>Time</th>
<th>Bird Group</th>
<th>1 ml kg⁻¹</th>
<th>2 ml kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>Broiler male</td>
<td>No death</td>
<td>3 out of 9</td>
</tr>
<tr>
<td></td>
<td>Broiler female</td>
<td>2 out of 9</td>
<td>1 out of 9</td>
</tr>
<tr>
<td></td>
<td>Laying hens</td>
<td>2 out of 9</td>
<td>1 out of 9</td>
</tr>
<tr>
<td>Total</td>
<td>No death</td>
<td>11 out of 27 (40%)</td>
<td>4 out of 27 (15%)</td>
</tr>
</tbody>
</table>

G1: not intoxicated with salinomycin and no treatment
G2: intoxicated with salinomycin and received isotonic saline
G3: intoxicated with salinomycin and received a single dose (1ml kg⁻¹) of HD.
G4: intoxicated with salinomycin and received a double dose (1ml kg⁻¹) of HD.
G5: intoxicated with salinomycin and received a single dose (2ml kg⁻¹) of HD.
G6: intoxicated with salinomycin and received a double dose (2ml kg⁻¹) of HD.

### Table 2: Activity of AST in chickens after oral administration of salinomycin (85 mg kg⁻¹) in various groups of birds

<table>
<thead>
<tr>
<th>Time</th>
<th>Bird Group</th>
<th>AST Activity (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Broiler male</td>
<td>386± 106 aAI</td>
</tr>
<tr>
<td></td>
<td>Broiler female</td>
<td>324± 118aAll</td>
</tr>
<tr>
<td></td>
<td>Laying hens</td>
<td>191± 12aBIII</td>
</tr>
<tr>
<td>Day 2</td>
<td>Broiler male</td>
<td>203± 19aAI</td>
</tr>
<tr>
<td></td>
<td>Broiler female</td>
<td>203± 17aAI</td>
</tr>
<tr>
<td></td>
<td>Laying hens</td>
<td>197± 22aAII</td>
</tr>
<tr>
<td>Day 3</td>
<td>Broiler male</td>
<td>196± 19aAI</td>
</tr>
<tr>
<td></td>
<td>Broiler female</td>
<td>195± 14aAI</td>
</tr>
<tr>
<td></td>
<td>Laying hens</td>
<td>197± 22aAII</td>
</tr>
<tr>
<td>Day 4</td>
<td>Broiler male</td>
<td>270± 64aAI</td>
</tr>
<tr>
<td></td>
<td>Broiler female</td>
<td>194± 40aAI</td>
</tr>
<tr>
<td>Total</td>
<td>No death</td>
<td>11 out of 27 (40%)</td>
</tr>
</tbody>
</table>

Number of birds=3-9; Different letters indicate significant (p<0.05); small letters compared groups in rows; Large letters compared groups in columns for various birds on a specific day; Italic number compared broiler male, broiler female and layers in columns on various days.

### Table 3: Activity of ALT in chickens after oral administration of salinomycin (85 mg kg⁻¹) in various groups of birds

<table>
<thead>
<tr>
<th>Time</th>
<th>Bird Group</th>
<th>ALT Activity (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Broiler male</td>
<td>386± 106 aAI</td>
</tr>
<tr>
<td></td>
<td>Broiler female</td>
<td>324± 118aAll</td>
</tr>
<tr>
<td></td>
<td>Laying hens</td>
<td>191± 12aBIII</td>
</tr>
<tr>
<td>Day 2</td>
<td>Broiler male</td>
<td>203± 19aAI</td>
</tr>
<tr>
<td></td>
<td>Broiler female</td>
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<td></td>
<td>Laying hens</td>
<td>197± 22aAII</td>
</tr>
<tr>
<td>Day 3</td>
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</tr>
<tr>
<td></td>
<td>Broiler female</td>
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</tr>
<tr>
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<td>Laying hens</td>
<td>197± 22aAII</td>
</tr>
<tr>
<td>Day 4</td>
<td>Broiler male</td>
<td>270± 64aAI</td>
</tr>
<tr>
<td></td>
<td>Broiler female</td>
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<tr>
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</tr>
</tbody>
</table>

Number of birds=3-9; Different letters indicate significant (p<0.05); small letters compared groups in rows; Large letters compared groups in columns for various birds on a specific day; Italic number compared broiler male, broiler female and layers in columns on various days.
moderately efficient in the present study. This is reflected in the results depicted in Table 1 as the number of enzymes was obtained 616±40.8, 76.2±7.6 and 98.6±4.3, respectively. Increase in the levels of these enzymes increases the level of serum AST, ALT and CK (Rajaian et al., 2009c). The highest value of these enzymes is found in broiler chickens and laying hens not treated with HD. In contrast, these effects are reversed in those groups receiving treatment (Thrall, 2004).

Intoxication with salinomycin caused an increase in the serum levels of AST, ALT and CK in broiler chickens (Thrall, 2004), which is different from the values obtained in the control group in the present study. The high CK values in the control group may be due to the stress of bleeding and handling of birds. A lower decrease in AST and a higher decrease in CK at days 7 and 14 may be due to their half life and not because of the treatment. Basically, a decrease in the activities of the above enzymes is not valuable in clinical pathology and metastasis (Bergen and Bates, 1984). As a result of cell membrane damage, an influx of sodium and calcium ions into cells may lead to cell death. Ionophore may also directly reduce mitochondrial oxidative phosphorylation in myocytes, resulting in a decrease in cellular respiration (Van Vleet and Frans, 1983). The primary target tissues are cardiac and skeletal muscles (Nicpon et al., 1997; Thrall, 2004), which is similar to the values found by others (Bradley, 1992; Neuschl et al., 2001; Neuschl et al., 2002; Vaczi et al., 2006; Rajaian et al., 2009a). The minor differences could be explained by differences in the age of the chickens used in each study and the method of analysis as four week old birds have been employed in other investigations and LD50 has been calculated using a double dose interpolation method according to Roth (1962). Potter et al. (1986) also reported a higher toxicity of salinomycin for younger turkeys compared to older ones. Although there has been no report of an antidote or proven treatment regimen against ionophore toxicity (Jones, 2001), the use of emetics (in small animal only) and activated charcoal or mineral oil in combination with saline cathartics, may decrease the absorption of ionophore drugs (Haward, 1993). Moreover, salinomycin induces toxicity by generating free radicals and disturbing the antioxidant defense, which could be effectively prevented by the use of zinc as an antioxidant (Kamashi et al., 2004).

The use of HD in the treatment of experimental salinomycin toxicity in chickens was shown to be moderately efficient in the present study. This is reflected in the results depicted in Table 1 as the number of mortalities was decreased in groups receiving HD (Table 1). However, the difference in the mortalities were only significant (P<0.05) when all birds (broiler male and female and layers) were collectively analyzed statistically (Table 1).

Salinomycin, an ionophore coccidiostat widely used in chicken feed (Johansen et al., 2007), is a compound that acts by transporting alkali metal ions, resulting in an altered ionic gradient and a disturbed physiological process in coccidia (Pressman, 1976). Over dosage or accidental exposure of a non target species to the compound can lead to toxic syndromes (Novilla, 1992) that probably relate to the disturbance of the metabolism of ions within the tissues or to the oxidative damage (Kamashi et al., 2004).

One of the effects of ionophore is seen on energy metabolism. Alternation of the cellular ionic gradient by ionophores can deplete intracellular ATP levels (Bergen and Bates, 1984). As a result of cell membrane damage, an influx of sodium and calcium ions into cells may lead to cell death. Ionophore may also directly reduce mitochondrial oxidative phosphorylation in myocytes, resulting in a decrease in cellular respiration (Van Vleet and Frans, 1983). The primary target tissues are cardiac and skeletal muscles (Nicpon et al., 1997; Mendes et al., 2003).

Normal serum enzyme activity in birds is less than 230 IU/L for AST and less than 20 IU/L for ALT (Kaneko, 1997; Thrall, 2004), which is similar to the values found in this study in the control groups (Tables 2 and 3). Normal serum CK activity in birds is reported to be between 100 and 200 IU/L (Kaneko, 1997; Thrall, 2004), which is different from the values obtained in the control group in the present study. The high CK values in the control group may be due to the stress of bleeding and handling of birds. A lower decrease in AST and a higher decrease in CK at days 7 and 14 may be due to their half life and not because of the treatment. Basically, a decrease in the activities of the above enzymes is not valuable in clinical pathology (Thrall, 2004).

Intoxication with salinomycin caused an increase in the serum levels of AST, ALT and CK in broiler chickens and laying hens not treated with HD. In contrast, these effects are reversed in those groups receiving treatment with HD (Tables 2, 3 and 4).

Oral administration of various doses (1, 2 and 4 mg/kg) salinomycin in sheep has been shown to cause an increase in the level of serum AST, ALT and CK (Rajaian et al., 2009c). The highest value of these enzymes was obtained 616±40.8, 76.2±7.6 and 98.6±4.3, respectively. Increase in the levels of these enzymes was statistically (Table 1).
are probably due to hepatic and myocardial damages and the serum activity of CK may increase because of myopathy (Rajaian et al., 2009c). Rajaian et al. (2009b) also reported that salinomycin significantly increased the activities of ALT, AST and CK from 19.0±1.9, 301±16.6 and 409±34.7 IU/L to 74.7±9.4, 301±16.5 and 409±34.7 IU/L, respectively, 4 days following drug administration in female calves. In male calves receiving a larger dose (5 mg/kg) of salinomycin the enzyme activity of ALT, AST and CK was significantly increased from 21.3±0.5, 92.3±2.5, and 123.6±10.1 IU/L to 78.0±11.5, 316.0±24.6, and 385.0±38.4 IU/L respectively. The activities of ALT and CK were returned to normal values after 4 days, while AST activity remained high, even till the end of the experiment. Chickens receiving salinomycin with or without the administration of phenobarbital and chloraphenicol, showed no significant differences in the activities of AST, ALT and CK. (Rajaian et al., 2009a).

ALT is a cytoplasmic enzyme and AST is both a cytoplasmic and mitochondrial enzyme. The increase in the serum level of ALT and AST may indicate hepatic and/or myocardial damage (Stockham and Scott, 2002). Elevated levels in AST and ALT in toxicosis with salinomycin in broilers might be due to oxidative damage by free radicals resulting in hepatocellular injury (Kmashi et al., 2004, Novilla, 1992, Lehel et al., 1995). The serum activity of CK was increased, probably due to the characteristic myopathy (Stockham and Scott, 2002; Valberg, 1996). A variety of insult (pathologic and iatrogenic) may damage muscle fibers and release CK from the muscle fiber (Stockham and Scott, 2002).

Values of serum AST and ALT in turkey affected by salinomycin are usually not elevated early in the clinical syndrome and should not be considered useful as an early indicator of muscle damage (Neufeld, 1992). However, Neufeld (1992) reported that the serum CK level is markedly elevated in clinically ill turkey (receiving 15.5 ppm salinomycin in their feed) and, therefore, may be a useful tool to confirm early muscle damage (Stokowsky, 2003).

Conclusion:
It is generally concluded that first, the administration of HD is partially effective in the treatment of salinomycin poisoning in chickens and second, serum AST, ALT and CK levels are increased due to salinomycin toxicosis, and treatment with HD counteracts these effects.

ACKNOWLEDGMENTS

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REFERENCES


