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Effect of Salinomycin on Immunity of Broiler Chicks

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Abstract: Broilers (n = 80) were given salinomycin at the rate of 0 ppm (control), 60, 120 and 180 ppm in feed from day-old to eight weeks of age. Broilers showed no clinical signs throughout the study. Non significant effect of various doses of salinomycin was observed on indices of lymphoid organs. Grossly and histologically lymphoid organs of broilers receiving various doses of salinomycin appeared normal. Total leucocyte and lymphocyte count was significantly ($p < 0.001$) decreased in broilers receiving 180 ppm salinornycin. GMT against ND and SRBCs was decreased in broilers receiving higher doses of salinomycin. Wattle response to mallein and wing web response to bovine serum albumin was significantly ($p < 0.001$) decreased in broilers at 120 and 180 ppm salinornycin.

Key words: Broiler chicks, immunity, salinomycin

Introduction

Coccidiosis is still a common problem in poultry (Muneer, 1997) although preventive medication is used for management of coccidiosis. Feed manufacturing companies do not declare type and quantity of anticoccidial used. In case of an outbreak, poultry farmers treat coccidiosis using anticoccidial drugs such as sulfa arid amprolium in drinking water and also some anticoccidial feed premix. The medication may continue for 3-4 weeks. In this way, there is a likelihood of over-medication with anticoccidial drugs. The most commonly used anticoccidial in feed in Pakistan is salinomycin (Hayat *et al.,* 1996). It acts by transporting alkali metal ions which results in altered ionic gradients and disturbed physiological processes in coccidia (Kinashi *et al.,* 1973; Pressman, 1973, 1976). Their toxicity at high dietary level probably relates to disturbance of metabolism of ions within the tissues of the host animals.

Keeping in view the extensive use of salinomycin in poultry and probability of prolonged over medication, this project was designed to investigate the effects of salinomycin on immunity of broilers.

Materials and Methods

Effect of salinornycin was studied experimentally on immunity of broilers. Eighty, day old broiler chicks were divided into four groups having 20 chicks in each group. The chicks in all groups were fed commercial broiler starter mash (CP 21-22%, ME 2850-2900 KCal/Kg) upto 4 weeks and broiler finisher ration (CP 19%, ME 3000 KCal/Kg) thereafter. Chicks in group A served as control, chicks in groups B, C and D were given coxistac in feed, respectively, at 60 ppm (the recommended preventive dose), 120 and 180 ppm concentrations. Ten birds from every group were slaughtered at 4 and 8 weeks of age for ascertaining any effect of the drug on lymphoid organs of the body. Since organ weight is directly related to body weight, organ index i.e., percent weight of the organ to the body weight was calculated to create uniformity and avoid influence of variation in body weight. Organ index was calculated as follows:

$$\text{Organ index} = (\text{Organ weight(g)}/\text{Live body weight(g)}] \times 100$$

The blood was collected at 8th week of age from wing Vein from 10 randomly selected birds from each group for leucocyte count (Natt and Herrick, 1952) And differential leucocyte count as described by Benjamin (1978) using EDTA as an anticoagulant. At the same time blood was collected without anticoagulant for separation of serum for antibody titer determination.

Immunological studies:

a) Newcastle disease vaccine virus (Lasota strain Bioteke, (Italy) was administered at 7 (eye drop), 31 (0.5 ml injection subcutaneously) days of treatment. Antibodies were measured on 28th and 56th day of treatment following the technique described in MAFF (1984)
b) Sheep 8BCs were administered (subcutaneously) at 11 and 30 days of treatment. Antibodies were measured on 28th and 56th day of treatment following the method of Ismail *et al.* (1987) and Dohms and Jaeger (1988)
c) Mallein antigen was injected (subcutaneously) for sensitization at 19 days of age. Primary wattle response was measured at 26th and secondary response was measured at 39th days of treatment as described by Hussain (1991)
D) Bovine serum albumin (BSA) was injected (subcutaneously) at 15 days of age for sensitization. Primary wing web response was measured at 22nd and secondary response was measured at 35th days of treatment following the method of Parmentier *et al.* (1993)

Results and Discussion

Lymphoid organs and leucogram: Salinomycin is an inophore antibiotic (Kinashi *et al.,* 1973) used extensively in the prevention of coccidiosis in poultry.

Table 1: Effect of salinomycin on lymphoid organs indices in broiler chicks

		Doses of salinomycin		
Organs and Age	Control	60	120	180
Bursa of Fabncus (4w)	0.20±0.01	0.18±0.01	0.19±0.02	0.21±0.03
Thymus (8W)	0.42±0.04	0.41±0.03	0.39±003	0.40±0.04
Spleen (8w)	0.11±0.01	0.12±0.01	0.09±10.01	0.09±0.02

Table 2: Effect of salinomycin on total leucocyte and differential leucocyte count in broiler chicks

		Doses of salinomycin (ppm)		
Parameter	Control	60	120	180
TLC (103/µl)	48.70 ± 1.47	50.90 ± 1.97	51.20 ± 2.05	40.10 ± 1.30***
Heterophil (%)	27.20 ± 2.34	27.14 ± 1.22	29.65 ± 1.79***	31.91 ± 1.63***
	(13246.40)	(13814.26)	(14180.80)	(12795.91)
Lymphocyte (%)	67.27 ± 1.46	66.44 ± 1.12	64.19 ± 1.27***	62.61 ± 1.67***
	(32760.49)	(33817.96)	(32865 ± 28)	(25106.61)
Monocyte (%)	3.68 ± 1.8)	4.20 ± 1.34	3.23 ± 1.34	4.34 ± 1.59
	(1792.16)	(2137.80)	(1653.76)	(1740.34)
Eosinophil (%)	1.85 ± 0.74	2.02 ± 1.04	2.56 ± 0.75	1.64 ± 0.47
	(900.95)	(1028.18)	(1310.72)	(657.64)

Each figure represents mean (±Standard error of the mean) of 10 chicks. Figures in parentheses indicate absolute number. Data subjected to analysis of variance revealed significant differences between treatments
Significant difference ($p < 0.001$) compared with the control group

It has growth depressing effect from 80 to 160 ppm in poultry (Yvore *et al.*, 1980). This growth depressing effect may depresses the immune system of chicken. The primary organs of immune system are bursa of Fabricus and thymus, reach their maximum size in chicks about four weeks after hatching and then undergo gradual involution (Tizard, 1992). Early damage to bursa and thymus may lead to immunosuppression. Therefore, effect of salinomycin on immune system was assessed through weight of lymphoid organs, their structure, total and differential leucocyte count and antibody production against various antigens. In broilers there was no significant effect of salinomycin On bursa, thymus or spleen index (Table 1). Grossly the lymphoid organs appeared normal. In contrast to this finding Shalaby *et al.* (1993) reported a decrease in relative weight of lymphoid organs in chicks given 120 mg salinomycin/Kg of feed. Similarly, Githkopoulos and Lekkas (1990) observed decreased spleen volume and necrosis of iyMphocytes in white pulp in turkeys given salinomycin at a dose of 30 mg/Kg feed. Circulating leucocytes are part of peripheral lymphoid organs and are integral part of defense in the- chicken body. Amongst leucogram, heterophils and snonocytes. phagocytose and disintegrate particulate matter such as bacteria whereas lymphocytes are involved in humoral and cellular immune mechanism of the body (Roitt, 1988). In the present study, salinomycin did not affect the total leucocyte count (TLC) at 60 and 120 ppm dose salinomycin but it was significantly reduced in broilers receiving 180 ppm salinomycin (Table 2, $p < 0.001$) The absolute heterophil and lymphocyte count was substantially reduced at 120 and 180 ppm salinomycin (Table 2). This suggests that the decrease in TLC was mainly due to decrease in circulating lymphocytes rather than other types of leucocytes.

Immune response: Lymphocytes consists of B- and T-lymphocytes. B-lymphocytes are responsible for humoral immune response i.e., antibody production and T-lymphocytes are responsible for cell-mediated immune response (Roitt, 1988). There is likelihood that the decrease in lymphocytes could lead to reduced antibody production or reduced cell-mediated immune response. To investigate this possibility birds were challenged with various antigens including Newcastle disease (ND) vaccine virus, sheep red blood cells (SRBCs), bovine serum albumin (BSA) and mallein.

Geometric mean titre (GMT) in primary and secondary responses against ND vaccine virus was decreased in broilers receiving 60, 120 and 180 ppm salinomycin (Table 3). It indicates that salinomycin depressed humoral response against ND vaccine virus in broilers. In congruence with the present study, Shalaby *et al.* (1993) reported a drastic immunosuppressive effect of salinomycin at the dose of 120 mg/Kg of feed as characterized by lower titres of antibodies to ND vaccine virus in chicks.

Salinomycin did not affect primary and secondary responses to SRBCs in broilers at 60 ppm dose. In broilers primary and secondary responses were decreased at 120 and 180 ppm salinomycin (Table 4). The production of antibody to SRBCs requires cooperation between B cells and T-helper cells (Purchase, 1983). Therefore, the study indicates that the decrease in antibody production to SRBCs could be due to decrease in 8-cells function, decrease in T-helper, cells funbtion or decrease.in function of both.

Table 3: Effect of salinomycin on geometric mean titre IGMT1 against Newcastle disease (ND) vaccine in broiler chicks

		Doses of salinomycin (ppm)		
Response	Control	60	120	180
Primary	111.40	73.30	64.00	60.20
Secondary	147.00	85.70	75.70	64.00

Each figure represents GMT of 10 broiler chicks. ND vaccine virus was administered on 7th (lst) and 31st (2nd) day of age. Antibody titre was determined on 28th and 56th day of age

Table 4: Effect salinomycin on geometric mean titer (GMT) against sheep red blood cells (SRBCs) in broiler chicks

		Doses of salinomycin (ppm)		
Response	Control	60	120	180
Primary Secondary	113.00	106.00	61.70	57.00
	149.00	113.40	61.20	53.40

Each figure represents GMT of 10 broiler chicks. Sheep red blood cells(SRBCs) were administered on 11th (lst) and 30th (2nd) day of age. Antibody titre was determined on 28th and 56th day of age

Cutaneous delayed-type hypersensitivity using mitogens and T cell-dependent antigens is often used as model for in *vivo* T-cell reactivity in birds (Giambrone *et al.,* 1984; Parmentier *et al.,* 1993). The primary response against mallein was decreased significantly at 180 and secondary response was decreased significantly at 120 and 180 ppm salinomycin (Table 5, $p < 0.001$).

Wing web response against BSA differ non-significantly in broilers receiving 60 ppm salinomycin. However the primary and secondary responses were decreased significantly at 120 ppm and 180 ppm salinomycin (Table 6; $p < 0.001$). It

Table 5: Effect of salinomycin on wattle reaction Imm) against mallein antigen

		Doses of salinomycin (ppm)		
Reaction	Control	60	120	180
Primary	0.238 ± 0.013	0.272 ± 0.008	0.233 ± 0.008	0.162 ± 0.006
Secondary	0.336 ± 0.014	0.310 ± 0 010	0.2721 ± 0.015	0.206 ± 0.013

Sensitizing dose of mallein was injected at the age of 19 days of treatment. Mallein was injected into one wattle on 26th and 39th day of age. The contralateral wattle served as control. Thickness of both wattles was measeured 48 hours after each inoculation. Each figure represents mean ± Standard error of the men) difference in thickness of inoculated and contralateral un-inoculated wattle of 10 chicks. ¨Significant difference ($p < 0.001$) compared with the control group

Table 6: Effect of salinomycin on wing web reaction (mm) against bovine albumin serum (BSA) in broiler chicks

		Doses of salinomycin (ppm)		
Reaction	Control	60	120	180
Primary	0.418 ± 0.025	0.571 ± 0.081	0.277 ± 0.014	0.287 ± 0.031
Secondary	0.825 ± 0.059	0.750 ± 0.055	0.541 ± 0.036	0.330 ± 0.018

Sensitizing dose of bovine serum albumin (BSA) was injected at the age' 15 days of treatment. BSA was injected into one wing web on 22nd. and 35th day of age. The contralateral wing web served as control. Thickness of both wing webs was measured 6 hours after each inoculation. Each figure represents mean (± Standard error of the mean) difference in thickness of inoculated and contralateral un-inoculated wing webs of 10 chicks
$p < 0.001$ compared with the control group

indicates that higher doses of salinomycin has depressed the response against BSA in broilers. According to Cotter *et al.* (1985) broiler-type chickens may develop a strong wattle response to a challenge injection with bovine serum albumin. In conclusion salinomycin at higher doses i.e., 120 and 180 ppm reduced the weight of lymphoid organs and depressed the humoral and cellular response to various antigens in broilers.

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