IMMUNOMODULATORY EFFECTS OF ETHANOL IN BROILERS

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ABSTRACT

An experiment of three week duration was conducted to study the effects of ethanol through crop tube on lymphoid organs and immune system in broilers. A total of ninety 21-days old broiler chicks were divided in to five equal groups (A to E). Groups A, B and C were given 40% ethanol 6ml, 4ml and 2ml respectively. The group D was kept as positive control given 6ml tap water through crop tube and group E was negative control. Ethanol through crop tube was given from day 21 to 42 days of age, continuously for three weeks. All groups were vaccinated against Newcastle Disease (ND) and Infectious Bursal Disease (IBD) and 3% sheep red blood cells were injected intravenously. At the end of each week, six birds were randomly selected and sacrificed. Blood samples were collected and serum was separated. Serum antibodies titers against New Castle Disease, Infectious Bursal Disease and sheep red blood cells were determined. Cell mediated immune response against the mallein antigen was also determined. No gross abnormality was observed in all lymphoid organs in treated groups as compared with controls. Relative weights of Bursa of Fabricus was non-significantly different in treated and non treated groups while relative weight of spleen and thymus were significantly ($p<0.05$) higher in treated groups as compared with control groups. Histopathological studies revealed decreased cellular density in spleen and thymus and decreased follicular diameter and epithelial height in Bursa of Fabricus in treated groups as compared with controls. The Geo mean titer (GMT) against ND, IBD and washed sheep RBCs was lowered in treated groups as compared with controls.

Key words: Ethanol, Broiler, Histopathology, Immunity.

INTRODUCTION

Although poultry industry has progressed to one of the giant industry, but it is still facing problems of various kinds including disease stress. Farmers have gained quite an experience in this regard and have started to do many of the things on their own to overcome these problems. One of such a practice is the use of ethanol in broilers as a growth promoter, for the treatment of mild respiratory problems and for better-feed conversion. Ethanol affects many body systems including reproductive system, respiratory system, cardiovascular system, nervous system and immune system (Klassen and Persaud, 1978). Ethanol primarily got absorption through oral route. The absorption starts right from the oral mucosa, but maximum absorption occurs in small intestine. The absorption from gastrointestinal tract is reported to be rapid in fasting state with peak blood levels attained within 30-60 minutes (Hagger and Forney, 1963). Body starts to dispose it immediately upon its absorption. Liver metabolizes greater than 90% of absorbed ethanol. Remaining 10% of the absorbed ethanol is metabolized and excreted out chiefly in the urine, measurably in expired air and detectably in sweat (Haggard and Greenberg, 1934). In the liver, there are three mains pathways of ethanol metabolism but the major one is the Alcohol Dehydrogenase (ADH) pathway. Absorbed ethanol is converted into acetaldehyde which is further converted into acetate and H-ions and then into carbon dioxide and water (Forney and Hughes, 1963). When there is higher concentration of ethanol in blood and ADH system is not sufficient to metabolize the ethanol, and then other pathways starts working. As a result of metabolism, ethanol is converted into Acetaldehyde. Acetaldehyde is a very toxic substance (Forney and Hughes., 1963). Ethanol has direct effects on heart muscle, thyroid and hepatic tissue. On central nervous system, ethanol has depressing effect. The degree of depression depends upon the concentration of ethanol in the blood. It suppresses certain brain functions that may lead to respiratory depression which may lead to hypoxia. The toxic effects of ethanol mainly depend on amount of ethanol taken, time of intake, genetics and the physical status of individual. It has been reported that due to excessive intake of ethanol host immunity decreases (Braulio *et al.* 2001). It also affects the phagocytic and microbial killing activity of phagocytes and impairs the functioning of helper T lymphocytes. Spleen is one of the functioning sites of immune cells and it has been reported that ethanol consumption impairs the antigen presentation in spleen and also decreases the splenic follicular diameter and volume density (Mikszta *et al.* 1995); keeping in view the above the present study was planned to investigate the immunomodulatory effects of ethanol on broilers.

MATERIALS AND METHODS

The experiment was executed on 90 broiler birds of 21days of age to investigate the effects of ethanol through crop tube. These chicks were reared in cages under optimal conditions of management and offered ethanol through crop tube once in a day. The birds were fed *ad libitum* with constant access to feed. These birds were randomly divided irrespective of sex in five equal groups i.e., A, B, C, D and E. The groups A, B and C were administered 40% ethanol v/v in drinking water through crop tube @ 6, 4 and 2ml respectively. The group D was kept as positive control and was administered 6ml drinking water through crop tube. The ethanol was administered from 21 day to 42 days of life once early in the morning daily. The group E was kept as negative control. All the birds were vaccinated against Newcastle Disease using Hitchner B1 and Lasota vaccines at 4^{th} and 21^{st} day of life respectively. Infectious Bursal disease vaccine (Fort Dodge Animal Health, USA) was used at day 9^{th} and 24^{th} day of age. Birds were injected 3% washed sheep red blood cells intravenously at day 20 and 35 of age to observe humoral immune response against sheep red blood cells (Qureshi *et al.,* 1997). Cell mediated immune response was also observed against mallein antigen, at wing web the primary dose of mallein antigen was injected at day 24 and secondary dose was injected at day 39 in the toe web (Hussain, 1991).

Sample Collection: Six birds randomly selected from each group were sacrificed at the end of each week, Blood samples and lymphoid organs were collected. The blood was collected without anticoagulant to obtain serum by centrifuging the whole blood at 5000 rpm. Lymphoid organs including thymus, bursa of Fabricius and spleen were weighed and relative weights of organs were calculated. They were subjected to gross and histopathological examination under light microscope. The sections were stained with haematoxylin and eosin stain (Bancroft and Steven, 1990).

Live Body Weight: Birds were weighed individually before sacrifice, for the calculation of relative weights of lymphoid organs.

Relative Weight of Lymphoid Organs: Lymphoid organs including spleen, bursa of Fabricius and thymus were weighed and their relative weights were calculated.

Humoral Immune Response: Haemagglutination Inhibition test was performed according to the technique described by Buxton and Fraser, (1977) for the antibody titer against New Castle disease vaccine virus. Indirect Haemagglutination test was performed to access the antibody titer against IBD vaccine virus as described by Rehman *et al.,* (1994). Antibody titre to sheep red blood cells was determined by the method described by Ismail *et al.* (1987) and Dohms and Jaeger (1988).

Cell mediated immune response: It was determined by measuring the swelling amplitude by injecting 0.2ml mallein in the wing web (primary dose) at day 24 and secondary dose in the toe web at day 39 (Hussain, 1991).

Statistical Analysis: Data thus obtained in the experiment was subjected to analysis of variance technique and means were compared by using LSD by using SAS 6.12 statistical software (Anonymous, 1996).

RESULTS AND DISCUSSION

Broiler farmers make use of ethanol through drinking water to boost the broiler particularly against respiratory pathogens in local conditions of husbandry. Present study was planned to investigate the effects of ethanol in broilers at a specific dose as all the birds do not drink equal amount of water so the birds were given a fixed volume of ethanol through crop tube to observe the effect on lymphoid organs and immune response. During the treatment trial, clinical signs in group received ethanol (40%) at dose level of 6mL showed decreased responsiveness, increased depression and some birds were drowsy and went in deep sleep for 2 to 4 hours. Clinical signs and symptoms of general weakness, dullness, depression, emaciation, and staggering gait, loss of appetite, convulsion, comma and death in the birds of group A (30%) and birds of group B (26%) were recorded. Broilers received 4mL ethanol through crop tube showed less drowsiness than birds receiving 6mL ethanol. The birds received 2mL ethanol showed no signs. Klassen and Persaud (1978) reported that ethanol has depressing effects on central nervous system. Similarly, it has been reported that ethanol is CNS depressant and at high dose levels it acts as a general anaesthetic (Forney and Hughes 1963).

1. Mortality: During 1st week of treatment, two broilers died from each group given 4 and 6 mL ethanol through crop route, while one bird from each group received 2 mL, positive and negative control. During 2nd week of experiment, three birds from each group given 2, 4 mL and positive control, two from negative control, while 5 from group given 6 mL ethanol. During 3rd week, three birds died from each group given 4 and 6 mL, and two broilers from each group given 2 mL ethanol and positive control, while one bird from negative control. Total mortality in group A was 30 %, 26 % in group B, 20 % in each group C & group D, while in negative control was 17 %. Mortality in the treated groups might be due to loss of consciousness decrease in feed intake, depression as a result of ethanol ingestion, while in control it could be due to some unknown reason.

2. Live body weight: Effect of ethanol through crop route on the live body weight of broiler is shown in Table 1. There was non-significant difference (P< 0.05) in the live weight of broilers received different doses of ethanol (40%) throughout the trial. At the start of the trial, body weight of treatment groups was higher as compared with controls. In the middle of the trial, body weight of treatment and control groups were almost same but at the end of the trial, body weight was less in groups A and C received 6ml and 2ml ethanol (40%), respectively through crop route as compared with control groups. Peebles *et al.* (1996) studied effects of ethanol on live weight in juvenile, meat-type chickens. Their findings were inline with the findings of present study. Similarly, a study in guinea pigs revealed non-significant difference between ethanol treated and control groups (Roselle and Mendenhall, 1984). Relatively lower weight gain in treated broilers could be due to dose dependent effect of ethanol causing depression as seen during present study which was responsible for low feed intake resultant low weight gain. Ethanol causes depression of certain brain centers with resultant unconsciousness (Forney and Hughes., 1963).

TABLE 1: Comparison of means ± SD of live body weight (gm).

Group	Body weights (gms) at different Age		
	28 days	35 days	42 days
A	609.63 ± 151.58	912.50 ± 194.45	1150.00 ± 636.39
B	504.67 ± 128.68	910.00 ± 52.20	1446.67 ± 83.86
C	575.35 ± 199.32	886.67 ± 206.78	1266.67 ± 488.83
D	616.32 ± 80.44	773.33 ± 353.42	1483.33 ± 670.93
E	543.28 ± 44.84	916.00 ± 31.75	1461.25 ± 69.81

3. Lymphoid Organs: The primary organs of immune system of chicken consist of spleen, Bursa of Fabricius and thymus. They reach their maximum size in chicks in about one to two weeks of age and then undergo gradual involution (Tizard, 1992). After 5 weeks of age, there is normal regression of these organs. Early damage to spleen, bursa and thymus may lead to immunosuppression. Effect of ethanol through crop tube on relative weight of spleen of broilers is shown in Table 2. At the age of 28 days relative weight of spleen of broilers of group B and C was significantly higher (P< 0.05) as compared with positive control group D. Significant increase was observed in group D as compared with negative control group E at 35 days of age. At the age of 42 days, a significant increase was observed in groups B and D as compared with negative control group E. However, it was also significantly higher in positive control than negative control. This indicates that the change in spleen weight is not due to ethanol but due to stress of tubing and filling of crop some how leading to increase in spleen weight. The results of present study contradict with the findings of Khan *et al.* (2006) who reported a decrease in relative weight of spleen in ethanol treated groups through drinking water. No gross abnormality was observed in the spleen of treatment groups. Histopathology revealed slightly lower cellularity in treated groups which was comparable. These findings were inline with the results of Khan *et al.* (2006). However, decrease in cellularity of the lymphoid organ has been reported due to ethanol consumption by Jerrells *et al.* (1986) and Faunce *et al.* (1998), which has been related with inhibition of splenocyte proliferation (Wang *et al*. 1994).

Relative weight of bursa of Fabricus is shown in the Table 3. Relative weight of bursa of Fabricus of broilers in all treated groups were non-significantly different as compared with controls throughout the trial (28, 35 and 42 days of age). There were similar findings in the bursa of Fabricius which suggest that there was similar effect of ethanol on the bursa of Fabricius as on the other lymphoid organs No gross abnormality was observed in bursa of Fabricius of the treated birds. These findings were inline with the findings of Khan *et al.* (2006). Histopathologically no change was observed in the treated groups. Change in diameter was also significant in positive control which confirms no effect of ethanol on bursa of Fabricius through crop tube and the volume of ethanol given. Comparison of Follicular diameter and epithelial height of Bursa of Fabricus studies in treated and controls are shown in Table 4. There was a significant decrease (P< 0.05) in epithelial height in all the groups as compared with negative control group. A significant decrease (P< 0.05) in follicular diameter in group A and B was observed as compared with positive control while, a significant increase in follicular diameter of broilers of positive control was observed as compared with negative control. These findings were contrary with the findings of Khan *et al.* (2006) who reported non significant difference in treated and non treated groups in follicular diameter and epithelial height.

Relative weight of thymus of broilers received different treatments are shown in Table 5. A significant increase in relative weight of thymus was observed in birds of positive control as compared with negative control at the age of 28 days. A significant increase in all the groups was observed as compared with negative control at the age of 35 days. While, at the age of 42 days, a significant increase was observed in group B, C and D as compared with negative control group. These findings were otherwise than the findings of Khan *et al.*

(2006). There were no gross changes in the thymus of the treated birds as compared with control groups. There was a decrease in the cellular density of thymus in the treated groups received higher doses of ethanol (40%) as compared with the control groups. These findings were inline with the findings of Khan *et al.* (2006). Jerrells *et al.* (1986) and Han *et al.* (1993). Wang *et al.* (1994) reported that ethanol significantly inhibits the thymocyte proliferation. Decrease in cellularity has been related to increase in the endogenous glucocorticoids, as high corticosterone levels in ethanol treated animals induces apoptosis thus causes thymus atrophy which results in the decrease in the cellular density and relative weight of thymus (Han *et al.* 1993). It can be deduced from the present results that ethanol through crop tube at these levels has no effect on spleen and bursa of Fabricius but on thymus.

TABLE 2: Comparison of means ± SD of relative weight of spleen.

Group	lymphoid organs		
	28	35	42
A	0.1436 a ±0.0375	0.1360 ±0.0375	0.1179 ±0.0091
B	0.1518 a ±0.0768	0.1264 ±0.0441	0.1488 b ±0.0483
C	0.1854 a ±0.1344	0.106 ±0.0354	0.0950 ±0.0462
D	0.1475 ±0.0256	0.1428 b ±0.0123	0.1719 b ±0.0517
E	0.0999 a ±0.0108	0.0626 a ±0.0024	0.0461 a ±0.0026

(a) Significantly different (P< 0.05) compared with positive control group (D).
(b) Significantly different (P< 0.05) compared with negative control group (E).

TABLE 3: Comparison of means ± SD of relative weight of Bursa of Fabricus.

Group	Age of birds in days		
	28	35	42
A	0.0481 ±0.0138	0.1121 ±0.0353	0.0309 ±0.0215
B	0.0925 ±0.0768	0.0757 ±0.0158	0.0495 ±0.0176
C	0.1344 ±0.1113	0.1065 ±0.0526	0.0460 ±0.0127
D	0.1285 ±0.0639	0.0476 ±0.0187	0.0533 ±0.0189
E	0.1118 ±0.0098	0.0673 ±0.0053	0.0434 ±0.0020

Table 4: Comparison of means ± SD of epithelial height (µm) and follicular diameter (µm) of Bursa of Fabricus.

Groups	Epithelial height	Follicular diameter
A	27.68±7.51 b	265.45±49.44 a
B	25.62±6.22 b	250.61±57.60 a
C	25.62±7.05 b	300.07±48.92
D	28.70±7.86 b	340.81±58.49 b
E	49.71±17.99a	269.32 ±73.87a

(a) Significantly different (P< 0.05) compared with positive control group (D).
(b) Significantly different (P< 0.05) compared with negative control group (E).

4. Humoral Immune Response: The effect of ethanol through crop tube on Geo mean titer (GMT) against the New Castle Disease vaccine virus in broilers is shown in Table 6. The Geo mean titer (GMT) against the New Castle Disease vaccine virus was low in all treatment groups as compared with both control groups throughout the trial. It was almost same in all the treatment groups and control groups at the age of 28 and 42 days. At the age of 35 days, it was low in groups A and B as compared with the positive control (group D). At 42 days of age, it was relatively low in group A as compared with the control groups.

TABLE 5: Comparison of means ± SD of relative weight of thymus.

Group	Age of birds in days		
	28	35	42
A	0.2905 ±0.0902	0.4479 b ±0.0130	0.2201 ±0.1234
B	0.3087 ±0.0863	0.3169 b ±0.0249	0.2318 b ±0.0245
C	0.2288 ±0.1382	0.3353 b ±0.1297	0.2910 b ±0.0310
D	0.3833 b ±0.1500	0.3486 b ±0.1233	0.3713 b ±0.1202
E	0.1260 a ±0.0144	0.0766 a ±0.0084	0.0521 a ±0.0020

(a) Significantly different (P< 0.05) compared with positive control group (D).
(b) Significantly different (P< 0.05) compared with negative control group (E).

Geo mean titer (GMT) against the Infectious Bursal Disease vaccine virus in broilers is shown in Table 7. The GMT against IBD vaccine virus was low in group A, B and C as compared with control groups at the age 28 days. At the age of 35 days, Geo mean titer (GMT) against the IBD vaccine virus was low in group

A and at the age of 42 days, very low in group A and low in group B as compared with control groups.

Geo mean titer (GMT) against the washed sheep red blood cells in broilers is shown in the Table 8. The Geo mean titer (GMT) against the washed sheep red blood cells was low in group A as compared with the control groups at the age of 28 days. At the age of 35 days, variable results were obtained i.e., groups A and C showed very low antibody titre as compared with controls while, group B showed low antibody titre as compared with controls. At the age of 42 days, group A showed very low while, group B showed low antibody titer as compared with controls. The findings of present study was in accordance with the findings of Khan *et al.* (2006) It has been reported that ethanol consumption produces a decrease in T-helper cells and B-lymphocyte due to increase in concentration of immunosuppressive cytokines (TGF-ß & IL-10) (Pacifici *et al.*2001), which may be true at dose level of 6 mL in broilers. Szabo (1999), also reported that chronic ethanol consumption increases immunoglobulin levels in human, which sounds true at 2 and 4ml levels in broilers as observed during present study, while at higher levels i.e., 6 ml, there appeared a depressing effect which is in line with findings of Xu *et al.* (1998) and Padgett *et al.* (2000), who reported that ethanol causes increase in glucocorticoids in the blood which are responsible for decrease in humoral immune response by depress B-cell activity.

TABLE 6: Comparison of geo mean titer (GMT) against Newcastle Disease vaccine virus.

Group	28 days	35 days	42 days
A	2.52	3.17	2.83
B	2.52	3.99	3.4
C	2.82	5.04	3.4
D	3.66	5.66	3.17
E	3.36	6.73	3.17

ND vaccine virus was administered on 4th and 24th day of age.

TABLE 7: Comparison of Geo mean titer (GMT) against IBD vaccine virus.

Group	28 days	35 days	42 days
A	161.06	107.60	63.97
B	152.10	152.10	101.54
C	168.80	255.85	180.92
D	180.92	255.85	161.18
E	180.90	203.10	180.90

IBD vaccine virus was administered on 9th and 21st day of age.

TABLE 8: Comparison of Geo mean titer (GMT) against washed sheep red blood cells.

Group	28 days	35 days	42 days
A	22.62	11.31	38.30
B	45.23	25.20	63.90
C	63.90	11.31	90.46
D	90.46	45.23	90.46
E	63.90	16.00	90.50

Washed sheep red blood cells were administered on 22nd and 37th day of age.

TABLE 9: Comparison of means ± SD of swelling amplitude (mm) against the mallein antigen.

Group	Time after inoculation			
	0 hrs.	24 hrs.	48 hrs.	72 hrs.
A	0.474 ± 0.040	0.618 ± 0.053	0.525 ± 0.078	0.475 ± 0.078
B	0.458 ± 0.011	0.580 ± 0.042	0.448 ± 0.060	0.435 ± 0.014
C	0.509 ± 0.015	0.602 ± 0.025	0.595 ± 0.098	0.528 ± 0.095
D	0.482 ± 0.060	0.590 ± 0.007	0.592 ± 0.025	0.548 ± 0.017
E	0.528 ± 0.025	0.648 ± 0.011	0.680 ± 0.056	0.605 ± 0.007

Primary dose of mallein antigen was injected at 24th and the booster dose at 39th day of age.

5. Cell mediated immune response: Swelling amplitude against the mallein antigen is given in Table 9. There was non-significant difference in swelling amplitude against the mallein antigen between the treatment groups and control though a dose dependent decrease in swelling amplitude was observed. It has been reported that ethanol consumption inhibits Th1-associated interleukin-12 and interferon-gamma cytokine production and delayed type of hypersensitivity (Waltenbaugh *et al.* 1998). Th-1 cells are the cells which are mainly involved in cell mediated immunity and they are regulated by the Gamma interferon and IL-12 cytokines, so decrease in cell mediated immunity might be due to decrease in production of these regulatory cytokines as a result of ethanol consumption. Similarly, suppression of cell mediated immune response after ethanol exposure was mediated by increased presence of proinflammatory cytokine (IL-6) this cytokine decreases the production of IL-4 which is known to be a potent stimulant of cell mediated immune response (Faunce *et al.* 1998; Messingham *et al.* 2002).

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