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EFFECTS OF ETHANOL ON DIFFERENT ORGANS AND ON FCR IN QUAILS (*COTURNIX JAPONICA)*

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ABSTRACT

A study was planned to investigate the effects of different doses of ethanol on body organs of Japanese quails. A total of 120 quails were randomly divided into five groups, A, B, C, D and E. Quails of groups A, B, C and D were given ethanol at concentrations of 2, 4, 8 and 16%, respectively in drinking water for four weeks, while birds of group E served as untreated control. The results at the end of 4th week revealed a significant effect on relative weight of heart, kidney and lungs in most treated groups. The increase in heart and lung weight was significant ($P<0.05$) in quail given 4% and higher ethanol, of kidney given 2 to 8% ethanol, while statistically no effect was observed on relative weight of liver. The relative weight of the proventriculus and the intestine at 4th week also showed statistically no difference compared to control group. However, the weight of the gizzard at 4th week increased significantly (P0.05) in groups given 8 to 16% ethanol and the increase was 42% in these groups compared with control group. The lymphoid organs at the end of 4th week revealed significant difference in weight of the bursa of Fabricius in quails given 16% ethanol and of the thymus in quails given 4 to 16% ethanol. Statistically, no difference was observed in spleen weight of treated groups compared to control group. The gross and light microscopic examination failed to reveal significant changes in these organs with routine methods of examination. Ethanol showed a significant effect on feed conversion ratio which was poor in ethanol treated groups; at the end of 4th week, it varied from 232 to 442% in groups given 8 and 16% ethanol, respectively. These data suggest that ethanol has significant effects on relative weight of heart, kidney, lungs, thymus, and on feed conversion ratio in the Japanese quails.

Key words*:* Ethanol, quail, lymphoid organs, visceral organs, FCR.

INTRODUCTION

In Pakistan, investment in Poultry sector is about 1 billion US dollars (Badar *et al*., 2006). The government extended its support to farmers and exempted this industry from income tax and sales tax and managed export of table eggs, day old chicks and broilers on subsidized rates, but the productivity of local birds in terms of eggs or returns has been low (Abedullah *et al*., 2007). One of the reasons of this low productivity is that most of the poultry farmers are illiterate and are using layman practices for growth of birds and treatment of disease.

Ethanol is used by some broiler farmers as presumptive growth promoter, for avoiding stress and in milder respiratory infections in winter season. This coumpound has varied effects on many systems of the body (Klassen and Persaud, 1978) and is toxic at higher dose level or at moderate dose level when used for long periods. The major toxic metabolites of ethanol are acetaldehyde and free radicals (Suzuki and Cherian, 2000). Chronic ethanol ingestion induces changes in intestinal brush-border membrane (Bikle *et al*. 1986), cardiomyopathies (Martinez *et al*. 2000), brain, pancreas, serum electrolytes and haematology (Bashir and Javed, 2005). It has been reported that chronic ethanol administration ameliorated and/or delayed the development of nephrotic syndrome in adriamycin nephropathy in rats (Tesar *et al*., 1995). In turkey poults, ethanol caused increased relative heart weight purely due to decrease in body weight (Ali and Czarnecki, 1987). These wide range of known effects of ethanol, and its use in broilers, prompted the authors to carry out this study to determine any adverse effects of ethanol on brain, pancreas, haematology, serum electrolytes (data already published; Bashir and Javed, 2005), visceral organs, lymphoid organs and feed conversion ratio (data presented in this paper) in an avian model system using Japanese quails.

MATERIALS AND METHODS

The experimental procedure was the same as published earlier (Bashir and Javed, 2005). In this experiment, 120 quails of 39 days of age and of mixed sex were purchased from the local market, kept for four days to acclimatize and were randomly divided into five equal groups (A, B, C, D and E). Ethanol was given to first four groups at concentrations of 2, 4, 8 and 16%, respectively for four weeks, through drinking water, while group E

served as the control. All the birds were offered feed and drinking water *ad libitum*. Commercial broiler finisher feed was given to all the groups. The experiment was conducted during the summer season. Six birds from each group were slaughtered at weekly intervals. The live weights of the bird and weight of heart, liver, kidneys, lungs, proventriculus, gizzard, intestine, bursa of Fabricius, spleen and thymus were recorded and the relative weight of each organ was calculated. Gross and light microscopic examination of each organ was performed using the routine haemotoxylin and eosin staining technique. Daily feed consumption was recorded for each group and then weekly feed conversion ratio was calculated by dividing the total feed consumed by total weight of the bird. The study was formally approved by the faculty scrutiny and advisory committee, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan. Data obtained in the experiment were analysed using analysis of variance technique and the means were compared by least significant difference test using SAS 6.12 statistical software (SAS Institute Inc., 1996).

RESULTS

Effects on heart, liver, kidneys and lungs

The effects of ethanol on relative weights of heart, liver, kidney and lungs are given in Table 1. A significant ($P<0.05$) difference in relative weight of heart was observed at weeks 1, 3 and 4. At first week, the relative weight of heart increased ($P<0.05$) in group offered 2% ethanol, while at 3rd week, it increased ($P<0.05$) in groups offered 8 and 16% ethanol. At the end of 4th week, it increased ($P<0.05$) in groups offered 4 to16% ethanol. An increase in relative weight of heart at the end of 4th week varied from 16 to 52%.

At first week, the relative weight of liver increased ($P<0.05$) in group offered 2% ethanol but at 2nd week, it decreased ($P<0.05$). At 4th week, an increase of 12 and 6% in relative weight of liver was seen in the groups given 8 and 16% ethanol, respectively, while a decrease of 4 and 7% was found in the groups offered 2 and 4% ethanol, respectively. The relative kidney weights decreased ($P<0.05$) in all treated groups compared to the control at week 1 and increased ($P<0.05$) in weeks 3 and 4. At the end of 4th week, the relative kidney weight increased ($P<0.05$) by 50 to 67% in groups offered 2 to 8% ethanol and there was 34% increase in group given 16% ethanol. The relative weight of the lungs varied significantly ($P<0.05$) at 2nd, 3rd and 4th week. At week 2, there was an increase ($P<0.05$) in relative weight of the lungs in groups offered 2 and 4% ethanol. At week 3, a significant increase ($P<0.05$) was observed in groups offered 4 and 16% ethanol. At week 4, an increase in weight varied from 7 to 69%. Although the results were significant in the groups offered 4 to 16% ethanol.

Effects on proventriculus, gizzard and intestine

The effects of ethanol on relative weight of proventriculus, gizzard and intestine are presented in Table 2. At week 2, the proventriculus weight decreased ($P<0.05$) in groups offered 2 or 8% ethanol, and in week 3, it increased ($P<0.05$) in groups offered 8 or 16% ethanol. The results at the end of the 4th week, however, were non-significant and showed increase varying from 0 to 33% in the treated groups. The weight of the gizzard significantly increased ($P<0.05$) at the 3rd (16% ethanol group) and 4th (8 and 16% ethanol groups) weeks. At 4th week, an increase in the weight of the gizzard varied from 31 to 42% in groups offered 4% to 16% ethanol, while it showed a fractional decrease in group offered 2% ethanol. The weight of the intestine showed significant ($P<0.05$) decrease at week 2 in groups offered 2 and 8% ethanol but the results at other weeks were non-significant and a negligible decrease of 2 to 10% was observed at the end of 4th week in treated groups.

Effects on bursa of Fabricius, spleen and thymus

The relative weights of bursa of Fabricius, spleen and thymus in birds of different groups are presented in Table 3. It shows a non-significant difference in relative weights of these organs at all weeks in all treated groups, except at 4th week where a significant ($P<0.05$) increase in weight of bursa of Fabricius and thymus was observed. For the bursa, the increase was 50% ($P<0.05$) in the group offered 16% ethanol, whilst the other groups showed no difference. For the thymus, a 50% increase ($P<0.05$) was recorded in groups offered 4 to 16% ethanol and the group given 2% ethanol showed a non-significant increase of 33%. The weight of spleen showed non-significant difference at all weeks in all groups. However, at week 4, an increase of 40% was observed in groups offered 2 and 16% ethanol, while an increase of 60 % was observed in groups offered 4 and 8% ethanol.

Other than changes in weight of organs, the gross and light microscopic examination of each organ revealed no significant change.

Feed conversion ratio (FCR)

The results of feed conversion ratio (FCR) are presented in Table 4. A significant difference was recorded in FCR between the groups at all weeks. The FCR was significantly ($P<0.05$) poor in the groups offered 4 to 16% ethanol at week 1 compared with the control group. At week 2, FCR was better ($P<0.05$) in the groups offered 2 and 16% ethanol. At week 3 the FCR was poor in groups offered 4 to 16% ethanol ($P<0.05$) and at week 4 in groups offered 8 and 16% ethanol ($P<0.05$) compared with control group. The results at the end of 4th week showed that groups offered 2 and 4% ethanol consumed 36 and 32% more feed, respectively, to convert to body mass, while groups offered 8 and 16% ethanol consumed 232 and 442% ($P<0.05$) more feed, respectively, to convert to body mass compared with control group.

Table 1: Relative weights of heart, liver, kidney and lungs (mean ± SD) in quails given ethanol at various concentrations through drinking water

Organs	Treatment groups	Weeks				Difference from control (%)
		1	2	3	4	
Heart						
	A	1.19 ± 0.24*	0.57 ± 0.15	0.66 ± 0.07	0.73 ± 0.07	+ 16
	B	0.71 ± 0.09	0.77 ± 0.10	0.89 ± 0.09	0.87 ± 0.13*	+ 38
	C	0.71 ± 0.06	0.57 ± 0.16	0.96 ± 0.04*	0.96 ± 0.03*	+ 52
	D	0.94 ± 0.14	0.74 ± 0.20	1.07 ± 0.23*	0.93 ± 0.07*	+ 48
	E	0.69 ± 0.04	0.65 ± 0.23	0.72 ± 0.04	0.63 ± 0.06	
Liver						
	A	3.54 ± 0.71*	1.73 ± 0.15*	2.56 ± 0.61	2.09 ± 0.72	- 04
	B	2.51 ± 0.16	2.29 ± 0.25	2.75 ± 0.95	2.03 ± 0.65	- 07
	C	2.54 ± 0.33	2.31 ± 0.20	2.95 ± 0.90	2.45 ± 1.10	+ 12
	D	2.78 ± 0.10	2.88 ± 0.30	3.05 ± 1.06	2.32 ± 1.03	+ 06
	E	2.32 ± 0.36	2.54 ± 0.36	2.63 ± 0.25	2.18 ± 0.78	
Kidney						
	A	0.43 ± 0.12*	0.46 ± 0.09	0.68 ± 0.17	0.87 ± 0.04*	+ 50
	B	0.43 ± 0.14*	0.70 ± 0.04	0.81 ± 0.12	0.90 ± 0.19*	+ 55
	C	0.52 ± 0.04*	0.63 ± 0.10	0.79 ± 0.12	0.97 ± 0.25*	+ 67
	D	0.42 ± 0.07*	0.56 ± 0.21	0.89 ± 0.28*	0.78 ± 0.24	+ 34
	E	0.73 ± 0.13	0.52 ± 0.08	0.66 ± 0.30	0.58 ± 0.05	
Lungs						
	A	1.07 ± 0.12	0.65 ± 0.10*	0.68 ± 0.10	0.62 ± 0.07	+ 07
	B	0.86 ± 0.11	0.96 ± 0.09*	1.02 ± 0.12*	0.94 ± 0.19*	+ 62
	C	1.02 ± 0.14	0.58 ± 0.23	0.86 ± 0.10	0.89 ± 0.24*	+ 53
	D	0.95 ± 0.06	0.49 ± 0.07	1.09 ± 0.13*	0.98 ± 0.23*	+ 69
	E	0.98 ± 0.13	0.40 ± 0.09	0.70 ± 0.08	0.58 ± 0.04	

*Significant difference (P< 0.05) compared with control group.

Table 2: Relative weights of proventriculus, gizzard and intestine (mean ± SD) in quails given ethanol at various concentrations through drinking water

Organs	Treatments groups	Weeks				Difference from control (%)
		1	2	3	4	
Proventriculus						
	A	0.41 ± 0.57	0.23 ± 0.04*	0.36 ± 0.10	0.33 ± 0.04	0
	B	0.38 ± 0.07	0.33 ± 0.06	0.45 ± 0.09	0.37 ± 0.05	+ 12
	C	0.44 ± 0.06	0.28 ± 0.06*	0.50 ± 0.15*	0.44 ± 0.14	+ 33
	D	0.46 ± 0.07	0.37 ± 0.06	0.48 ± 0.04*	0.44 ± 0.13	+ 33
	E	0.41 ± 0.06	0.42 ± 0.05	0.32 ± 0.05	0.33 ± 0.07	
Gizzard						
	A	2.01 ± 0.24	1.15 ± 0.40	2.02 ± 0.22	1.56 ± 0.25	- 02
	B	1.90 ± 0.11	2.05 ± 0.35	2.00 ± 0.65	2.08 ± 0.36	+ 31
	C	2.39 ± 0.35	2.22 ± 0.81	2.13 ± 0.28	2.25 ± 0.77*	+ 42
	D	2.21 ± 0.13	2.82 ± 0.59	2.58 ± 0.49*	2.25 ± 0.46*	+ 42
	E	2.18 ± 0.16	2.06 ± 0.44	1.70 ± 0.42	1.59 ± 0.30	
Intestine						
	A	5.10 ± 0.64	2.57 ± 0.71*	5.18 ± 1.58	3.83 ± 1.36	- 05
	B	4.00 ± 0.66	4.40 ± 1.12	4.87 ± 1.12	3.62 ± 0.87	- 10
	C	4.52 ± 0.51	3.36 ± 0.37*	4.14 ± 2.13	3.62 ± 0.98	- 10
	D	4.60 ± 0.28	5.40 ± 0.76	3.85 ± 1.28	3.96 ± 1.75	- 02
	E	4.86 ± 0.69	5.48 ± 1.03	4.46 ± 0.59	4.03 ± 1.08	

*Significant difference (P< 0.05) compared with control group.
The weights of GIT were for flushed out pieces of GIT.

Table 3: Relative weights of bursa of Fabricius, spleen and thymus (mean ± SD) in quails given ethanol at various concentrations through drinking water

Organs	Treatment groups	Weeks				Difference from control (%)
		1	2	3	4	
Bursa						
	A	0.07 ± 0.02	0.04 ± 0.01	0.06 ± 0.00	0.04 ± 0.01	
	B	0.06 ± 0.03	0.03 ± 0.02	0.05 ± 0.02	0.04 ± 0.02	
	C	0.08 ± 0.06	0.05 ± 0.00	0.06 ± 0.02	0.04 ± 0.01	
	D	0.09 ± 0.01	0.04 ± 0.03	0.07 ± 0.02	0.06 ± 0.02*	+ 50
	E	0.05 ± 0.03	0.06 ± 0.03	0.04 ± 0.01	0.04 ± 0.01	
Spleen						
	A	0.05 ± 0.01	0.04 ± 0.02	0.06 ± 0.02	0.07 ± 0.03	+ 40
	B	0.11 ± 0.06	0.05 ± 0.02	0.08 ± 0.04	0.08 ± 0.03	+ 60
	C	0.11 ± 0.04	0.06 ± 0.03	0.08 ± 0.02	0.08 ± 0.05	+ 60
	D	0.13 ± 0.03	0.08 ± 0.03	0.09 ± 0.01	0.07 ± 0.04	+ 40
	E	0.06 ± 0.03	0.06 ± 0.02	0.06 ± 0.02	0.05 ± 0.01	
Thymus						
	A	0.37 ± 0.12	0.09 ± 0.07	0.14 ± 0.05	0.16 ± 0.06	+ 33
	B	0.33 ± 0.10	0.15 ± 0.08	0.24 ± 0.07	0.18 ± 0.03*	+ 50
	C	0.31 ± 0.08	0.16 ± 0.03	0.19 ± 0.05	0.18 ± 0.04*	+ 50
	D	0.34 ± 0.11	0.16 ± 0.02	0.23 ± 0.08	0.18 ± 0.01*	+ 50
	E	0.22 ± 0.04	0.22 ± 0.19	0.16 ± 0.05	0.12 ± 0.02	

*Significant difference (P< 0.05) compared with control group.

Table 4: Feed conversion ratio (FCR) (mean ± SD) in quails given ethanol at various concentrations through drinking water

Treatment groups	Weeks				Difference from control (%)
	1	2	3	4	
A	0.48 ± 0.04	0.29 ± 0.02*	0.48 ± 0.02	0.38 ± 0.01	+ 36
B	0.66 ± 0.12*	0.35 ± 0.03	0.65 ± 0.09*	0.37 ± 0.08	+ 32
C	0.69 ± 0.18*	0.35 ± 0.01	1.10 ± 0.10*	0.93 ± 0.02*	+ 232
D	0.74 ± 0.18*	0.29 ± 0.01*	1.92 ± 0.35*	1.52 ± 0.29*	+ 442
E	0.37 ± 0.06	0.38 ± 0.02	0.31 ± 0.01	0.28 ± 0.01	

*Significant difference (P< 0.05) compared with control group.

DISCUSSION

The data on relative weights of organs including heart, kidney and lungs showed an increase which was significant in most groups. After 4 weeks of treatment, the increase in weight of heart and kidney was highest in quails offered 8% ethanol, while the increase in the lungs weight was highest in quails offered 16% ethanol. The relative liver weight, however, showed no statistical difference from control, although increases varied from 4 to 12% in treated groups. The significant increase in weight of the heart, kidney and lungs at 4th week suggests an effect of ethanol on these organs. In a previous study, a 60% reduction in heart weight at the dose rate of 2 g/kg in senescent mice has been reported (Shi *et al*., 2001), which may be due to species (quail and mice) variation. However, our results confirm the results in turkey poults given 4-5% ethanol in drinking water for 6 weeks, where a significant (P<0.05) increase in relative weight of heart was observed (Czarnecki *et al*., 1985; 1987) with ultrastructural changes of glycogen accumulation, swollen mitochondria, myofibrillar lysis, increased number of lysosomes, dilated sarcoplasmic reticulum and dense myofibers. Dilatation of heart and congestive cardiomyopathy in turkey poults (Edes *et al*., 1987), left ventricular dilatation and left ventricular dysfunction in chicken with myocyte hypertrophy, interstitial fibrosis and myocytolysis have been reported by 20% ethanol in drinking water (Morris *et al*., 1999); these changes were also observed by non-invasive methods in birds given ethanol (Edes *et al*., 1987; Soos *et al*., 1991). Alcoholic cardiomyopathy has been shown to develop more rapidly with high doses of ethanol and long-lasting alcoholization (Tsyplenkova and Sholts, 1988). We were unable to spot these changes in the myocardium by ordinary microscopy during the present study. Our results of decreased kidney weight at week 1 in all the treatment groups and increase in the weight at week 4 in quails offered 2-8% ethanol

differed from non-significant difference in the weight of kidney in male Wister rats (McNeil *et al*., 1988). Our results for the liver weight were in congruence with those observed in meat-type chicken administered 20% ethanol at a dose rate of 2 ml/kg from 21 to 28 days (Peebles *et al*., 1996). Mild to moderate hepatocellular fatty change in a few birds has been reported with 95% ethanol at a dose rate of 1 ml for 7 days (Allen *et al*., 1981). The increase in lungs weight observed in our study might be associated with changes in heart or vice versa. However, we were unable to find gross pathological or histopathological changes in these organs, although some insignificant findings at occasion in some birds were seen which were thought of meaningless as we could not correlate the gross and histopathological alterations. The changes with immuno- histochmical methods or electron microscopic method might have helped but we were lacking these facilities.

The effects on gastrointestinal organs were non-significant at the final week, except for the gizzard weight which increased (42%) in quails offered 8 to 16% ethanol. The increase in gizzard weight might be associated with hypertrophy and/or hyperplastic changes which could not be seen by subjective methods of microscopic examination. A study in male rats showed no effect of ethanol on the length of the small intestine, 5 and 15% ethanol inhibited cell production in jejunum and distal aspect of the ileum (Lansdown and Dayan, 1987). This seems quite relevant to our results as we found a non-significant decrease in relative weight of the intestine that varied from 2 to 10%. Reversible effect on integrity of small intestinal villi without significantly affecting gastrointestinal permeability due to chronic alcoholism has also been reported (Keshavarzian *et al*., 1994). Ethanol has been reported to cause reduced intestinal blood flow with gastrointestinal haemorrhages and gastrointestinal ulceration (Horie and Ishii, 2001), increase in number of chronic inflammatory cells in mucosa and increase in goblet cells in rats (Vaquera *et al*., 2002) and alteration in intestinal brush border in chicken (Bikle *et al*., 1986). These changes were not observed in the present investigation in quails which may be due to species variation. However, further experiments may be conducted to confirm these effects in quails.

The data on lymphoid organs revealed no statistical effect, but there was a consistent increase in spleen weight, while the effect on weight of bursa of Fabricius was only at 16% ethanol. The thymus weight increased at week 4 but the values were lower than the control ones for weeks 1 and 2, and in week 2 all the treated values were less than the control. These changes in weight of the thymus might also have been associated with functional changes in the immune system. However, these findings may be linked with impaired function of T-cells with particular reference to abnormalities in antigen presentation (Mikszta *et al*., 1995).

The FCR increased at week 1, 3, and 4, while decreased at week 2. This change at week 2 is difficult to explain. Ethanol at levels higher than 4% showed a significant effect on FCR, although levels of 4% and lower also showed considerable effect. These results confirm the earlier findings of low feed efficiency in alcohol treated animals (Larue-Achagiotis *et al*., 1989). A lower growth rate in chicks given 15% ethanol through drinking water has previously been reported (Bikle *et al*., 1986). The poor FCR in ethanol treated quails might be due to microcirculatory disturbance in gastrointestinal mucosa (Horrie and Ishii, 2001) and/or due to alterations in intestinal mucosa (Bikle *et al*., 1986).

Conclusion

Based on these results, it can be concluded that treatment of Japanese quails with 2, 4, 8 or 16% ethanol in drinking water for 4 weeks affected relative weight to body weight of heart, kidney, lungs, gizzard, lymphoid organs (especially thymus) and FCR. The latter was very poor at higher doses of ethanol. Therefore, broiler farmers are advised not to use ethanol in broilers for any purpose.

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