TOXICO-PATHOLOGICAL EFFECTS OF FORMALIN (37% FORMALDEHYDE) FEEDING IN BROILER CHICKS

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ABSTRACT

One hundred day-old broiler chicks kept in five equal groups (A-E) were given formalin (37% formaldehyde) at dose levels of 20, 10, 5, 2.5 and 0 ml / kg feed for 7 weeks. Body weight and feed intake of the birds fed 2.5 ml formalin / kg feed was non-significantly different from control whereas higher formalin levels groups had significantly lower values. Formalin at 2.5 and 5.0 ml / kg feed lavel did not produce clinical signs in the birds but Higher doses of formalin resulted in depression, somnolence and a staggering gait. Necrotic and ulcerative areas on the mucosa of crop, proventriculus and petechial hemorrhages were observed on the mucosa of small intestine. Liver and kidneys did not show any specific microscopic changes. The no observed adverse effect lavel (NOAEL) of formalin appeared to lie between 2.5 and 5 ml/ kg feed.

Key Words: Formalin, Broiler chicks, Toxico-pathological effects.

INTRODUCTION

Formaldehyde (FA) is a colorless gaseous chemical commercially available as 37% aqueous solution known as formalin. It is highly germicidal and used as a disinfectant in poultry and livestock industry. Food and Drug Agency (FDA) in USA has approved the addition of formalin in poultry feed for keeping it salmonella free (Brown, 1996). However, FA is highly irritant to mucous membranes, its incorporation may prove toxic to the chicken. There are reports concerning toxic effects of FA given per-orally to rats, mice, calf, (Restani and Corredor, 1991). However, no report describing per-oral toxicity of FA or formalin in chicken is available. The present study was designed to investigate the toxico-pathological effects of different levels of formalin fed to the broiler chicks.

MATERIALS AND METHODS

Broiler Chicks and Feed

One hundred day-old broiler chicks procured from a local hatchery were kept on wood shavings under standard conditions of brooding and management. Fresh water and basal feed (broiler chick starter mash, 22% total protein) was provided *ad libitum*.

Experimental Procedure

On first day of the experiment, chicks were divided into five groups (A through E) of 20 birds each. Formalin (37% aqueous solution of formaldehyde) was fed to these groups by mixing in their daily rations for 7 weeks at dose rates of 20.0, 10.0, 5.0, 2.50 and 0.0 ml/kg feed. After 7 weeks, the birds were slaughtered and specimens were collected to study the different parameters.

Clinical Evaluation

Broiler chicks were examined twice daily for clinical signs and behavioral alterations. The criteria were based on subjective evaluation of (i) alertness, (ii) response to disturbance, and (iii) attraction towards feed.

Body Weight, Feed and Formaldehyde Intake

Chicks in each group were individually weighed at weeks 1, 2, 3, 4, 5, 6, and 7. Daily feed intake of birds in each group was measured and calculated as feed consumed per bird per day for each week. FA intake for each group was calculated as mg/kg body weight / day from the average daily feed intake of birds each week.

Gross lesions and Histopathology

Following slaughtering, the visceral organs of each chick were examined for the presence of gross lesions. Different organs including heart, liver, bursa of Fabricious and spleen were weighed and their relative weights were calculated as percent of body weight in individual birds.

Liver and kidney tissues of about 5 mm thickness fixed in 10% neutral buffered formalin were processed by routine paraffin embedding procedure. Section of 5 µm thickness were stained with hematoxylin and eosin.

Statistical analysis

The data obtained from different parameters were subjected to statistical analysis of variance and group means were compared by Duncan's multiple range test using MSTAT-C statistical computer package. The level of significance was 0.05 or lower.

RESULTS

Clinical Signs

No clinical signs and behavioral alterations were observed in chicks of groups D & E. In groups A and B the clinical signs begun to appear after 1 week of experiment and included decreased attraction towards feed, somnolence, dullness, sitting with closed eyes and decreased response on disturbance. These signs were more pronounced in morning for about one hour soon after ingestion of feed compared with rest of the day. At weeks 6 and 7 of experiment, a few birds in group C also exhibited similar but less pronounced signs.

Feed Intake

Feed intake (g/bird/day) of different groups has been presented in Table 1.

Table 1: Feed intake (g/bird/day) of Broiler chicks fed different levels of formalion.

	Groups*				
Period (Weeks)	**A**	**B**	**C**	**D**	**E**
1	9.32 ± 2.46	10.32 ± 2.34	10.36 ± 2.40	11.50 ± 2.61	11.84 ± 2.88
2	23.04 ± 6.80	28.64 ± 12.92	31.26 ± 13.33	32.67 ± 14.65	33.62 ± 15.98
3	36.12 ± 6.39b	59.79 ± 5.57a	59.12 ± 3.40a	68.16 ± 11.67a	68.06 ± 9.46a
4	44.39 ± 5.56c	64.74 ± 13.82b	70.84 ± 6.15b	88.34 ± 5.90a	86.50 ± 7.25a
5	51.04 ± 1.00c	76.71 ± 4.22b	83.07 ± 3.10b	104.60 ± 6.04a	106.60 ± 4.76a
6	52.79 ± 2.52b	81.29 ± 2.60c	91.82 ± 3.33b	121.10 ± 7.97a	125.00 ± 5.52a
7	71.57±10.61b	95.43 ± 7.83c	114.60 ± 15.01b	174.30 ± 24.09a	168.60 ± 20.40a

* Groups A, B, C, D and E were given 20, 10, 5, 2.5 and 0 ml formalin / kg feed, respectively.
Values in each row followed with different letters are significantly different (≤0.05).

Feed intake in first two weeks remained non-significantly different between all groups. The difference between groups D and E remained non-significant throughout the experiment. On week 3 it was significantly lower in group A compared with all other groups. During weeks 5-7 feed intake was lowest in group A followed by B and C, the differences being significant and these groups were also significantly lower from groups D and E.

Formaldehyde Intake

Average formaldehyde intake of chicks in each group is presented in Table 2. FA intake in groups A-E in first week was 1359, 752, 392, 211 and 0, mg /kg body weight/ day, respectively. In second week it inceased to 2319, 1164, 655, 322 and 0, mg /kg body weight/ day, respectively. Average FA intake in all groups decreased with increase in age and became lowest in week 6. In week 7 it slightly increased and was 531, 201, 106, 75 and 0 mg/kg body weight /day, respectively.

Body Weight

The body weight of broiler chicks in different groups has been presented in Table 3.

On week 2 the body weight was significantly lower in group A compared with all other groups. During weeks 5-7 the lowest body weight was observed in group A followed by groups B and C and the differences between these groups were significant. These groups also had significantly lower body weight than groups D and E. The difference between groups D and E remained nonsignificant till the end of experiment except on week 5 when group D had significantly higher body weight than group E.

Organ Weights

The absolute and relative weights of different organs have been presented in Table 4.

The absolute weight of liver was significantly lower in group A than in group B and C which in turn were significantly lower than in groups D and E. The relative weight of liver in groups B and C was significantly lower than in all other groups.

The absolute weight of spleen differed significantly between all groups, being lowest in group A followed by B, C and E. It was the highest in group D. The relative weight was also lowest in group A and it differed significantly from groups B, D and E.

Table 2: Formaldehyde intake (mg / kg body weight / day) of broiler chicks given different levels of formalin in feed

Period (weeks)	Groups* A	B	C	D	E
1	1359	752	392	211	0
2	2319	1164	655	322	0
3	1825	760	360	196	0
4	1370	603	252	135	0
5	966	415	151	95	0
6	400	188	97	57	0
7	531	201	106	75	0

* Groups A, B, C, D and E were given 20, 10, 5, 2.5 and 0 ml formalin/kg feed, respectively.

hemorrhages were present in the breast and thigh muscles of the chicks in group A but not in other groups. Petechial hemorrhages were present on the epicardium in groups E (0/20), D (0/20), C (2/20), B (4/20) and A (10/20). Liver in groups A and B were congested and had petechial hemorrhages on the surfaces.

Histopathological changes

In groups E, D, C and B hepatocytes had foamy cytoplasm. Aggregates of lymphoid cells were scattered in the liver parenchyma. In group A, cytoplasm had multiple small vacoules. Sinusoidal spaces contained erythrocytes.

In group E, D and C kidney did not show any morphological alteration. In group B and A the kidneys showed a mild degree of congestion but no degeneration or necrotic changes were observed.

Table 3: Body weight of Broiler chicks fed different levels of formalin.

Period (Weeks)	Groups * A	B	C	D	E
1	50.74 ± 3.86	50.73 ± 3.89	48.93 ± 9.49	50.43 ± 3.84	51.25 ± 3.95
2	73.40 ± 9.55	91.02 ± 11.73	88.34 ± 9.59	93.73 ± 5.26	87.69 ± 8.42
3	146.50 ± 6.41b	291.10 ±38.04a	303.60 ±37.42a	323.10 ±24.09a	322.80 ±26.27a
4	240.10 ±51.98d	397.40 ±41.26c	520.30 ±54.66b	597.70 ±49.43a	550.20±43.04 ab
5	391.70 ±90.78d	683.80 ±91.53c	851.20 ±80.33b	1018.00±110.00a	1032.00±96.57a
6	976.50± 133.78e	1605.00±201.25d	1737.00±113.06c	1960.00±200.65a	1885.00±139.64b
7	997.80±124.19d	1758.00±172.37c	2000.00±118.09b	2122.00±139.65a	2123.00±58.85a

* Groups A, B, C, D and E were given 20, 10, 5, 2.5 and 0 ml formalin / kg feed, respectively.
Values in each row with different letters are significantly different (≤0.05).

The absolute weight of heart differed significantly in all groups and was lowest in group A followed by Group B, C E and D. The relative weight of heart in group A was highest and significantly different from groups B and E. Group E had lowest relative weight of heart.

The absolute weight of bursa was lowest in group A followed by group B and these groups were significantly different from each other and all other groups. Group D had the highest weight of bursa which was significantly different from group E. The relative weight of bursa was highest in group A and it differed significantly from group E.

Gross Lesions

No visible lesions were observed in oral cavity of the chicks fed different levels of formalin except that the whitish area on the tip was more prominent in birds of group A compared with other groups. Areas of necrosis and ulcers were present on the mucosal surface of crop in birds of group A but not in other groups. Proventriculus in group A also showed some raised circular areas of necrosis. Small intestine in groups C, B, A had petechiation on the mucosal membrane in 3/20, 7/20 and 10/20 birds, respectively. Petechial

DISCUSSION

In the present study depression, somnolence, staggering gait and decreased water intake appeared as prominent clinical signs in broiler chicks fed 10 ml or higher levels of formalin / kg feed. More pronounced signs soon after ingestion of feed suggested a local irritant effect of formaldehyde on mucosa of upper digestive tract. No report is available about clinical signs or behavioral alterations in chicken following per-oral ingestion of FA. Rats administered FA in water for 60 days showed decreased responsiveness (Bhatt and Panchal, 1992). FA toxicosis in chicks following inhalation of FA vapors caused irritation of upper respiratory tract resulting in gasping and photophobia (Sander, 1996, Julian and Browm, 1997). Absence of signs of respiratory irritation in the present study suggested no involvement of respiratory tract in per-oral FA toxicity.

Significantly lower body weight in birds fed 5 ml and higher levels of formalin /kg body weight was also accompanied by significant decrease in feed intake suggesting that later could be the cause of lower body

weights in these groups. Schutte and Smith (1991) reported that broilers fed on a diet containing FA treated soybean meal showed an improvement in weight gain and feed efficiency up to 21 days old. Spears *et al.* (1980) reported no effect of 0.3 percent FA treatment of soybean meal on body weight and feed efficiency of broiler chicks for 21 days but higher levels decreased the body weight. In the present study 10 ml formalin / kg feed also had non significant effect on body weight and feed intake for up to 21 days of age which could be due to less intake of feed and hence less intake of FA. Per-oral administration of formaldehyde for various periods has been shown to result in deceased body weight in rats (Til *et al.*, 1989, Tobe *et al.*, 1989, Til *et al.*, 1989) and dog (Johannsen *et al.*, 1986). In the present study, 5 and 2.5 ml formalin / kg feed had no observable adverse effects on body weight and feed efficiency up to 6 and 7 weeks of the experiment. Rather an improvement in body weight was observed at week 6 of the experiment suggesting a beneficial effect.

A decrease in the absolute weights of organs like liver, spleen, heart and bursa of Fabricious in the present study could be due to decreased body weight of the chicks in 10 or 20 ml formalin/ kg feed groups. Increase in the relative weight of heart and bursa suggested a variable susceptibility of different organs to FA.

Presence of inflammatory lesions in different segments of digestive tract are suggestive of a local irritant effect of formaldehyde. Similar inflammatory lesions in digestive system following oral administration of formaldehyde has been reported in calves (Preston *et al.*, 1960) and rats (Til *et al.*, 1989, Tobe *et al.*, 1989, Til *et al.*, 1988). Petechial hemorrhages on epicardium and muscles could be due to a systemic effect of formaldehyde.

The no observed adverse effect level (NOAEL) of formaldehyde given in drinking water to rats have been reported to vary with the length of exposure being 24 mg/kg body weight/day for 4 weeks to 10 mg/kg/day for 24 months (Restani and Corredor, 1991). Other reports mentioned a NOAEL of 100 mg/kg/day for 90 days exposure (Johanessen *et al.*, 1986) and 15-21 mg/kg/day for 24 months (Till *et al.*, 1989). In the present study a NOAEL for 7 weeks exposure was between 2.5 and 5 mg/kg feed / day. However, this effect could not be compared to the studies in rats where FA was administered on body weight basis. While in the present study the formaldehyde intake /kg body weight did not remained constant and decreased with age. Had the formaldehyde intake per kg body weight been constant during experiment the resulting NOAEL probably would had been different. Further work is needed to collect more data on toxicity of FA in chicken.

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