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Furazolidone Toxicosis in Male Japanese Quails: Effect on Testes and Reversibility of Effect after Cessation of Treatment

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Abstract: Male Japanese quails of 7 weeks of age, divided into different equal groups, were fed furazolidone (Fz) at rate of 0(control), 400, 600 mg per kg feed. After four weeks of Fz feeding the birds in all groups were switched to Fz free basal feed for another 4 weeks. A pair-fed (PF) group was maintained and given daily ration as much as consumed by quails of group fed 600 Fz/kg feed. Fz fed quails showed significantly ($P<0.05$) lower feed consumption, body weights, testes weights, testes volume and diameter of seminiferous tubules than control quails. Microscopically seminiferous tubules of Fz fed quails showed decreased population of germinal epithelial cells, absence of round and elongate spermatids, pyknotic nuclei of round spermatids and round heads of elongate spermatids and young spermatozoa. After cessation of Fz feeding the feed consumption, body weights, testes weights and volume gradually increased and became non-significantly different from those fed 0 mg Fz /kg feed. The microscopic picture of the testes of Fz fed quails also became similar to that of control group. The pair fed group also exhibited significant decrease in body weight, testes weight and volume compared with control. Microscopically the seminiferous tubules had decreased population of germinal epithelial cells, however, no abnormal morphology of spermatids was observed. After increase in feed consumption all parameters of pair-fed group became non-significant from control group earlier than Fz fed groups. This observation suggested that Fz induced decreased intake was not the sole cause of changes in testes of Fz fed quails.

Key words: Furazolidone, toxicity, testes, quail

Introduction

Furazolidone (Fz), an antibacterial agent is used for the prevention and treatment of several common diseases in poultry. Its therapeutic level recommended for poultry, 0.04% w/w in feed, is reportedly toxic for quails (Arbid *et al.*, 1990). Fz has been known to induce degenerative changes in testes of chicken (Siddique *et al.*, 1996; Zermeno and Martinnez, 1984), ducklings (Webb and Vleet, 1990), and rats (Satoru *et al.*, 1977; Ludewig *et al.*, 1995). Its effect on testes of japanese quails have not been investigated. Also in male japanese quails no information is available about the reversibility of Fz induced changes after cessation of its administration. In poultry Fz administration causes anorexia (Ali and Bartlet, 1982). It is not known whether Fz induced pathological changes are a consequence of anorexia or anorexia plus toxic metabolites of Fz in body tissues.

With these considerations, the present study describes the pathological effects of Fz in male Japanese quails and reversibility of the induced changes after cessation of its administration.

Materials and Methods

Quails and Feed: A total of 150 male Japanese quails (*Coturnix coturnix Japonica*) of 5 wks of age having similar body weight and free from any apparent clinical ailment were purchased from a local quail farm. The quails were placed in wire cages. Basal feed (Broiler starter mash, 22% protein) and clean drinking water was offered *ad libitum* to these birds.

Experimental Procedure: At the age of 7 wks, quails were divided into 4 equal groups viz. F0, F4, F6 and PF, each having 36 birds. The remaining 6 quails were used to obtain zero day observations of different parameters.

Furazolidone (Furazole, 24.4% Fz, Hilton Pharma, Pakistan) was mixed in the daily ration of groups F0, F4, F6 and PF at a dose rate of 0, 400, 600 and 0 mg/kg feed.

After 4 wks administration of Fz was stopped and all the groups were switched to basal feed for another 4 wks. The quails of pair fed (PF) group were given daily ration equal to that consumed by group F6.

Individual quails from each group were weighed at days 0, 7, 14, 21, 28, 42, and 56 of the experiment. Daily feed consumption of quails in each group was recorded. Six birds randomly selected from each group were slaughtered at days 7, 14, 21, 28, 42 and 56 of the experiment.

Testes of slaughtered quails were weighed and examined for the presence of gross lesions. Volume of the testes was measured by fluid displacement technique.

Slices of about 5 mm thickness obtained from middle portion of testes were fixed in 10 per cent neutral buffered formalin and processed for histopathological examination by paraffin embedding method. Sections of 6 micrometer thickness were stained with hematoxylin and eosin.

The data obtained were subjected to the analysis of variance and different group means were compared by Duncan's multiple range test using MStat-C statistical computer package. The level of significance was $P\leq0.05$.

Results

Feed consumption and body weights: Feed consumption and body weights of quails in different groups is presented Table 1. The groups F4 and F6 showed a significant decrease in feed consumption on week 1 compared with group F0. After

Table 1: Feed consumption (g/bird/day) and body weights (g) of Japanese quails fed different levels of furazolidone (FZ) and after its withdrawal (mean ± SD).

Wks	Fz in feed		Groups* F0	F400	F600	PF
0	No	Body wt	159.0 ± 21	159.0 ± 21	159.0 ± 21	159.0 ± 21
1	Yes	Feed	21.2 ± 3.1	21.7 ± 3.2	21.6 ± 3.3	22.2 ± 3.1
1	Yes	Body wt	168.0 ± 11a	161.0 ± 11a	151.0 ± 12b	152.0 ± 17b
2	Yes	Feed	27.2 ± 2.1a	18.5 ± 2.0b	19.3 ± 1.5b	19.5 ± 1.4b
2	Yes	Body wt	155.0 ± 9a	148.0 ± 20a	137.0 ± 9b	149.0 ± 7a
3	Yes	Feed	27.4 ± 2.5a	17.6 ± 2.4b	17.4 ± 1.3b	17.6 ± 1.2b
3	Yes	Body wt	170.0 ± 1a	150.0 ± 11b	120.0 ± 19b	157.0 ± 19ab
4	Yes	Feed	28.1 ± 3.6a	17.1 ± 1.7b	16.6 ± 1.3b	16.8 ± 1.1b
4	Yes	Body wt	174.0 ± 11a	143.0 ± 15 b	136.0 ± 17b	164.0 ± 12a
6	No	Feed	27.1 ± 2.0a	27.7 ± 2.4a	25.9 ± 2.8ab	25.7 ± 4.2a
6	No	Body wt	190.0 ± 18 a	170.0 ± 13b	164.0 ± 11b	170.0 ± 15 b
8	No	Feed	28.7 ± 1.7a	28.7 ± 2.6a	27.6 ± 5.2a	27.2 ± 3.6a
8	No	Body wt	177.0 ± 24 ab	183.0 ± 4 a	163.0 ± 8b	170.0 ± 10ab

Table 2: Absolute weight (g) Relative weight (% body wt) and volume (ml) of testes of Japanese quails fed different levels of furazolidone (FZ) and after its withdrawal (mean ± SD)

Wks	FZ in feed		Groups* F0	F400	F600	PF
0	No	Abs wt	2.53 ± 0.37	2.54 ± 0.37	2.54 ± 0.37	2.53 ± 0.37
		Rel wt	1.59 ± 0.11	1.59 ± 0.11	1.59 ± 0.11	1.59 ± 0.11
		Vol	2.43 ± 0.26	2.43 ± 0.26	2.43 ± 0.26	2.43 ± 0.26
1	Yes	Abs wt	2.38 ± 0.24	2.33 ± 0.51	2.13 ± 0.30	2.43 ± 0.33
		Rel wt	1.38 ± 0.11	1.54 ± 0.38	1.41 ± 0.15	1.60 ± 0.12
		Vol	2.23 ± 0.28a	2.20 ± 0.31a	1.82 ± 0.32b	2.27 ± 0.31a
2	Yes	Abs wt	2.29 ± 0.38a	2.02 ± 0.43a	1.39 ± 0.27b	1.98 ± 0.23a
		Rel wt	1.54 ± 0.18a	1.36 ± 0.25a	1.01 ± 0.18b	1.32 ± 0.11a
		Vol	2.37 ± 0.40a	2.03 ± 0.35ab	1.33 ± 0.33c	1.92 ± 0.20b
3	Yes	Abs wt	2.46 ± 0.29a	1.55 ± 0.23bc	1.30 ± 0.38c	1.91 ± 0.61b
		Rel wt	1.53 ± 0.19a	1.03 ± 0.17b	1.06 ± 0.16b	1.19 ± 0.26b
		Vol	2.50 ± 0.32a	1.47 ± 0.22bc	1.25 ± 0.48c	1.83 ± 0.63b
4	Yes	Abs wt	2.34 ± 0.32a	1.50 ± 0.52b	1.16 ± 0.30b	2.38 ± 0.34a
		Rel wt	1.35 ± 0.18a	1.03 ± 0.30b	0.86 ± 0.24b	1.45 ± 0.19a
		Vol	2.22 ± 0.31a	1.30 ± 0.42b	1.15 ± 0.30b	2.17 ± 0.26a
6	No	Abs wt	2.46 ± 0.24a	2.22 ± 0.55ab	1.82 ± 0.17b	2.16 ± 0.26ab
		Rel wt	1.40 ± 0.13a	1.31 ± 0.33ab	1.10 ± 0.06b	1.28 ± 0.3 ab
		Vol	2.33 ± 0.28a	2.01 ± 0.48b	1.82 ± 0.15b	2.17 ± 0.26ab
8	No	Abs wt	2.02 ± 0.25	1.91 ± 0.19	1.90 ± 0.19	1.99 ± 0.34
		Rel wt	1.45 ± 0.15	1.35 ± 0.09	1.27 ± 0.15	1.42 ± 0.25
		Vol	2.43 ± 0.26a	2.07 ± 0.17b	1.97 ± 0.15b	2.33 ± 0.39a

Table 3: Diameter of seminiferous tubules (μm) of Japanese quails fed different levels of furazolidone (FZ) and after its withdrawal (mean ± SD)

Wks	FZ in Feed	Groups* F0	400	F600	PF
0	No	294.08 ± 7.03	294.08 ± 7.03	294.08 ± 7.03	294.08 ± 7.03
1	Yes	291.29 ± 3.42a	286.03 ± 5.14a	250.72 ± 4.93b	283.56 ± 1.60a
2	Yes	287.04 ± 3.59a	235.82 ± 4.59b	202.56 ± 10.87c	280.16 ± 9.47a
3	Yes	290.40 ± 4.97a	194.54 ± 6.64b	177.28 ± 5.35c	196.16 ± 3.66b
4	Yes	276.96 ± 2.54a	182.56 ± 10.00c	176.45 ± 4.23c	197.16 ± 7.53b
6	No	281.36 ± 3.66a	254.08 ± 11.11b	248.48 ± 4.73b	278.48 ± 7.28a
8	No	291.91 ± 2.66a	287.14 ± 7.34a	277.84 ± 2.09b	288.80 ± 13.23a

*Groups F0, F400, F600 and PF were given 0, 400, 600 and 0 mg FZ/kg feed respectively. PF group was given ration equal to that consumed by group F600. Values in each row with different small letters are significantly different ($P \leq 0.05$).

cessation of Fz feeding, the feed intake increased and at week 8 Fz fed groups became non significantly different from group F0.

Body weights, comparing with group F0, decreased significantly in groups F6, F4 and PF at week 1, 3 and 3, respectively. After cessation of Fz feeding the body weights increased and at 8 weeks quails in groups F4, F6 and PF became non-significantly different from those of group F0.

Testes weight and volume: The absolute and relative weights and volume of testes of quails of different groups is presented in Table 2. In comparison with group F0, the absolute weight significantly decreased in groups F4, F6 and PF at weeks 3, 2 and 3, respectively. The weight in group PF became non-significantly different from group F0 at week 4 till the end of the experiment. After cessation of Fz feeding the weight in groups F4 and F6 increased and became non significantly different from group F0 at weeks 6 and 8.

The relative weight of testes (percent of body weight), comparing with group F0, significantly decreased in group F6 at week 2 and in group F4 and PF at week 3. After cessation of Fz feeding the testes weight increased and in groups F4, F6 and PF it became nonsignificant from group F0 at weeks 6, 8 and 4, respectively.

In comparison with group F0, the volume of testes decreased significantly in groups F4, F6 and PF at weeks 2, 1 and 3 of the experiment. The testes volume in group PF became non-significant at week 4 till the end of the experiment. After cessation of Fz feeding the testes volume increased in groups F4 and F6 but remained significantly lower than that group F0 till the end of the experiment.

Pathology: In group F0 the spermatogenic activity was present throughout the duration of the experiment. Tubules were lined by spermatogonial cells followed by primary spermatocytes, round spermatids and bundles of elongate spermatids facing towards basement membrane. Spermatozoa were present in many tubular lumeni.

Feeding of Fz resulted in changes in seminiferous tubules of groups F6 and F4 from weeks 1 and 3 of the experiment. These changes started with the vacuole formation in the germinal epithelial layer of the tubules. In group F6 on week 3 and in group F4 on week 4, the germinal epithelial layer of seminiferous tubules in some quails consisted of spermatogonial cells and primary spermatocytes and did not show round and elongate spermatids. In other quails of these groups the round spermatids had pyknotic nuclei. The elongate spermatids decreased in number and were scattered facing towards the lumen of the tubules. Many of the elongate spermatids and young spermatozoa had rounded heads. After cessation of Fz feeding these changes reversed gradually and at week 6 seminiferous tubules of quails from group F4 had aggregates of round spermatids and the bundle of elongate spermatids facing towards basement membrane. In group F6 round spermatids with pyknotic nuclei and elongate spermatids having rounded heads were present at week 6 but at week 8 the histological picture of tubules of this group became indistinguishable from that of group F0.

In group PF there was no variation in the histological picture from group F0 at week 1 and 2 but at week 3 the germinal epithelial layer showed vacuole formation between the cells. The round and elongate spermatids were present in lesser number than those in group F0 but their arrangement and morphology did not differ from those in group F0. There were no pyknotic nuclei of round spermatids and round heads of elongate spermatids and spermatozoa. After increase in feed of these quails the vacuole formation in germinal layer disappeared and tubular picture became indistinguishable from control group.

Diameter of seminiferous tubules: The mean diameter of seminiferous tubules of Japanese quails of different groups is shown in Table 3. The diameter decreased significantly in groups F4, F6 and PF on weeks 2, 1 and 3, respectively, compared with group F0. The maximum decrease in diameter of seminiferous tubules occurred in group F6 at week 4 of the experiment. After cessation of FZ feeding, the diameter of seminiferous tubules increased and in groups F4 and F6 it became non-significantly different from group F0 at week 8 of the experiment. In PF group it was non-significant from group F0 at week 6 of the experiment.

Discussion

A decrease in the feed consumption and body weight of Fz fed quails as observed in the present study has also been reported in quail (Arbid *et al.*, 1990) and chicken (Siddique et al., 1996; Khan *et al.*, 1995). Furazolidone is known to induce anorexia in chicken (Ali and Bartlet, 1982) A decrease in body weight of Fz fed quails in the present study could be a consequence of reduced feed intake. After cessation of Fz feeding, an increase in feed consumption and body weight of group F6 and a non-significant difference between body weights of quails in group F6 and its counterpart pair fed group (PF) also suggested that lower body weight during Fz

feeding period were due in part at least to anorexic effect of this drug.

Feeding of both levels of Fz in the present study resulted in a decrease in weight and volume of testes of quails. This observation is suggestive of a gonadotoxic effect of Fz in male quails. Degenerative effects of Fz upon ovary and oviduct of quails has been reported (Arbid *et al.*, 1990; Ullah *et al.*, 1998) but there is no report of Fz induced degenerative changes in testes of quails. Nevertheless Fz induced testicular degenerative changes have been reported in chicken (Siddique *et al.*, 1996; Khan *et al.*, 1995; Hernadez-Jauregui *et al.*, 1983; Andrabi *et al.*, 1998), rat (Satoru *et al.*, 1977; Ludewig *et al.*, 1995). and mice (Hernandez-Jauregui *et al.*, 1985). Smaller diameter of seminiferous tubules and empty spaces in germinal epithelial layer reflecting a reduced number of germinal cell in the present study were suggestive of a suppressed spermatogenesis which has been reported to occur in Fz fed male chicken (Siddique *et al.*, 1996; Zermeno Hernandez and Paasch Martinnez, 1984) and rat (Satoru *et al.*, 1977; Ludewig *et al.*, 1995). Presence of primary spermatocytes, absence of round and elongate spermatids, and pyknotic nuclei of early round spermatids in the present study indicated an arrest of spermatogenesis at primary spermatocyte stage. Spermatogenesis arrest at primary spermatocyte stage have been reported in mice (Hernandez-Jauregui *et al.*, 1985) and rats (Ludewig *et al.*, 1995). In Fz fed roosters testicles showed a blockage of spermatogenesis in pachytene phase and primary spermatocytes showed nuclear vacuolation (Hernandez-Jauregui *et al.*, 1983). Round heads of late elongate spermatids and spermatozoa in Fz fed quails in the present study are similar to Fz induced degenerative changes described in other avian and mammalian species (Ali *et al.*, 1988; Zamora *et al.*, 1986; Gehlaut *et al.*, 1989; Dixon *et al.*, 1992). Cessation of Fz feeding in the present study resulted in increased weight of testes and restoration of the histological picture which was indistinguishable from control birds. However, the testes volume remained smaller than those of control group suggesting that the 4 week period was probably not sufficient for complete recovery. Reversibility of Fz induced decrease in egg production and hatchability has been reported (Dixon et al., 1992) but there is no report concerning reversibility of Fz induced changes in testes of quails. However, Fz induced changes in testes are reportedly reversible in chicken (Siddique *et al.*, 1996) and mice (Hernandez-Jauregui *et al.*, 1985). The finding of present study suggested that like in chicken and mice, Fz induced testicular degeneration is also reversible in quails.

Pathological changes observed in testes of quails in group PF, given Fz free basal feed equal to that consumed by one of Fz fed groups (F600), also showed smaller testes, smaller diameter of seminiferous tubules and reduced number of germinal epithelial cells but no abnormal morphology of spermatids was observed. This observation suggested that reduced feed intake induced degenerative changes in the testes of the quails but the toxic metabolites of Fz played an important role in producing abnormal morphology of germinal cells in testes.

In conclusion, Fz induced changes in testes of mature japanese quails comprising of decreased weight and volume, smaller diameter of seminiferous tubules, less population of germinal epithelial cells, absence of round and elongate spermatids and pyknotic nuclei of round spermatids and abnormally round heads of elongate spermatids and young spermatozoa. The reduced feed intake resulted in decreased population of germinal cells but did not effect the morphology of spermatids.

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