



Effect of dietary T-2 toxin levels on liveability, organs weight, immunity and histopathology of organs in Japanese quails

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Received: 16 May 2020; Accepted: 21 July 2021

ABSTRACT

To establish the tolerance level of T-2 toxin, day-old Japanese quail chicks (n=225) were divided into five dietary treatments: T₁, control; T₂, T₁+50 ppb T-2 Toxin; T₃, T₁+100 ppb T-2 Toxin; T₄, T₁+150 ppb T-2 Toxin; T₅, T₁+200 ppb T-2 Toxin. Each diet was fed to 3 replicated groups of 15 birds each from 1 to 35 days of age. The results showed that the overall liveability percentage, at fifth week of age in T₁ was statistically similar to T₂ and T₃; and higher than T₄ and T₅. The relative weight of liver, kidney and spleen in T₁ was lower than T₄ and T₅; and statistically similar to T₂ and T₃. The relative weight of bursa in T₁ was higher than T₄ and T₅; and statistically similar to T₂ and T₃. The CMI and HA titre values in T₁ was higher than T₄ and T₅. The CMI and HA titre value in group T₁ was statistically similar to T₂ and T₃. In group T₂, mild necrosis of mucosa in the proventriculus and gizzard and in T₃, dystrophy and granular degeneration in the liver and kidney and necrosis of mucosa in the gizzard and proventriculus was observed. In T₄ and T₅, severe histopathological lesions including hepatocyte necrosis with discrete foci, necrosis and inflammation of gallbladder mucosa having mild proliferation of bile ductules, necrosis of intestinal epithelium following transient shortening of villi and mitotic figures in crypt epithelium; necrosis in feather epithelium and mucosa of the proventriculus and gizzard was observed. In addition, dystrophy and granular degeneration in the liver and kidney; interstitial nephritis, kidney sclerosis and glomerulonephritis was also observed. It was concluded that Japanese quails can tolerate up to 100 ppb of T-2 toxin in their diet without any adverse effects on their liveability percentage, organs weight, immunity and histopathology of organs during 0-5 weeks of growth period.

Keywords: Histopathology, Immunity, Japanese quail, Liveability, Organ weight, Tolerance level, T-2 toxin

Mycotoxins are toxic secondary metabolites produced by various fungi which causes toxic effect called mycotoxicosis. The economic losses associated with mycotoxicoses include poor growth, reduced egg production, reduced feed conversion, increased morbidity and mortality, carcass condemnation, poor egg shell quality, reduced fertility, leg problems and increased susceptibility to disease (Singh *et al.* 2013). Mycotoxins pose a grave public health hazard due to their deleterious side effects and the fact that they pose a severe threat to humans upon the consumption of residual traces in animal-derived food products originating from animals feeding on contaminated feedstuff (Kalantari and Moosavi 2010). The T-2 toxin is a naturally occurring trichothecene (TCT) mycotoxin produced by several species of fungi in the genus *Fusarium* (Diaz 2005). T-2 toxin also have severe adverse effects and continue to poison farm animals worldwide. The toxicological characteristics of TCT mycotoxins (depends on the animal species) include vomiting, irregular

heartbeats, and diarrhea; haemorrhaging, edemas, cutaneous tissue necrosis; haemorrhaging of the stomach and intestinal epithelial mucosa; destruction of hematopoietic tissues; decrease of white blood cells and circulating platelets; haemorrhaging meninges (cerebral); alteration of the central nervous system; rejection of food; necrotic lesions on different parts of the mouth; pathological deterioration of bone marrow, lymphatic nodules, and intestinal cells (Singh *et al.* 2013). T-2 toxicosis in poultry caused reduced feed intake, growth depression (Singh 2020), oral lesions (Kubena *et al.* 1990, Leeson *et al.* 1995, Girish and Devegowda 2006), abnormal feathering (Raju and Devegowda 2000), decreased egg production, thinner egg shells (Leeson *et al.* 1995), impaired hatchability and coagulopathy (Girish and Devegowda 2006). T-2 toxicosis in broiler chickens resulted in abnormal positioning of the wings (Chi *et al.* 1977). An outbreak of T-2 toxin mycotoxicosis in a commercial flock of broiler chickens was reported by Bitay *et al.* (1981) wherein altered feathering, depression, necrosis of the oral and oesophageal mucosa and visible atrophy of lymphoid organs was observed. The objective of the present investigation was to study the effect of T-2 toxin on

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Table 1. Experimental groups and treatments

Group	Dietary treatment
T ₁	Control (Basal diet)
T ₂	T ₁ + 50 ppb T-2 Toxin
T ₃	T ₁ + 100 ppb T-2 Toxin
T ₄	T ₁ + 150 ppb T-2 Toxin
T ₅	T ₁ + 200 ppb T-2 Toxin

liveability, organs weight, immunity and histopathology of organs in Japanese quails.

MATERIALS AND METHODS

T-2 Toxin production and analysis: T-2 toxin was produced on maize substrate using the pure culture of *Fusarium sporotrichioides* NRRL 3510 which was obtained from the USDA/ARS Culture Collection (NCAUR, Peoria III). Then T-2 toxin was estimated as per the method of thin layer chromatography (TLC) as described by Mandal *et al.* (2018).

Experimental design: Experimental design was completely randomized design (CRD). There were five dietary treatments. Each dietary treatment had 3 replicates and each replicate had 15 chicks. The experiment was conducted in Japanese quails from day-old to 5 weeks of age. The various dietary treatments were prepared by mixing the required quantity of mycotoxin to get the desired concentration of T-2 Toxin in basal diet (Table 1).

Ingredients and chemical composition of basal feed: A basal diet with maize 54.2, rice bran (dehulled) 2, soybean meal (solvent extracted) 31.15, sunflower meal 2, rapeseed meal 4, fish meal 4, limestone 0.75, dicalcium phosphate 1.4, salt 0.15, DL-methionine 0.06, trace mineral (TM) premix 0.1, vitamin premix 0.165, and choline chloride 0.03 as per cent was formulated. The TM premix supplied Mg 300 mg/kg, Mn 55 mg/kg, I 0.4 mg/kg, Fe 56 mg/kg, Zn 30 mg/kg and Cu 4 mg/kg diet. The vitamin premix supplied vit. A 8250 IU, vit. D₃ 1200 IU, vit. K 1 mg, vit. B₁ 2 mg, vit. B₂ 4 mg, vit. B₁₂ 10 mcg, niacin 60 mg, pantothenic acid 10 mg, choline 500 mg, vit. E 40 IU per kg diet. The control diet so formulated contained crude protein 23.95%, metabolisable energy 2795 kcal/kg, calcium 1.05%, available phosphorus 0.47%, lysine 1.2% and methionine 0.50%. The crude protein content as per AOAC (1995) and calcium content as per Talapatra *et al.* (1940) were estimated, while the concentrations of lysine,

methionine, available P and metabolizable energy values were calculated. Mortality was recorded as and when occurred. The cell mediated immune response to PHA-P antigen was evaluated by the method described by Carrier and DeLoach (1990). The microtitre haemagglutination procedure as described by Siegel and Gross (1980) was followed to measure total HA antibody titres. At the end of the experiment, organ samples were collected, weighed and fixed in 10% formal saline. The formal saline fixed samples were cut into pieces of 2–3 mm thickness and washed thoroughly in tap water overnight before dehydrating the tissues in ascending grades of alcohol (50%, 60%, 70%, 80%, 90% absolute alcohol I and II). The dehydrated tissues were cleared in benzene and embedded in paraffin blocks. Serial sections of 5 micron thickness were cut and stained with hematoxyline and eosin (Culling 1968) and examined for various histopathological changes.

Statistical analysis: The collected data was subjected to statistical analysis using Statistical Package for Social Sciences (SPSS Version 16.0). The recorded data were subjected to one-way analysis of variance with comparison among means was made by Duncan's multiple range test with significance level of P<0.05.

RESULTS AND DISCUSSION

The data pertaining to week-wise liveability percentage of Japanese quails fed on various dietary treatments are presented in Table 2. The data pertaining to relative organ weights (liver, kidney, heart, spleen and bursa of Fabricius) expressed as per cent live weights; and the results pertaining to cell mediated immune response to PHA-P measured as foot web index and humoral immune response measured as haemagglutination titre (HA) against SRBCs in Japanese quails fed on various dietary treatments was statistically analyzed and presented in Table 3.

Liveability percentage (LP): During first week of age, no mortality was recorded as the dead chicks were replaced with chicks of equal weights. During second week of age, the LP did not vary significantly (P<0.05) among various dietary treatments. During third and fourth weeks of age, the LP varied from 88.89 (T₅) to 95.56 (T₁) and 86.67 (T₅) to 95.56 (T₁), respectively. The LP, at third and fourth weeks, in T₁ was statistically similar to those of T₂, T₃ and T₄. The LP in T₁ was higher (P<0.05) than that of T₅. The overall LP (0-5 weeks), at fifth week of age, in T₁ was statistically similar to those of T₂ and T₃. The overall LP in

Table 2. Effect of varying levels of T-2 Toxin on liveability percentage of Japanese quails fed during 1 to 35 days of age

Treatment	1 wk	2 wk	3 wk	4 wk	5 wk
T ₁	100.00±0.00	100.00±0.00	95.56±2.22 ^b	95.56±2.22 ^b	95.56±2.22 ^c
T ₂	100.00±0.00	100.00±0.00	95.56±2.22 ^b	95.56±2.22 ^b	95.56±2.22 ^c
T ₃	100.00±0.00	100.00±0.00	93.34±0.00 ^{ab}	93.34±0.00 ^b	93.34±0.00 ^{bc}
T ₄	100.00±0.00	97.78±2.22	93.34±0.00 ^{ab}	91.11±2.22 ^{ab}	88.89±2.22 ^{ab}
T ₅	100.00±0.00	97.78±2.22	88.89±2.22 ^a	86.67±0.00 ^a	86.67±0.00 ^a

Values bearing different superscripts in a column differ significantly (P<0.05).

Table 3. Effect of T-2 toxin on relative organ weights (% of live weight) and immune response of Japanese quails fed during 1 to 35 days of age

Treatment	Liver	Kidney	Spleen	Bursa	Heart	CMI (mm)	HA titre
T ₁	2.26±0.03 ^a	0.57±0.02 ^a	0.06±0.01 ^a	0.15±0.00 ^b	0.59±0.02 ^a	0.30±0.01 ^b	12.34±0.15 ^b
T ₂	2.27±0.05 ^a	0.61±0.02 ^a	0.05±0.00 ^a	0.16±0.01 ^b	0.60±0.01 ^a	0.31±0.01 ^b	12.05±0.03 ^b
T ₃	2.27±0.03 ^a	0.59±0.01 ^a	0.05±0.00 ^a	0.16±0.01 ^b	0.61±0.01 ^a	0.30±0.00 ^b	12.27±0.15 ^b
T ₄	2.82±0.01 ^b	0.85±0.03 ^b	0.09±0.00 ^b	0.10±0.01 ^a	0.62±0.01 ^a	0.21±0.00 ^a	8.56±0.27 ^a
T ₅	3.01±0.00 ^c	0.90±0.02 ^b	0.09±0.00 ^b	0.09±0.00 ^a	0.59±0.01 ^a	0.20±0.00 ^a	8.32±0.17 ^a

Values bearing different superscripts in a column differ significantly ($P<0.05$).

T₁ was higher ($P<0.05$) than those of T₄ and T₅. The overall LP between T₂ and T₃; and between T₄ and T₅ was statistically similar.

In the present study, dietary inclusion of T-2 toxin at 150 or higher level resulted in significant ($P<0.05$) decrease in LP of growing Japanese quails. This result was in agreement with those of Hoerr *et al.* (1982), Ziprin and Elisalde (1990) wherein reduced liveability due to exposure to T-2 toxin in chickens was reported.

Organ weights

Liver: The relative weight of liver (% of live weight) in T₁ was lower ($P<0.05$) than those of T₄ and T₅. The relative weight of liver in T₁ was statistically similar to those of T₂ and T₃. The relative weight of liver amongst groups T₁, T₂ and T₃ did not vary significantly. The relative weight of liver in T₄ was lower ($P<0.05$) than that of T₅. In the present study, inclusion of T-2 toxin in the feed at 150 or higher level resulted in increased ($P<0.05$) relative weight of liver. This result was in agreement with those of Curtui (2000), Indresh and Umakantha (2013), wherein increased liver weight due to T-2 toxicosis in broiler chickens was reported. Contrary to this, Kumar (2009) reported that the relative weight of liver was not altered due to feeding of 500 ppb T-2 toxin in the diet of broiler chickens. Diaz *et al.* (2005) who too observed no alteration in the relative liver weights of broiler chicks fed 2 ppm of T-2 toxin from 1 to 28 days of age. The increase in relative weight of liver could be attributed to increased lipid metabolism in liver due to impaired fat metabolism which brings appreciable changes in the general functioning and gross appearance of liver. The study further revealed that quails can tolerate upto 100 ppb of T-2 toxin in their feed without affecting relative weight of liver.

Kidney: The relative weight of kidney in T₁ was lower ($P<0.05$) than those of T₄ and T₅. The relative weight of kidney in T₁ was statistically similar to those of T₂ and T₃. The relative weight of kidney between groups T₁, T₂; and between T₄ and T₅ did not vary significantly. The results revealed that incorporation of T-2 toxin in the feed at 150 ppb or higher level resulted in increase ($P<0.05$) in the relative weight of kidney of Japanese quails. This result was in agreement with that of Edrington *et al.* (1997) who also reported increased kidney weight due to T-2 toxin contamination of feed in growing broilers. However, Bailey *et al.* (1998) reported non-significant effect on kidney

weight due to T-2 toxicosis in broiler chickens. The present study revealed that quails can tolerate upto 100 ppb T-2 toxin in their feed without affecting relative weight of kidney.

Spleen: The relative weight of spleen in T₁ was lower ($P<0.05$) than those of T₄ and T₅. The relative weight of spleen in T₁ was statistically similar to those of T₂ and T₃. The relative weight of spleen amongst groups T₁, T₂ and T₃ did not vary significantly. The relative weight of spleen in group T₄ was statistically similar to that of T₅. In the present study, contamination of T-2 toxin in feed at 150 ppb or higher level resulted in significant increase in relative weight of spleen. This result was in agreement with that of Wyatt *et al.* (1973), however, Indresh and Umakanta (2013) reported no significant effect on relative weight of spleen due to T-2 toxicosis in broiler chickens. Diaz *et al.* (2005) did not observe any change in the relative weights of spleen of broiler chicks fed 2 ppm of T-2 toxin from 1 to 28 days of age. Kumar (2009) also reported that the relative weights of spleen was not altered due to feeding of 500 ppb T-2 toxin in the diet of broiler chickens.

Bursa of fabricius: The relative weight of bursa of fabricius in T₁ was higher ($P<0.05$) than those of T₄ and T₅. The relative weight of bursa in T₁ was statistically similar to those of T₂ and T₃. The relative weight of bursa amongst T₁, T₂ and T₃; and between T₄ and T₅ did not differ significantly. In the present study, dietary inclusion of T-2 toxin at 150 or higher level resulted in decreased ($P<0.05$) relative weight of bursa of growing Japanese quails. This result was in agreement with those of Indresh and Umakantha (2013) and Wyatt *et al.* (1973). However, Kumar (2009) reported that the relative weights of bursa was not altered due to feeding of 500 ppb T-2 toxin in the diet of broiler chickens. Diaz *et al.* (2005) also did not find significant change in the relative weight of bursa of Fabricius in broiler chicks fed 2 ppm of T-2 toxin from 1 to 28 days of age. Mycotoxins are known to cause immuno-suppression in chickens and concomitantly reduce the relative sizes of bursa of fabricius responsible for immunological competence (Kubena *et al.* 1989). The reduction in size of bursa might have been due to necrosis and cellular depletion by the mycotoxin.

Heart: The relative weight of heart in various dietary treatments varied from 0.59 (T₁) to 0.62 (T₄). There was no significant difference in relative weight of heart among various dietary treatments. Addition of T-2 toxin (50-200

ppb) to the feed did not produce any significant effect on relative weight of heart. This finding was in concurrence with that of Diaz *et al.* (2005) who reported that feeding broiler chicks with 2 ppm of T-2 toxin from 1 to 28 days of age did not affect the relative weight of heart.

Cell mediated and humoral immune response: The cell mediated immune response (CMI) value in T₁ was higher ($P < 0.05$) than those of T₄ and T₅. The CMI value in T₁ was statistically similar to those of T₂ and T₃. The CMI value amongst T₁, T₂ and T₃ did not vary significantly. The CMI value between T₄ and T₅ did not vary significantly. Thus, dietary inclusion of T-2 toxin at 150 ppb or higher level resulted in significant decrease in CMI value in growing Japanese quails. Significant decrease in CMI response due to T-2 toxicosis was also reported earlier by Corrier and Ziprin (1986). However, Kumar (2009) reported no significant effect on CMI due to feeding of 500 ppb T-2 toxin in the diet of broiler chickens. The HA titre value in T₁ was higher ($P < 0.05$) than those of T₄ and T₅ and in T₁ was statistically similar to those of T₂ and T₃. The HA titre value amongst T₁, T₂ and T₃ did not vary significantly and between T₄ and T₅ was statistically similar. In the present study, dietary inclusion of T-2 toxin at 150 or higher level resulted in significant decrease in HA titre value. This depression in titre values are clear indication of immunodepressing effects of mycotoxin on humoral antibody response. The reduction of antibody titers could be due to mycotoxin inhibiting DNA and protein synthesis through impairment of amino acid transport and m-RNA transcription, resulting in lowered level of antibody production (Kamalavenkatesh *et al.* 2005). The reduced antibody titers in T-2 toxicosis was in also reported by Wang *et al.* (2009) in commercial broilers fed 3 ppm T-2 toxin. Similar result was also reorted by Indresh and Umakanta (2013). Weber *et al.* (2006) also observed that feeding of chickens with T-2 toxin contaminated feed for 14 days at a dose of 2.32 mg/kg decreased the antibody formation. Gounalan *et al.* (2006) reported that layer chicks fed 0.5 ppm T-2 toxin from 0 to 12 weeks of age showed significant reduction in the HA titre to NDV. Also, Prasath *et al.* (2006) reported a significant reduction in ELISA titre of Japanese quail fed with diets containing 1, 2 and 3 ppm of T-2 toxin for six weeks from day of hatch. However, Kumar (2009) reported no significant effect on humoral immune response due to feeding of 500 ppb T-2 toxin in the diet of broiler chickens. Sklan *et al.* (2001) also did not find significant alterations in the humoral immune response in broiler chicks fed 1 ppm of T-2 toxin. The study further revealed that Japanese quails can tolerate upto 100 ppb of T-2 toxin in their diet without adversely affecting immune response.

Histopathology of organs: In group T₂, mild necrosis of mucosa in the proventriculus and gizzard was observed. In T₃, dystrophy and granular degeneration in the liver and kidney, distension of glands, interglandular fibrosis, MNC infiltration and necrosis of mucosa in the gizzard; necrosis of mucosal epithelium, diffuse infiltration of MNC in the lamina propria and occasional infiltration of MNC in the

glands in proventriculus was observed. In T₄ and T₅, severe histopathological lesions including hepatocyte necrosis with discrete foci, necrosis and inflammation of gallbladder mucosa having mild proliferation of bile ductules, necrosis of intestinal epithelium following transient shortening of villi and mitotic figures in crypt epithelium; necrosis in feather epithelium and mucosa of the proventriculus and gizzard was observed. In addition, dystrophy and granular degeneration in the liver and kidney; interstitial nephritis, kidney sclerosis and glomerulonephritis was also observed. In crop, hyperplasia and keratinization of the mucosal epithelium was reported. Similar histopathological lesions due to T-2 toxicosis in poultry were also reported by Hoerr *et al.* (1981), Calnek *et al.* (1997), Narayanaswamy (1998), Krishnamoorthy (2007) and Stoev *et al.* (2010).

It was concluded that Japanese quails can tolerate up to 100 ppb of T-2 toxin in their diet without any adverse effects on their liveability percentage, organ weights, immunity and histopathology of organs during 0-5 weeks of growth period.

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