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GROSS PATHOLOGICAL CHANGES AND RELATIVE ORGAN WEIGHTS IN TURKEY POULTS (*Meliagridesgallopavo* - Beltsville Small White variety) FED EXPERIMENTALLY WITH AFLATOXIN AND T-2 TOXIN

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Abstract: Experimental mycotoxicoses were induced singly and in combination in 48 newly hatched turkey poults (*Meleagridisgallopavo* - Beltsville small white) for a period of 0 to 13 weeks by feeding diets containing 100 ppb AF and 1 ppm T-2 toxin. The AF was produced on rice while T-2 toxin was produced on corn grits using *Aspergillus parasiticus* NRRL 2999 and *Fusarium sporotrichioides* respectively. The AF and T-2 toxin from cultures were quantified by TLC. Weighed amounts of powdered culture material was incorporated into the toxin free diet and were adjusted to 100 ppb AF and 1 ppm T-2 toxin. A detailed post mortem was conducted on sacrificed bird during the 7^{th} week and 13^{th} week of experimental period. Weights of liver, spleen and bursa of Fabricius and gross lesions were recorded. Only relative spleen weights significantly ($P<0.05$) decreased in the toxin treated groups except T-2 when compared to the control. The AF fed birds during the 7^{th} and 13^{th} week revealed enlarged and congested, pale or patchy yellowish discoloured liver and translucent gall bladder distended with thin, light green bile. The kidneys were mildly congested. Duodenum contained excess mucus mixed contents and bursa of Fabricius was mildly enlarged. The study revealed pale to congested liver in the T-2 toxin fed poults yellowish necrotic plaques in the hard palate and ventral surface of the tip of the tongue and milder oral lesions during the 7^{th} and 13^{th} week respectively. The results observed during the 7^{th} and 13^{th} week in AF-T-2 toxin treated birds were poor bodily condition, liver was reduced in size, pale to yellowish discoloured, soft and friable, congested kidneys, swelling, encrustations, necrosis and plaque lesions in the lateral commissures, of the hard palate and tongue. Kidneys were mildly congested. Mucus mixed contents was seen in intestine. Spleen appeared shrunken. Bursal plicae were oedematous. Gross lesions observed in AF treated birds could be due to direct and indirect effects of

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toxins. In T-2 toxin treated birds, the gross lesions in the oral cavity and intestine could be due to direct effect of toxins in the epithelial cells causing necrosis. In combined treatment group, the lesions observed were severe in organs like liver, kidney, bursa of Fabricius which might be due to additive effect of toxins.
Keywords: Turkey poults, aflatoxin, T-2 toxin, gross pathological changes, organ weights.

INTRODUCTION

The contamination of food and feed by mycotoxins has been characterized by the World Health Organization (WHO) as significant sources of food-borne illnesses.Food safety and security have generally remained basic human needs globally. Among the very many hazards contamination of food and feed by mycotoxins (toxic metabolites of fungi) in the form of multiple mycotoxicoses is the current problem faced by the poultry farmers. Aflatoxin (AF) and T-2 toxin are the most frequently encountered mycotoxins. AF is a potent hepatotoxin with dihydrofuran-coumarin moiety and is of importance in producing the biological effects and is produced by *Aspergillus flavus* and *A. parasiticus*. T-2 toxin is a 3 hydroxy 4, 15 diacetoxy 8 (3-methylbutryloxy), 12, 13 epoxy trichothec-9-ene metabolite. It is a potent irritant, inflammatory (dermotoxic, alimentary toxic, hepatotoxic and growth inhibitory agent) and radiomimetic agent produced by *Fusarium* species. The AF, by binding to both RNA and DNA blocks transcription whereas, T-2 toxin blocks initiation of translation. The studies on aflatoxicosis in turkey poults were limited owing to the potential sensitivity of the species, the same on T-2 was scant and there were none on AF-T-2 combined toxicity for a period of 91days. Hence, the present study was conducted.

MATERIALS AND METHODS

AF was produced on rice (Shotwell *et al*., 1966) by using A. *Parasiticus* NRRL 2999 strain. The T-2 toxin was produced on corn grits (Burmeister, 1971) by using *F. sporotrichoides* MTCC 1894 strain (Microbial Type Culture Collection, Chandigarh, India). The mycotoxin content in cultured material was analysed at Pharmacovigilance Laboratory for Animal Feed and Food Safety (PLAFFS), Centre for Animal Health Studies, TANUVAS, Madhavaram Milk Colony, Chennai, Tamil Nadu, India. Known amounts of AF and T-2 toxin containing powdered substrates were incorporated into turkey brooder mash both singly and in combination to yield 100 ppb AF and 1 ppm T-2 toxin. Forty eight newly hatched unsexed turkey poults obtained from standard hatcheries were wing banded, weighed and housed in battery brooders with *ad libidum* supply of feed and water. They were randomly distributed into four groups of twelve chicks each.

A biological trial was conducted with a total number of 48 newly hatched turkey poults. The turkey poults were wing banded, weighed and housed in battery brooders with ad-libitum supply of feed and water. They were randomly allotted to four groups namely group I control, group II aflatoxin, group III T-2 toxin and group IV combined AF T-2 of 12 birds each. The control and toxin mixed diets were fed to different groups for 91 days from the day of hatch.

A detailed post mortem was conducted on each sacrificed bird at the end of experimental period. Gross lesions observed were recorded. Weights of liver, spleen and bursa of Fabricius were recorded. The organ weights were converted into relative organ weights and expressed in percentage as mentioned below.

$$\text{Relative organ weight} = \frac{\text{Weight of the organ } (\text{g})}{\text{Body weight } (\text{g})} \times 100$$

The data generated from the experimental study were subjected to statistical analysis, as per Snedecor and Cochran (1989). The results of the study were subjected to two way analysis of variance (ANOVA).

RESULTS

Gross pathology - Seventh week sacrifice

The AF fed birds showed enlarged, congested, pale or yellowish patchy discolouration of liver, which were soft and friable. Gall bladder was translucent and distended with thin, light green bile. The kidneys were mildly congested. Duodenum contained excess mucus mixed contents and bursa of Fabricius was mildly enlarged.

The birds from T-2 toxin fed group were mildly dehydrated in condition. Swelling and encrustations were noticed in and around the lateral commissures of beak in a few birds. Yellowish plaques were seen adhering to the hard palate and tongue. Soft, pliable, enlarged and pale or yellowish liver was seen. Kidneys were mildly congested.

The AF-T-2 treated birds were poor in condition and dehydrated. Liver was reduced in size, soft and friable and showed pale to yellowish discolouration with distended gall bladder with greenish bile. Swelling and encrustations, necrosis and plaque lesions were observed in the lateral commissures, hard palate and tongue (Fig. 1). Kidneys were mildly congested. Mucus mixed contents was seen in intestine. Spleen appeared shrunken. Bursa was oedematous (Fig. 2-3).

Gross pathology -Thirteenth week sacrifice

The AF fed birds showed severe enlargement, congestion, pale or yellowish patchy discolouration of liver, which were soft and friable. Gall bladder was distended with thin,

greenish yellow bile. The kidneys were mildly congested. Duodenum contained excess mucus mixed contents and bursa of Fabricius was shrunken.

The birds from T-2 toxin fed group were mildly dehydrated in condition. Minute encrustations in the corners of the beak, yellowish plaques in two birds adhering to the tongue were seen. Soft, pliable, enlarged and pale or yellowish liver were seen. Kidneys were mildly congested. Duodenum contained excess mucus mixed contents and bursa of Fabricius was shrunken.

The AF-T-2 treated birds were poor in condition and dehydrated. Liver was firm and showed pale to yellowish discolouration (Fig. 4). Gall bladder was distended with thin, greenish bile (Fig. 5), while one of the birds had pouched gall bladder with thin greenish bile (Fig. 6). Among the toxin treated groups, the liver showed moderate to severe reduction in size when compared with the control (Fig.7). Kidneys were congested. Minute encrustations in the corners of the beak were observed. One bird revealed yellowish plaques seen adhering to the tongue. Kidneys were mildly congested. Mucus mixed contents was seen in intestine. Bursa of Fabricius was small and shrunken.

Relative organ weights

The relative mean (± SE) relative weights of liver, spleen and bursa of Fabricius in turkey poults fed AF and T-2 toxin singly and in combination are presented in Table 1-3 respectively. Comparison of overall means for relative liver weights and bursa of Fabricius revealed no significant differences between the control and mycotoxin treated groups. The relative spleen weights significantly ($P<0.05$) decreased in the toxin treated groups except T-2 when compared to the control.

DISCUSSION

Gross pathology The AF fed birds during the 7^{th} and 13^{th} week revealed enlarged and congested, pale or patchy yellowish discoloured liver and translucent gall bladder distended with thin, light green bile. The kidneys were mildly congested. Duodenum contained excess mucus mixed contents and bursa of Fabricius was mildly enlarged. The findings are in accordance with the earlier reports of congested liver and kidney, enlarged and firm, distended gall bladder and the duodenum distended with catarrhal contents (Siller and Ostler, 1961; Wannop, 1961; Calnek *et al*., 1997).

The study revealed pale to congested liver in the T-2 toxin fed poults yellowish necrotic plaques in the hard palate and ventral surface of the tip of the tongue and milder oral lesions during the 7^{th} and 13^{th} week respectively. Similar observations were also made in turkeys fed

1and 3ppm of T-2 toxin for four weeks (Babu Prasath, 2008), 5 ppm of T-2 toxin for eight and three weeks respectively (Allen *et al*., 1983; Kubena *et al*., 1995). Findings of this study agreed with Richard *et al*. (1978) who observed raised yellowish plaque in the oral cavity and severe glossitis in turkey poults fed 10 ppm T-2 toxin for four weeks.

The results observed during the 7^{th} and 13^{th} week in AF-T-2 toxin treated birds were poor bodily condition, liver was reduced in size, pale to yellowish discoloured, soft and friable, congested kidneys, swelling, encrustations, necrosis and plaque lesions in the lateral commissures, of the hard palate and tongue. Kidneys were mildly congested. Mucus mixed contents was seen in intestine. Spleen appeared shrunken. Bursal plicae were oedematous. No comparable literature could be cited in the combined toxicity in turkey poults. However, the observations were in accordance with the findings of Madheswaran *et al*. (2005) who reported similar findings in Japanese quail fed AF (3 ppm) and T-2 toxin (4 ppm) from 0 to 35 days of age.

In AF treated birds the gross lesions observed in this study could be due to direct and indirect effects of toxins on different organ systems. In T-2 toxin treated birds, the gross lesions in the oral cavity and intestine could be due to direct effect of toxins in the epithelial cells causing necrosis. In combined treatment group, the lesions observed were severe in organs like liver, kidney, bursa of Fabricius which might be due to additive effect of toxins.

Relative organ weights

Feeding 100 ppb AF and 1 ppm T-2 toxin in turkey poults from 0-13 weeks of age caused no significant differences in relative weights of liver and bursa of Fabriciusbut for numerical increase, while a significant decrease was observed in relative weight of spleen in mycotoxin treated group except T-2 group when compared to the control.

Aflatoxicosis

Comparison of overall means revealed no significant differences in relative weights of liver, bursa of Fabricius and a significant decrease in spleen between the control and AF treated group. On the contrary significantly decreased relative liver weights, numerically decreased spleen weights and no changes in bursa of Fabricius were observed by Quist *et al*. (2000) in 4-month-old wild turkey poults fed 100, 200 and 400 microgram aflatoxin/kg feed for 2 weeks. However, Elisângela Aparecida Guaiume (2005) reported significant increase in relative weights of spleen while numerical decrease in relative weight of liver in turkey poultsfed diets containing 0.15 mg/kg AFB1 from first week onwards in a 21 day trial. No other comparable results could be quoted. However similar results were reported by Smith

and Hamilton (1970) in birds fed 0.625, 1.25, 2.5, 5 and 10 ppm AFB_1 for three weeks which exhibited a dose dependent increase in relative liver weight. However, increasing the toxin level above 2.5 ppm appeared to have no significant effect upon liver weight. A dose of 1.25 ppm had to be exceeded for increase in pancreas and spleen weights. On the contrary, bursa of Fabricius revealed decreased weight with increased AF dose level. Theophilus (2006) observed significant increase in the weight of liver and spleen and decrease in the weight of bursa of Fabricius in broiler chicken fed birds AF (0.5 ppm) from 0 to 6 weeks of age while Nirmal Kumar Thapa (2008) reported that 27 week old White Leghorn layers fed with 1 and 2 ppm AF for six weeks had no significant differences in relative organ weights of liver and spleen in AF treated groups.

T-2 toxicosis

Comparison of overall means revealed no significant differences in relative weights of liver, bursa of Fabricius and spleen between the control and T-2 toxin treated group. The findings are in accordance with Babu Prasath (2008) who reported feeding diets containing 1 and 3 ppm T-2 toxin from 0 to 28 days of age to turkey poults revealed no significant differences in the weights of liver and bursa of Fabricius in T-2 toxin fed groups. While Ogunboet al. (2007) observed absolute decrease in the weight of spleen of turkey poults fed with diet containing T-2 toxin at 4 mg/kg of feed for a period of 21 days. Girish *et al.* (2008) reported significant increase in the relative weights bursa of Fabricius in hybrid turkey poults fed diet contaminated with *Fusarium* mycotoxins from day 1 to 12 weeks. Elisângela Aparecida Guaiume (2005) reported significant increase in relative organ weight of liver and spleen in turkey poultsfed diets containing 2 mg T-2 toxin /kg diet from first week onwards in a 21 day trial.

Combined effect of AF and T-2

Comparison of overall means revealed no significant differences in relative weights of liver, bursa of Fabricius and significantly decreased spleen weights between the control and AF-T-2 treated group. However, Elisângela Aparecida Guaiume (2005) reported significant decrease in relative organ weight of liver and a numerical increase in relative organ weight of spleen and bursa of Fabricius in turkey poultsfed diets containing mg T-2 toxin /kg diet singly or in combination with 0.15 mg/kg from first week onwards in a 21 day trial. Studies in other species revealedincreased relative weights of the liver and spleen as reported by Huff *et al.* (1988) while feeding 2.5 µg AF/g and 4 µg T-2 toxin/g in broiler chicks from 1 to 21 day of age. Significantly increased relative liver weight was observed by Raju and Devegowda

(2000) while feeding 0.3 mg AF/kg and 3 mg T-2 toxin/kg in broiler chicks from 1 to 35 days of age and Madheswaran *et al.* (2005) while feeding AF (3 ppm) and T-2 toxin (4 ppm) in combination from 0 to 35 days of age in Japanese quail.

The relative weights of liver decreased initially in toxin treated groups during the 7^{th} week and the findings correlated with the histopathological findings of degeneration and necrosis of hepatocytes. While a gradual increase in the weight is noticed during the 13^{th} week which corroborated with the histopathological findings of biliary hyperplasia, regeneration of surviving hepatic cells, megalocytosis, periductular fibrosis, generalized fibroplasias in the liver and increased collagen deposition which could have increased the relative liver weights in toxin treated groups. The decreased splenic weight observed in AF toxicosis might be attributed to lymphoid depletion and decreased weight in AF-T-2 group to regressive changes. The initial increase in the relative weight of bursa during 7^{th} week could be attributed to accumulation of inflammatory infiltrate which could be appreciated histologically. However, during 13^{th} week the relative weight of the bursa of Fabricius showed a decrease in toxin treated group which might be due to loss of lymphoid cells and atrophy of bursa of Fabricius. Hence the results of the present study reflect a dose and duration dependent change in relative organ weight of liver, spleen and bursa of Fabricius during 7^{th} week and 13^{th} week.

Conclusions

Hence, it may be concluded that feeding 100 ppb AF and 1 ppm T-2 toxin individually or in combination for 13 weeks can adversely affect the health of turkey poults. The study also indicated the potential of AF to interact significantly with T-2 toxin in causing gross pathological changes in treated group and severe lesions in combined group in vital organs which might be due to additive effect of toxins.

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TABLE 1
Mean (±SE) relative liver weights (%) of turkey poults fed aflatoxin and T-2 toxin singly and in combination

(n=6)

Groups	Liver (%)		
	7th week	**13th week**	**Overall mean**
Control	2.00±0.246	1.52±0.069	1.77±0.297
Aflatoxin (100 ppb)	1.57±0.130	2.07±0.081	1.82±0.282
T-2 toxin (1 ppm)	1.72±0.081	2.04±0.164	1.88±0.206
Aflatoxin (100 ppb) + T-2 toxin(1 ppm)	1.81±0.178	1.83±0.064	1.82±0.127

TABLE 2
Mean (±SE) relative spleen weights (%) of turkey poults fed aflatoxin and T-2 toxin singly and in combination

(n=6)

Groups	Spleen (%)		
	7th week	13th week	Overall means
Control	0.11±0.01	0.11±0.00	0.106^{a}±0.01
Aflatoxin (100 ppb)	0.10±0.01	0.10±0.01	0.093^{b}±0.01
T-2 toxin (1 ppm)	0.11±0.02	0.10±0.01	0.102^{a}±0.01
Aflatoxin (100 ppb) + T-2 toxin(1 ppm)	0.07±0.01	0.09±0.00	0.087^{c}±0.10

Means with different superscripts within a column differ significantly (P<0.05)

TABLE 3
Mean (±SE) relative bursa of Fabricius weights (%) of turkey poults fed aflatoxin and T-2 toxin singly and in combination

(n=6)

Groups	Bursa of Fabricius (%)		
	7th week	13th week	Overall means
Control	0.10±0.02	0.06±0.01	0.075±0.02
Aflatoxin (100 ppb)	0.10±0.01	0.05±0.01	0.075±0.03
T-2 toxin (1 ppm)	0.09±0.02	0.06±0.01	0.076±0.02
Aflatoxin (100 ppb) + T-2 toxin(1 ppm)	0.11±0.01	0.05±0.00	0.078±0.03

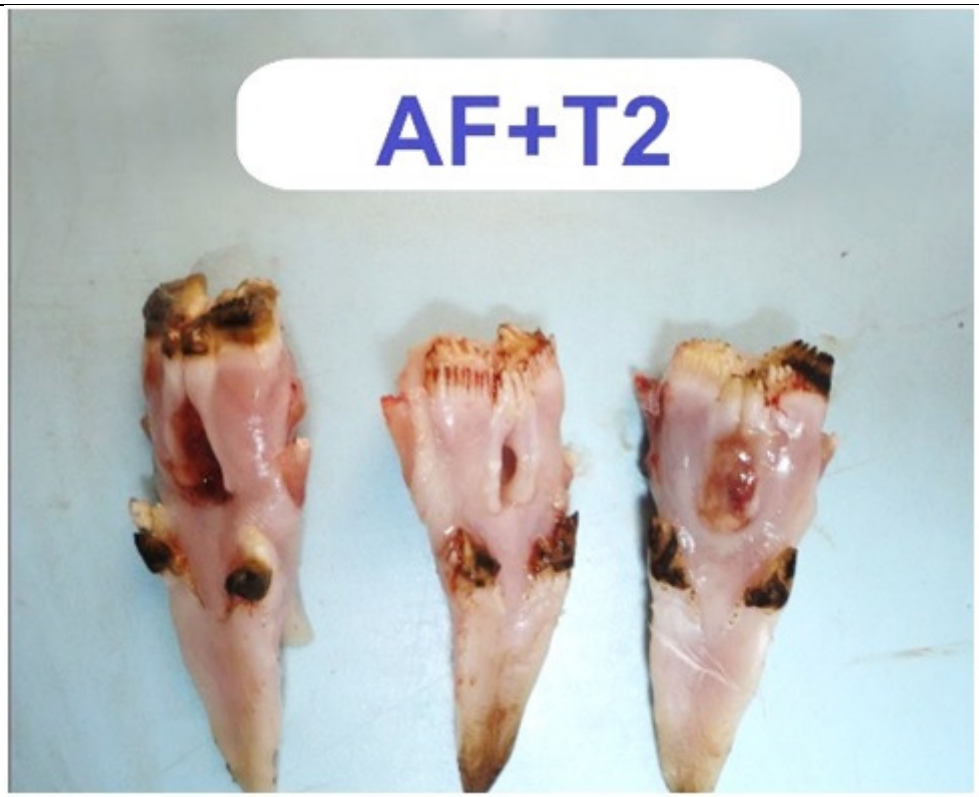

Figure 1:Turkey poult-7-Wk-AF+T-2 – 100pb – 1 ppm- Tongue – Necrotic plague lesions

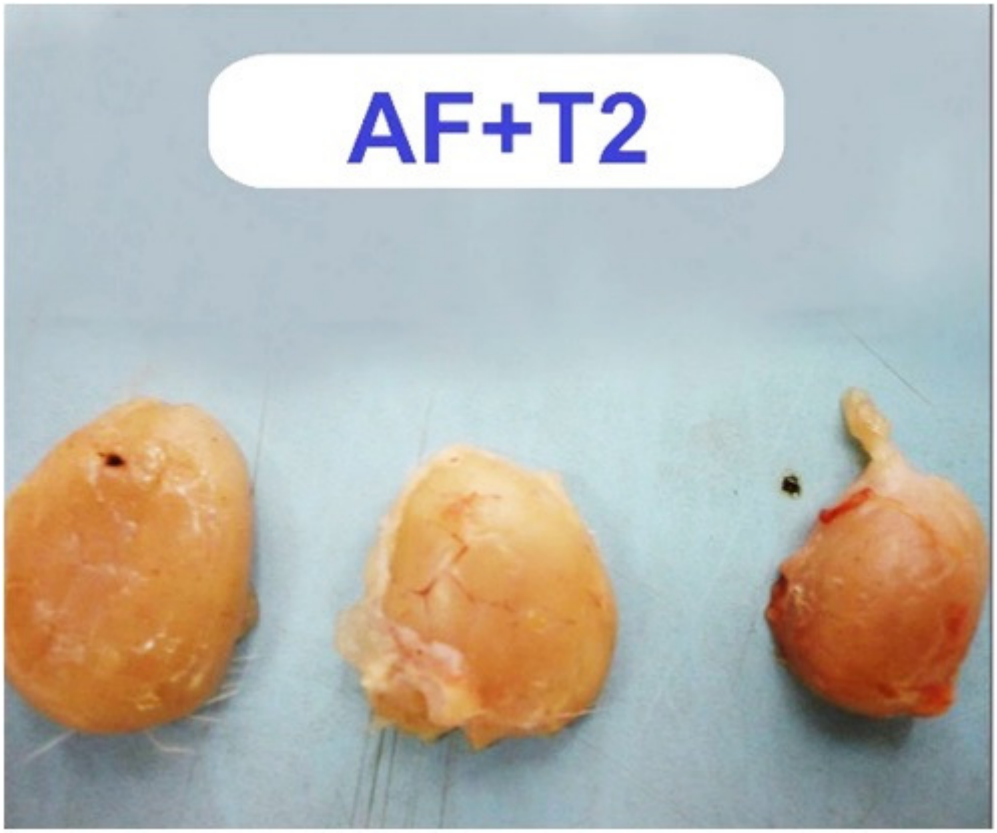

Figure 2:Turkey poult-7-Wk-AF+T-2 – 100pb – 1 ppm- Bursa of Fabricius – Oedematous

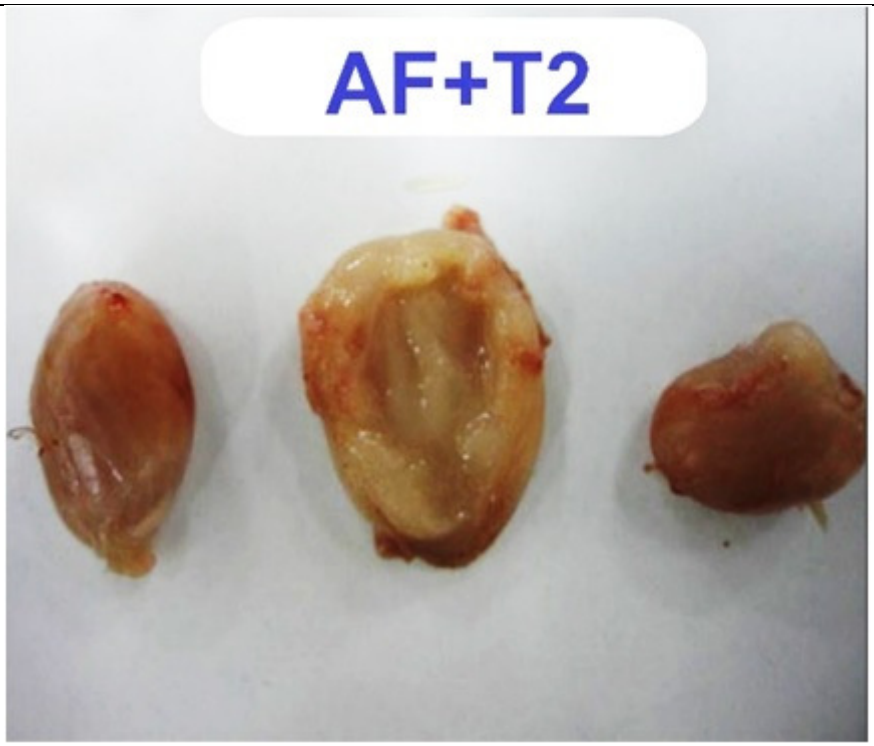

Figure 3:Turkey poult-7-Wk-AF+T-2 – 100pb – 1 ppm- Bursal plicae – Oedematous

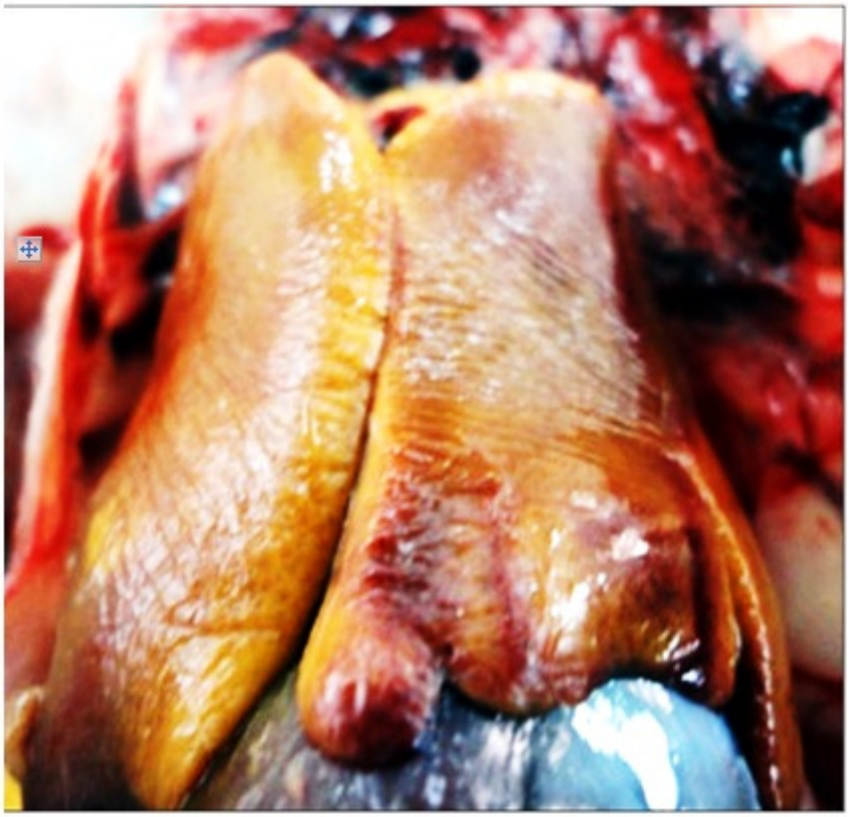

Figure 4:Turkey poult-13-Wk-AF+T-2 – 100pb – 1 ppm- Liver – Yellowish discolouration

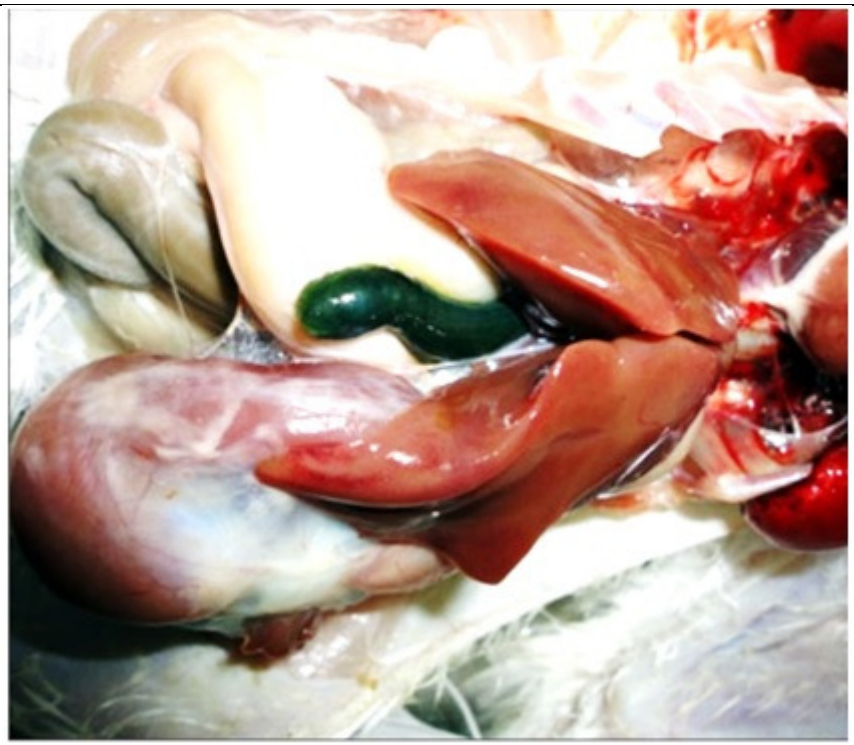

Figure 5:Turkey poult-7-Wk-AF+T-2 – 100pb – 1 ppm- Gall bladder – Distended with thin greenish bile

Figure 6:Turkey poult-7-Wk-AF+T-2 – 100pb – 1 ppm- Gall bladder – Thin greenish bile

Figure 7:Turkey poult-7-Wk- Liver – From left to right – Reduction in liver size from control to AF + T-2