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Effect of toxin binders on immunity and aflatoxin M1 residues in milk in buffaloes

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ABSTRACT

To investigate the effect of dietary toxin binders on AFM1 and immunity in buffaloes 48 Murrah buffaloes in same lactating phase were divided into 4 groups, viz. control (C), T1, T2 and T3 (toxin binder fed buffaloes) comprising of 12 animals each. The average milk AFM1 concentration decreased slightly in control group while marked decrease was found in T1 group throughout the experiment. The average milk SCC showed non significant increase in all the groups. Serum IgG concentration in control group decreased significantly from day 0 to day 45, while in groups T1, T2 and T3 there was significant increase, whereas no significant difference in serum IgG concentration was observed between the groups. The average serum total protein concentration in all the groups from day 7 through day 15, 30 and 45 was almost similar and within reference range. There was no significant difference between the groups. The average serum SGOT level was significant between the days in all the groups. The average SGPT concentration between the days was non significant in control and T1 groups and significant decrease was observed in T2 and T3 groups. The SGPT concentration was highly significant between control and T1, T2, T3 groups, respectively. The results suggested that feeding of toxin binder is effective in reducing AFM1 in milk at the dose rate of 50 mg/day having composition of exal 44.44% + bentonite 55.56% indicating that it provides a potential protective mechanism against aflatoxin exposure and also some alterations in biochemical parameters and IgG.

Key words: AFM1, Buffaloes, Enzymes, IgG, Toxin binders

Aflatoxicosis is the poisoning that results from ingestion of aflatoxin in contaminated food (Fatmi and Ruby 2011). It is primarily a hepatic disease. The effect of aflatoxins on animals varies depending on species, age, sex and nutritional status. The young ones of a species are more prone to aflatoxicosis. Gastrointestinal dysfunction, reduced reproductivity, reduced feed utilization, reduced efficiency, anemia and jaundice are the clinical signs of aflatoxicosis.The amount of AFM1 secreted in milk is directly related to the amount of AFB1 ingested by the cow. Although the amount of AFM1 secreted into milk depends on the individual cow and the experimental conditions, the levels are generally less than 5% of the AFB1 ingested in the feed. Elimination of an aflatoxin-contaminated diet results in a relatively rapid disappearance of the AFM1 in milk.

Several approaches have been investigated to reduce exposure of animals to aflatoxins in contaminated feeds. The addition of sequestering or binding agents to aflatoxin contaminated feedstuffs is one of the most used methods worldwide. Detoxification and inactivation methods include the use of binders or sequestering agents added to feed as an approach to reduce toxicity of mycotoxins by reducing reactivity of bound mycotoxins and reducing their intestinal absorption. This approach is seen as prevention rather than therapy. A binder must be effective at sequestering the mycotoxin(s) of interest. The binder should be physically usable in commercial feed manufacturing situations. Binder use and efficacy should be verifiable (Whitlow, 2006). The use of toxin binders or adsorbents may have the greatest application for routine avoidance of constant exposure to low level of multiple mycotoxins. Some toxin adsorbents are: silicate products (montmorillonite, bentonite and hydrated sodium calcium aluminosilicate, zeolites and clinoptilolite), carbon products (activated or superactivated charcoal), inorganic polymers (cholestyramine, polyvinylpyrrolidone). Among all these adsorbents, hydrated sodium calcium aluminosilicate (HSCAS) has been the most extensively studied *in vitro* and was selected for extensive *in vivo* application in a varied number of farm animals. HSCAS adsorb and retain 95% of aflatoxins. HSCAS are activated by heat drying process. These inorganic clays are thought to work by ion exchange interactions between free radicals on the clays and potentially charged groups on the toxins. That is why the clay binders are most effective against the polar toxins such as aflatoxins. Thus the aim of this trial was to evaluate

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AFM1, SCC in milk and monitor serum IgG, SGPT, SGOT, and total protein upto 45 days in different groups of buffaloes fed with and without toxin binders.

MATERIALS AND METHODS

The study was conducted on 48 Murrah buffaloes in same lactating phase maintained at private farm in Aarey milk colony, Goregaon, Mumbai. The animals were divided into 4 groups, viz. control (C), T1, T2 and T3 comprising of 12 animals each. The T1 and T2 groups were fed toxin binders at the dose rate of 50 g/day and T3 group at the dose rate of 25 g/day where as control group was not fed any toxin binder. In all the groups aflatoxin B1 level in the feed sample averaged 1771 pg/ml throughout the experiment. The composition of toxin binder for T1 group was exal 44.44% + bentonite 55.56% and for T2 and T3 group was talc 69.4% and chinal clay 30%, respectively. Pooled milk samples (morning and evening) from each group was collected on 0, 7, 8, 9, 10, 11, 12, 13, 14, 15, 21, 28, 35 and 45th day and blood samples were collected aseptically from the jugular vein on day 0, 7, 14, 21, 28, 35 and 45th day of experiment for IgG and at 15 days interval for serum enzymes.

Serum IgG was estimated by using Bovine IgG ELISA kit (Immunology Consultant Laboratory, Inc., USA), and milk aflatoxin M1 was measured using AFM1 ELISA kit (Europroxima BV Netherland) according to the manufacturer's instructions. Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), total bilirubin and total protein were estimated using autoanalyser and kits (Prietest Touch – Robonik (INDIA) Pvt. Ltd.). Statistical analysis of the data was done according to Snedecor and Cochran (1998) using complete randomized design between the days and randomized block design between groups. Differences in means were tested using critical difference (CD) test.

RESULTS AND DISCUSSION

Milk Aflatoxin M1 (AFM1): The average milk aflatoxin M1 concentration (pg/ml) is presented in Table 1.There was decrease in concentration of aflatoxin M1 in milk from day 0 to day 45 in all the groups. In control group the aflatoxin M1 concentration in milk ranged between 140 pg/ml to 150 pg/ml with highest concentration on day 0. The aflatoxin M1 concentration fluctuated between the days with lowest concentration of 113 pg/ml on day 11.The T1 group showed a specific trend of decrease in aflatoxin M1 concentration in milk with highest concentration on day 0 and then decreased to 128 pg/ml on day 8. Further it fluctuated and remained almost similar upto day 21 and then again decreased to lowest concentration of 117 pg/ml on day 45.

The T2 group also showed decrease in aflatoxin M1 concentration, recording highest concentration of 157 pg/ml on day 0 and decreasing to 112.5 pg/ml on day 8. Further it again increased and fluctuated between 116.5 pg/ml and 153.5 pg/ml from day 9 to day 45. The aflatoxin M1 concentration in milk remained almost similar between day 12 to day 45.

Table 1. Mean milk aflatoxin M1 concentration (pg/ml) from day 0 to day 45 in Murrah buffaloes

Day	C	T1	T2	T3
0	151.50	162.50	157.00	143.50
8	122.00	128.00	112.50	119.50
9	150.00	136.50	116.50	117.50
10	145.00	145.00	125.00	131.50
11	113.00	137.00	114.00	134.00
12	150.00	141.00	153.50	127.00
13	147.50	121.00	152.50	122.50
14	146.50	130.50	144.50	125.00
15	132.00	129.50	147.00	123.00
21	138.50	125.50	141.50	117.00
28	141.00	122.50	137.50	117.50
35	140.50	122.00	142.50	116.50
45	142.50	117.00	137.50	115.00
Treatment means	$140.00^{a}\pm$ 3.18	$132.15^{ab}\pm$ 3.42	$137.03^{a}\pm$ 4.25	$123.80^{b}\pm$ 2.32

The aflatoxin M1 concentration in milk in T3 group decreased from day 0 to day 8, which later slightly increased on day 10 and 11 and further decreased from day 12 through day 45.The statistical analysis revealed that there was no significant difference between control, T1 and T2 groups, while T3 group was highly significant ($P<0.01$) when compared to control and T2 groups, thus suggesting that T3 group toxin binder had significant effect on aflatoxin M1 in milk.

The AFM1 concentration recorded in buffalo's milk in control group which received the feed contaminated with aflatoxin B1 through the experiment in the present study are in agreement with Applebaum *et al.* (1982), Trucksess *et al.* (1983), Fremy *et al.* (1985), Pietri *et al.* (2003), Masoero *et al.* (2007), Sugiyama *et al.* (2008) and Britzi *et al.* (2013) in dairy cows and Battacone *et al.* (2003; 2009; 2012) in ewes. This measurable amount of AFM1 in milk provides an evidence that the toxins could enter the blood directly through rumen epithelium and are then available for distribution to the tissue. The slow release of the bound toxins from the tissue into the circulatory system could account for the uneven progression of elimination. The variations in toxin concentration also may be related to rumen movement (Trucksess *et al.* 1983). Masoero *et al.* (2007) opined that the adsorption and consequent transfer of toxins to blood and biological fluid is by passive diffusion of polar component into the liquid phase and by diffusion or active transport of the non polar component into the liquid phase. Because of the low molecular weight of AFB1 and AFM1 the toxins are rapidly adsorbed through membranes by passive mechanism. Upon adsorption the body's ability to AFB1 detoxification is associated with the action of the liver microsomal cytochrome P-450 enzyme and the enzyme S–glutathione transferase.

The data of the present study indicated that the interaction of toxin binders and aflatoxin provides a protective mechanism against aflatoxin exposure. This is based on the

reduced level of AFM1 excretion when toxin binders are administered in the aflatoxin contaminated feed, thus suggesting that the bioavailability of aflatoxin is reduced by toxin binders. The reduced levels of AFM1 may be due to a strong bond found between toxin binders and aflatoxin (Smith *et al.* 1994).

Milk somatic cell count (SCC): The average milk SCC ($\times 10^5$ cells/ml) is presented in Table 2.The average milk SCC was non significant in control group and ranged between 1.07×10^5 cells/ml to 1.17×10^5 cells/ml between day 0, 7, 14, 21, 28, 35 and 45. The SCC in T1 group ranged from 1.83×10^5 cells/ml to 1.88×10^5 cells/ml and was non significant from day 0 through day 45. Similarly the T2 group also showed non significant difference which ranged between 1.87×10^5 cells/ml to 1.94×10^5 cells/ml from day 0 to day 45. The average milk SCC of T3 group was also non significant throughout the experiment from day 0 to day 45 and ranged between 1.25×10^5 cells/ml to 1.39×10^5 cells/ml.

Table 2. Milk somatic cell count concentration ($\times 10^5$ cells/ml) from day 0 to day 45 in Murrah buffaloes (Mean±SE)

Day	C	T1	T2	T3
0	1.11±0.13	1.88±0.12	1.87±0.15	1.25±0.14
7	1.07±0.14	1.83±0.11	1.93±0.14	1.30±0.13
14	1.14±0.11	1.90±0.09	1.92±0.13	1.21±0.12
21	1.17±0.13	1.87±0.15	1.87±0.12	1.39±0.11
28	1.16±0.15	1.85±0.16	1.89±0.11	1.33±0.15
35	1.11±0.12	1.87±0.12	1.93±0.11	1.38±0.12
45	1.16±0.13	1.88±0.12	1.94±0.12	1.36±0.13
Treatment means	$1.13^{C\pm}$0.13	$1.86^{A\pm}$0.12	$1.90^{A\pm}$0.12	$1.31^{B\pm}$0.12

The milk SCC between the groups statistically revealed significant difference between control group with T1, T2 and T3 groups, whereas there was no significant difference between T1 and T2 group, while T3 group was statistically significant with T1 and T2 groups.

The observation of present study showing slight increase in count during the days of experiment is similar to those reported by Applebaum *et al.* (1982) in dairy cows. These values are too low to relate with any sub-clinical disorder associated with the mammary gland. The daily intake of aflatoxin may have had a slight effect on SCC but no appreciable effect was seen in treatment groups fed with toxin binders.These results of present study is also in accordance with Masoero *et al.* (2007) and Britzi *et al.* (2013) in cows, who also reported an average SCC of 1,00,000/ml and above, which is considered a threshold value for a healthy udder, thus indicating there is no effect of aflatoxin B1 on SCC with an average of 1771 pg/ml dose in feed. However Patel *et al.* (2011) found significant decrease in SCC in milk of cow.

Serum Immunoglobulin G (IgG): The mean±S. E. values of serum IgG levels is presented in Table 3. The difference

Table 3. Serum IgG (mg/ml) from day 0 to day 45 in Murrah buffaloes (Mean±SE)

Day	C	T1	T2	T3
0	$52.83^{bc}\pm$ 9.46	$25.66^{d}\pm$ 2.11	$27.83^{c}\pm$ 1.72	$30.58^{d}\pm$ 1.91
7	$42.25^{c}\pm$ 4.96	$49.50^{bc}\pm$ 6.71	$38.66^{bc}\pm$ 7.24	$33.58^{cd}\pm$ 1.85
14	$24.33^{d}\pm$ 4.18	$28.66^{d}\pm$ 5.82	$36.08^{bc}\pm$ 3.73	$32.00^{d}\pm$ 2.07
21	$59.41^{ab}\pm$ 7.21	$85.75^{a}\pm$ 2.15	$41.08^{bc}\pm$ 7.35	$33.16^{cd}\pm$ 2.37
28	$36.75^{cd}\pm$ 6.39	$40.16^{cd}\pm$ 7.53	$38.00^{bc}\pm$ 2.77	$38.66^{c}\pm$ 2.11
35	$46.25^{bc}\pm$ 4.71	$62.08^{b}\pm$ 6.48	$49.16^{ba}\pm$ 4.24	$48.66^{b}\pm$ 1.88
45	$75.58^{a}\pm$ 2.62	$93.83^{a}\pm$ 4.08	$62.08^{a}\pm$ 5.15	$66.83^{a}\pm$ 1.40
Treatment means	48.2± 4.77	55.23± 7.74	41.84± 3.16	40.49± 3.79

in serum IgG concentration between the days were highly significant ($P<0.01$) in control, T1, T2 and T3 groups. The values recorded in control group showed significant decrease in serum IgG concentration from day 0 to day 14 then it increased significantly from day 21 through day 35 and highest concentration was observed on day 45. The value recorded in group T1 showed a significant difference and fluctuations from day 0, 7, 14, 21 and 28. From day 35 through day 45 there was highly significant increase in serum IgG concentration. In group T2 serum IgG concentration increased non significantly from day 0 to day 28 whereas there was highly significant ($P<0.01$) increase between day 0 and day 35. Further it remained non significant up to day 45, but there was numerical increase in serum IgG concentration from day 35 to day 45. The values recorded in T3 group were non significant from day 0 to day 21 and then increased significantly ($P<0.01$) from day 28 through day 35 and day 45.There was no significant difference in serum IgG concentration between control, T1, T2 and T3 groups but numerically the serum IgG concentration was higher in control and T1 groups and lower in T2 and T3 groups.

This result of increase in serum IgG concentration in treatment groups from day 0 to day 45 is similar to those reported by Fernandez *et al.* (2000) in lambs. According to them the increase in serum IgG concentration is characteristic of aflatoxin intoxification whose target organ is liver and hepatic disease. They also reported that the main effect of aflatoxin is on cellular immunity thus indicating that the animal consuming aflatoxin may be susceptible to disease due to suppression of some humoral and cellular responses. However, Tripathi *et al.* (2008) observed that the immunity status of lamb was not influenced and also the serum IgG level due to low level of aflatoxin in diet. Similarly, Patterson *et al.* (1981) in Friesian calves and Meissonnier *et al.* (2008) in pigs reported no significant alteration in serum IgG concentration when exposed to

aflatoxin contaminated feed. In the present study the serum IgG concentration was higher in control group and T1 group as compared to T2 and T3 groups. This result is in accordance with Weaver *et al.* (2013) in pigs. According to them aflatoxin has stronger effect on immune system and also there is tendency to increase immunoglobulins and alter the immune system on feeding mycotoxins.

Total Protein: The mean ± S. E. values of serum total protein levels is presented in Table 4. The concentration of serum total protein in control, T1, T2 and T3 groups revealed that the difference in serum total protein between day 0 and day 15 was highly significant (P<0.01). In all the groups serum total protein concentration from day 7 through day 15, 30 and 45 was almost similar and within reference range. There was no significant difference between the control, T1, T2 and T3 groups.

Table 4. Serum total protein (g/dl) from day 0 to day 45 in Murrah buffaloes (Mean±SE)

Day	C	T1	T2	T3
0	9.60^{a}±0.72	9.66^{a}±0.52	9.88^{a}±0.37	10.0^{a}±0.56
7	7.73^{b}±0.39	8.26^{b}±0.37	7.83^{b}±0.21	6.72^{b}±0.25
15	7.49^{b}±0.24	7.39^{bc}±0.27	6.91^{c}±0.22	6.95^{b}±0.2
30	7.26^{b}±0.24	6.38^{cd}±0.24	6.44^{c}±0.2	6.09^{b}±0.2
45	7.61^{b}±0.41	6.36^{d}±0.22	6.63^{c}±0.23	6.01^{b}±0.28
Treatment means	7.94±0.27	7.61±0.4	7.54±0.4	7.16±0.47

These results are in accordance with Lynch *et al.* (1969) and Wyatt *et al.* (1985) in dairy calves, Fernandez *et al.* (1996) in lamb, Schell *et al.* (1993b), Marin *et al.* (2002) and Harper *et al.* (2010) in pigs and Battacone *et al.* (2009) in dairy ewes who observed no effect of aflatoxin or toxin binder on serum total protein. However, Lindemann *et al.* (1993) and Dilkin *et al.* (2003) in pigs, Edrington *et al.* (1994) in crossbreed lambs and Battacone *et al.* (2003) in ewes reported an increased activity of total protein in aflatoxin. Thus indicating impairment in activity of liver.

In contrast Tripathi *et al.* (2008) reported decreased serum total protein concentration in aflatoxin in lambs indicating the adverse influence of aflatoxin on liver.

Serum Glutamic Oxaloacetic Transaminase (SGOT / AST): The mean ± S. E. values of SGOT in control, T1, T2 and T3 groups is presented in Table 5. The SGOT levels between the days in all the groups were significant (P<0.05). In control group the highest SGOT concentration was recorded on day 0 which decrease significantly (P<0.05) on day 7 and then remained almost similar on day 15, 30 and 45. In T1 group the SGOT concentration decreased non significantly from day 0 to day 15. There was significant (P<0.05) difference in SGOT concentration between day 0 and day 30 and 45 respectively. Similar trend was also observed in T2 group. While in T3 group there was significant decrease in SGOT concentration between day 0 and day 15 which remained almost similar up to day 45.

Table 5. Serum SGOT/ALT (IU/L) from day 0 to day 45 in Murrah buffaloes (Mean±SE)

Days	C	T1	T2	T3
Day 0	111.81^{a}± 1.76	99.76^{a}± 6.06	107.00^{a}± 6.48	115.53^{a}± 6.78
Day 7	89.96^{b}± 6.16	88.29^{ab}± 4.89	98.11^{ab}± 4.57	100.58^{ab}± 7
Day 15	95.66^{b}± 5.68	85.37^{ab}± 4.44	93.48^{abc}± 4.79	94.73^{b}± 7.41
Day 30	86.12^{b}± 3.31	80.37^{b}± 4.94	87.59^{bc}± 4.78	90.98^{b}± 5.85
Day 45	87.33^{b}± 6.96	77.27^{b}± 5.18	83.30^{c}± 3.97	84.81^{b}± 5.03
Treatment Means	94.18^{A}± 3.03	86.21^{B}± 2.51	93.90^{A}± 2.67	97.33^{A}± 3.37

The SGOT concentration between the control, T1, T2 and T3 showed no significant difference between control and T2 and T3 groups whereas there was significant difference (P<0.05) between control and T1 group. The T1 group also showed significant difference with T2 and T3 groups respectively.

The results of the present study are in accordance with Weaver *et al.* (2013) in pigs that also showed minimal effect of mycotoxins and toxin binders or feed additives. The overall concentration of SGOT in all the groups was within normal range. Similar trend were observed by Rustemeyer *et al.* (2010), Chaytor *et al.* (2011) and Duan *et al.* (2013) in pigs. SGOT has been proposed as indicator of depressed liver function due to hepatocellular injury leading to change in plasma membrane permeability (Rustemeyer, 2010). Since, aflatoxin B1 induce liver damage, it will be expected that SGOT concentration would be increased in control group or before feeding toxin binders. However, the level of aflatoxin B1 in feed in this study averaged 1771 pg/ml but did not elicit substantial changes in SGOT concentrations. Similarly, Clark *et al.* (1984) in goats and Battacone *et al.* (2003) in ewes also found no significant difference in SGOT concentrations of the animal fed with aflatoxin B1 and without aflatoxin B1.

Serum Glutamic Pyruvic Transaminase (SGPT / ALT): The mean ± S. E. values of SGPT in control, T1, T2 and T3 groups is presented in Table 6. The SGPT concentration in control group between the days was within normal range and there was no significant difference between day 0, 7, 15, 30 and 45 respectively. On day 7 there was significant increase in SGPT concentration as compared to day 0, 15, 30 and 45. The SGPT concentration in T1 group indicated non significant difference between the day 0, 7, 15, 30 and 45 respectively and the concentration was within the normal range. The SGPT concentration in T2 group though it was within normal range decreased significantly from day 7 through day 15 and 30. A similar trend was also observed in T3 group showing a significant decrease but within normal reference range.However, the SGPT concentration was highly significant between control and T1, T2, T3

Table 6. Serum SGPT/AST (IU/L) from day 0 to day 45 in Murrah buffaloes (Mean±SE)

Day	C	T1	T2	T3
0	39.40^{ab}± 4.69	26.69± 1.1	34.09^{a}± 1.43	37.12^{a}± 1.67
7	51.20^{a}± 5.27	29.39± 1.59	33.24^{a}± 1.79	31.18^{b}± 1.71
15	35.14^{b}± 5.42	28.2± 1.57	28.38^{bc}± 1.81	30.56^{b}± 1.19
30	34.42^{b}± 2.28	26.26± 0.8	29.61^{ab}± 1.68	26.23^{c}± 1.2
45	36.60^{b}± 1.74	23.89± 2.04	24.24^{c}± 1.41	23.93^{c}± 0.98
Treatment means	39.35^{A}± 1.99	26.88^{B}± 0.6	29.91^{B}± 1.14	29.80^{B}± 1.46

groups respectively, whereas there was no significant difference between T1, T2 and T3 groups respectively.

The activity of SGPT concentration observed in control group are in agreement with those of Van Dijk *et al.* (1984) in Holstein calves, Tripathi *et al.* (2008) in lambs and Harper *et al.* (2010) in pigs. The levels of SGPT suggested that the levels of aflatoxin in this study in control group cause only low and transient negative effect on hepatocyte therefore the serum SGPT are frequently used to evaluate acute liver damage. Thus the toxin produced by aflatoxin intoxification in the feed was insufficient to induce toxicological manifestation.The results in T2 and T3 groups are in agreement with those of Schell *et al.* (1993a), Schell *et al.* (1993b) and Lindemann *et al.* (1993) in pigs who found decreased activity with advancing days of treatment. This suggested that the toxin binder prevented the serological abrasion normally observed with aflatoxin consumption (Schell *et al*. 1993a). Thus the data indicate aflatoxin indeed adsorbed in gastro intestinal track and the addition of toxin binder limits aflatoxin adsorption.

Irrespective of groups, the SGPT concentration was significantly higher in control group than T1, T2 and T3 groups respectively, whereas the three treatment groups did not show any significant difference amongst them. These results are similar to those reported by Clark *et al.* (1984) in goats, Brucato *et al.* (1986) in dairy calves, Edrington *et al.* (1994) in lambs, Battacone *et al.* (2003) in ewes, Dilkin *et al.* (2003), Rustemeyer *et al.* (2010) and Weaver *et al.* (2013) in pigs. In contrast Duan *et al.* (2013) observed no effect of toxin binders on SGPT in pigs.

Serum Total Bilirubin: The mean ± S. E. values of serum total bilirubin in control, T1, T2 and T3 groups is presented in Table 7. The mean ± S. E. serum total bilirubin concentration in control group was non significant throughout the experimental period from day 0 to day 45. It increased numerically on day 7 (0.80 ± 0.15) mg/dl and then decreased from day 15 (0.75 ± 0.17) mg/dl through day 30 and remained almost similar till day 45 (0.51 ± 0.13) mg/dl. The T1, T2 and T3 groups showed a significant increase in serum total bilirubin concentrations from day 0 to day 7 and remained almost similar till day 15. In T1 and T3 groups the serum total bilirubin concentrations decreased significantly from day 30 through day 45, where as in T2 group the concentration was non significant from day 7 through day 15, 30 and 45 respectively. The mean ± S. E. concentration between the groups control, T1, T2 and T3 was non significant.

Table 7. Serum total bilirubin (mg/dl) from day 0 to day 45 in Murrah buffalo (Mean±SE)

Day	C	T1	T2	T3
0	0.43±0.13	0.30^{c}±0.08	0.34^{b}±0.04	0.74^{b}±0.16
7	0.84±0.15	1.59^{a}±0.12	0.84^{a}±0.17	0.94^{b}±0.15
15	0.75±0.17	1.31^{a}±0.13	1.03^{a}±0.12	1.43^{a}±0.1
30	0.41±0.1	0.57^{bc}±0.07	0.86^{a}±0.14	0.65^{bc}±0.16
45	0.51±0.13	0.77^{b}±0.1	0.75^{a}±0.15	0.35^{c}±0.02
Treatment means	0.59±0.05	0.9±0.15	0.76±0.07	0.82±0.11

The findings of the present study that the concentration of serum total bilirubin increased on day 7 to day 15 and then decreased on day 30 in control, T1, T2 and T3 groups respectively is in accordance with Lynch *et al.* (1971) and Lynch *et al.* (1972) in dairy calves, Dilkin *et al.* (2003) and Rustemeyer *et al.* (2010) in pigs. According to them this increase is the result of an extra hepatic biliary blockage due to continuous presence of aflatoxin in the feed which may produce bile duct hyperplasia along with dilated intralobular lymphatic duct (Lynch *et al*. 1972). The significant decrease in serum total bilirubin concentrations in T3 group from day 30 through day 45 is in accordance with Lindemann *et al.* (1993) in pigs. This indicates that liver damage was occurring in initial days as a result of increasing aflatoxin. With the introduction of toxin binders in treatment groups, significant improvement was clearly provided. A decline in cell mediated immune response was evident with increasing aflatoxin level and toxin binders helped in restoring cell mediated responsiveness.

The average concentrations of serum total bilirubin between control, T1, T2 and T3 groups was non significant and is in accordance with Harper *et al.* (2010) and Weaver *et al.* (2013) in pigs. Thus indicating minimal effect of aflatoxin and the feed additives or toxin binders.

The most important factor for the AFM1 level in milk is the AFB1 concentration in feed component, though ruminants are more tolerant to dietary aflatoxins / mycotoxins and feeding of toxin binder is effective in reducing AFM1 in milk at the dose rate of 50 mg/day having composition of exal 44.44% + bentonite 55.56%. This indicates that toxin binders provide a potential protective mechanism against aflatoxin exposure which binds irreversibly in the gut to prevent absorption of the toxin across the intestinal wall. The feeding of aflatoxin contaminated feed and aflatoxin + toxin binder containing feed has caused some modification in SGOT, SGPT, total protein and total bilirubin and stronger effect on immune

system by increasing immunoglobulin i.e. IgG, but there was no change in milk SCC. However, proper toxin binder with specific dose rate is effective in controlling AFM1 in milk. Since AFM1 has been evaluated as a possible human carcinogen, the cancer risk arising from AFM1 contamination in milk is a serious problem in food safety. Thus there is the importance of periodically monitoring the level of AFM1 in milk as well routine inspection of AFB1 in feed of dairy animals.

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