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Assessment of Mycobiota and Aflatoxins in Poultry Feeds Collected from Poultry Farms

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Aflatoxins are mycotoxins produced by *Aspergillus* genus especially by *A. flavous* and *A. parasiticus.* Out of more than twenty known aflatoxins, aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2) are the most significant and are serious threat to poultry industry causing toxigenic diseases in poultry birds. They not only cause economic losses to the poultry industry but also affecting human health. A total of 396 samples of Starter and Grower poultry feed samples were studied for the involvement of aflatoxins and mycobiota in them with respect to regional variation effects on their growth and production. The results obtained were subjected to one way analysis of variance using single factor completely randomized design through statistical software package Statistix 8.1. The results showed significant variation among all the districts for total culturable fungi and aflatoxins levels in Starter and Grower poultry feeds samples. Minimum fungal viable counts (9.5×10^1 CFUs/g) were noted in the starter feed sample from Swat district, while maximum total culturable fungi (6.6×10^4 CFUs/g) were counted in the Grower feed samples collected from Chitral region. Starter and Grower samples from different districts of KPK, Pakistan were positive for the presences of AFB1 with mean quantities > 20μg/Kg, which was beyond WHO acceptable limit of < 20μg/Kg. Significant contamination of all -poultry rations by various species of fungi and elevated levels of AFB1 (> 20μg/Kg) is a serious health issue that needs proper attention and remedial measures.

Key words: Aflatoxins, CFU, Different zones, Cold and hot area, poultry rations.

Poultry industry is the important part of livestock sector which has 52.2% share in agro based economy of Pakistan that contribute 19% of total meat production in the country[1]. The present investment in poultry industry is more than 200 billion Rupees with an annual growth of 8-10%[2]. The production of total poultry, including domestic and commercial birds, was 518 million, and total meat production remained 601 thousand tons during 2007-08[3]. Poor quality and high cost of feeds and their ingredients are considered alarming problems for poultry sector. The growth of moulds in poultry feed is one of the main threats to the economy and health of poultry. They not only affect its nutritional and organoleptic properties but also produce mycotoxins[4]. More than hundred fungal species are known as natural contaminants of agricultural and foodstuff with some producing mycotoxins depending on their genetic makeup and environmental conditions[5].

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According to Leibetseder *et al*., (1989)[6] about 30% to 40% of the moulds can synthesize toxin in favorable environmental conditions. Aflatoxins are the mycotoxins produced by different species of *Aspergillus*. Out of twenty different aflatoxins, type B1, B2, G1 and G2 are the most significant. The toxic effects of aflatoxins in poultry birds have been studied for their immunosuppressive, carcinogenic, mutagenic, growth inhibition and teratogenic effects[7, 8] . Aflatoxins contaminated feed may cause aflatoxicoses in poultry birds that leads to low growth rate and weight gain, anorexia. It also increases their susceptibility to environmental and microbial stresses that ultimately causes increase in rate of mortality[9, 10].

Poultry feed is a mixture of cereals, pulses, cotton seed meal and other additives. In Pakistan commercial feed mills as well as manual grinding and mixing is used for feed preparation. In respective crop season, owners of feed mills buy large quantities of these ingredients and stock them for feed production all over the year. They purchase these ingredients directly from the farmers on site of their cultivar that contains high amount of moisture. Pakistan's tropical weather is ideal for mould growth and consequently for the production of aflatoxins. The poultry birds fed by toxin contaminated feed are not only responsible for the economic losses of the poultry industry but also pose threat to human health (Sahib *et al*., 2012)[11] been reported that high amount of aflatoxins are present in the liver than meat and kidneys in the poultry birds. This offal is sold and consumed by population separately, thereby, enhancing health hazards. According to international agency for research on cancer (IARC), in Asia and Africa some cases of the liver cancer in humans have relation with aflatoxin presence in food and food stuffs[12].

Though a lot of research have been carried out on toxigenic mycobiota and mycotoxins in poultry feeds throughout the world but little data is available on moulds and aflatoxin contaminating poultry feeds sold in Khyber Pakhthunkhwa (KPK) province of Pakistan[13, 14, 15]. Open shed poultry farms in different districts of KPK have unhygienic condition and poor environmental control (temperature, humidity, free air access) which are the detrimental factors in contamination of poultry feed. Furthermore, lack of basic knowledge and technical skills on part of poultry farmers, with respect to contamination and growth of toxigenic fungi during and after feed handling can also be a contributing factor in this respect.

The objective of this study was to analyze the poultry feeds collected from open shed poultry farms of selected districts of KPK, Pakistan, for the presence of mycoflora and aflatoxins levels.

MATERIALS AND METHODS

Sample Collection

A total of 396 samples of poultry feed were randomly collected on day 1st (both Starter and Grower), 7th (Starter) and 40th (Grower) from the feeding pans of local poultry farms from selected districts of KPK, Pakistan and brought to Mycotoxins Laboratory of Food Technology Centre, PCSIR Laboratories Complex Peshawar Pakistan where they were kept in polyethylene bags till mycofloral and aflatoxins analyses.

Chemicals

All the chemicals used in the present study were of analytical grade. Ferric chloride and copper carbonate were purchased from Merk (Darmstadt, Germany), sodium hydroxide, potassium hydroxide and benzene were purchased from BDH (Poole, England) while acetonitrile, sulphuric acid and chloroform were bought Sigma Chemical (ST.Louis, USA). Standards of aflatoxin B1, B2, G1 and G2 were procured from Biopure (Tecknopark Tullin, Austria).

Determination of Mycoflora

Malt extract agar (MEA) was used for the determination of total fungal counts. One gram from well ground poultry feed was mixed with 9ml of autoclaved distilled water containing 0.01% Tween 80 in universal glass bottles. The contents were then agitated for two minutes using a vortex mixer. A dilution series ($10^{-1} – 10^{-5}$) was made and 100 µl aliquot was spread on entire surface area of MEA plates. The plates so prepared were incubated for 7-10 days at 25°C, thereafter numbers of colonies of different fungi were counted[16-19].

Preparation of Aflatoxins Standard

Benzene/acetonitrile (98:2) *v/v* was used for the preparation of standard stock solutions of aflatoxin B1, B2, G1 and G2, which were stored at 4 ºC till use[20].

Determination of aflatoxins

Using standard guidelines of Association of Analytical Chemistry (AOAC) for the determination of aflatoxins; B1, B2, G1 and G2 levels were evaluated. . 50 gram of feed sample was blended with 250 ml of acetone/water (85:15) for three minutes and filtered using Whatman No.4 to yield a clear filtrate. One flask containing 170 ml NaOH (0.02N) was added with 30 ml ferric chloride solution (0.41M). While in other flask 3g of copper carbonate was added to 150 ml of filtrate. The contents of both flasks were mixed and filtered. 150 ml of the filtrate was transferred to a separating funnel already containing 250 ml of sulphuric acid (0.03%) and fractionated two times with 10 ml chloroform. The lower chloroform layers were separated and transferred to another separating funnel containing 100ml of potassium hydroxide (0.02 M) solution that was swirled for 30 seconds and then allowed to stand for layer separation. The chloroform fractions were pooled into a vial and subjected to evaporation till dryness at 45^0C under nitrogen stream. Dried residue so obtained was re-dissolved in 200 µl of benzene/acetonitrile (98:2 v/v) and spotted on pre coated silica gel plates along with standards. The prepared plates were eluted with solvent system consisting of chloroform/xylene/acetone (6:3:1) and the developed chromatograms were observed for identification of aflatoxins under long wavelength UV (»= 365 nm). The quantification aflatoxins were done by comparing the spots[20].

Statistical Analyses

The data obtained was subjected to one way analysis of variance (ANOVA) using single factor completely randomized (CR) design [21] through statistical software package Statistix 8.1.

RESULTS AND DISCUSSION

Effect of environmental zones on total fungal count

The ANOVA for total fungal viable counts (Table 1a) showed that all the samples from different

Table 1a. ANOVA for CFUs log10/g levels in poultry feeds collected from local farms of KPK districts.

Log 10 CFUs (Starter)				
Source	*df*[a]	MS	F[b]	P
Districts	5	10.524	40.2	0.0000
Error	192	0.261	-	-
Total	197	-	-	-
Log 10 CFUs (Grower)				
Source	*df*[a]	MS	F[b]	P
Districts	5	5.684	10.4	0.0000
Error	192	0.545	-	-
Total	197	-	-	-

a = degree of freedom, b = variance ratio.

Table 1b. Total fungal viable counts in sample of poultry feeds collected from local poultry farms of KPK districts.

Districts	Starter feed (n=33) Log10 CFUs/g dry weight				95% LC	
	No of sample +ve	Minimum	Maximum	Mean ± SEM	Lower	Upper
Charsadda	33	2.94	4.29	$3.39^{c} \pm 0.05$	2.949	4.297
Chitral	33	2.92	4.77	$3.69^{b} \pm 0.04$	2.929	4.778
Mansehra	33	2.04	4.65	$2.75^{d} \pm 0.10$	2.041	4.653
Mardan	33	2.11	5.34	$3.54^{bc} \pm 0.11$	2.113	5.342
Sawabi	33	2.92	4.81	$4.06^{a} \pm 0.08$	2.929	4.819
Swat	33	1.97	3.51	$2.59^{d} \pm 0.07$	1.977	3.518
Districts	Grower feed (n=33) Log10 CFUs/g dry weight				95% LC	
	No of sample +ve	Minimum	Maximum	Mean ± SEM	Lower	Upper
Charsadda	33	2.93	6.32	$4.14^{c} \pm 0.13$	2.939	6.322
Chitral	33	2.88	5.99	$4.67^{a} \pm 0.14$	2.886	5.991
Mansehra	33	2.61	4.47	$3.50^{d} \pm 0.08$	2.612	4.477
Mardan	33	2.93	6.41	$4.52^{ab} \pm 0.16$	2.934	6.415
Sawabi	33	2.93	5.92	$4.17^{bc} \pm 0.09$	2.934	5.929
Swat	33	2.86	5.88	$3.99^{c} \pm 0.12$	2.863	5.880

districts were significantly (Pd”0.01) differ in respect to the mould incidence in the feeds. The locations had significant effect (P<0.01) on total fungal counts in both types of the selected feeds (Starter and Grower) collected from different poultry farms.

From the table 1b it is clear that the lowest minimum and maximum number of fungal colonies were recorded in starter feed collected from Swat followed by Mansehra. For the rest of samples collected selected localities the numbers of fungal colonies were high. If we look to the climatic conditions of the selected localities the weather of Swat and Mansehra districts are comparatively colder than the rest of the districts. So it is concluded that fungal growth is lower in colder regions as compared to hot areas. In grower feed same trend was observed but this time the lowest minimum and maximum colonies were observed in feed collected from Mansehra followed by Swat.

Effect of environmental zones on aflatoxin B1 production (AFB1)

The ANOVA for AFB1 production revealed that all the localities were found significantly (Pd” 0.01) varied for AFB1 production in the feeds (Table 2a). The localities had significant effect (p<0.01) on aflatoxin B1 levels in both types of poultry rations. It was also noted that all the localities had greater effect (f=9.57) on B1 production in grower feeds.

The minimum contents (Table 2b) of AFB1 (5 µg/kg) was detected in the Starter feed samples from the non- conventional poultry farms of Swat region, while the maximum concentration (89 µg/kg) was found in the samples from Mardan district. The highest mean value (47.52 µg/kg) was noted for aflatoxin AFB1 in Starter rations collected from Sawabi district and the least mean value (22.06 µg/kg) was found for AFB1 in the samples from Mansehra district.

The grower feeds were found highly contaminated with AFB1 in which the minimum

Table 2a. ANOVA for AFB1 levels (µg/kg) in poultry feeds collected from local poultry farms of KPK districts

	Aflatoxin B1 (Starter)			
Source	*df*[a]	MS	F[b]	P
Districts	5	4880.32	30.1	0.0000
Error	192	162.35	-	-
Total	197	-	-	-
	Aflatoxin B1 (Grower)			
Source	*df*[a]	MS	F[b]	P
Districts	5	2977.23	9.57	0.0000
Error	192	311.22	-	-
Total	197	-	-	-

a = degree of freedom, b = variance ratio.

Table 2b. AFB1 levels in poultry feeds collected from local poultry farms of KPK districts. districts.

Districts	Starter feed (n=33) Aflatoxin B1(µg/kg)				95% LC	
	No of sample +ve	Minimum	Maximum	Mean ± SEM	Lower	Upper
Charsadda	33	15.87	36.37	25.57b±0.93	23.662	27.480
Chitral	33	10.12	55.45	26.13b±2.04	21.972	30.305
Mansehra	33	5.50	72.56	22.06bc±0.37	17.230	26.893
Mardan	33	9.99	89.10	42.05a ± 3.19	35.555	48.552
Sawabi	33	17.19	69.95	47.52a ± 2.53	42.372	52.685
Sawat	33	5.00	38.82	16.23c ±1.49	13.187	19.289
Districts	Grower Poultry Feed (n=33) Aflatoxin B1(µg/kg)95% LC					
	No of sample +ve	Minimum	Maximum	Mean ± SEM	Lower	Upper
Charsadda	33	20.20	99.50	49.74c±3.12	43.392	56.104
Chitral	33	21.60	88.20	60.81ab±3.32	54.038	67.797
Mansehra	33	18.90	71.30	39.00d± 2.32	34.263	43.736
Mardan	33	27.90	98.80	65.20a± 3.69	57.683	72.716
Sawabi	33	27.90	91.30	59.97ab±2.72	54.418	65.539
Swat	33	24.60	87.50	52.53bc±3.04	46.325	58.747

concentration (18.90 µg/kg) was examined in the samples from Mansehra, while the equal minimum concentration (27.90 µg/kg) was determined in the Grower rations collected from different farms of Mardan and Sawabi districts. The maximum concentration (99.50 µg/kg) of AFB1 was recorded in the feed sample from Charsadda followed by Mardan and Sawabi districts. The least mean value (39.00 µg/kg) was recorded for AFB1 in the Grower feed samples from Mansehra, while the other mean values were in the range of (49.74 to 65.20 µg/kg).

Table 3a. ANOVA for AFB2 levels (µg/kg) in poultry feeds collected from local poultry farms of KPK districts.

	Aflatoxin B2 (Starter)			
Source	dfa	MS	Fb	P
Districts	5	237.290	27.4	0.0000
Error	192	8.654	-	-
Total	197	-	-	-
	Aflatoxin B2 (Grower)			
Source	dfa	MS	Fb	P
Districts	5	132.182	8.30	0.0000
Error	192	15.921	-	-
Total	197	-	-	-

a = degree of freedom, b = variance ratio.

Effect of environmental zones on aflatoxin B2 production (AFB2)

The ANOVA for AFB2 (Table 3a) indicated that a significant difference (pd"0.01) were observed for AFB2 production among all the districts in both the starter and grower feeds samples. Location had significant effect (P<0.01) on AFb2 level while F= 27.4 for starter feed samples.

Table 3b data revealed that 13 Starter feeds samples were found negative for AFB2 collected from Mansehra region while 5 samples were from Swat area. The maximum contents of AFB2 (18.98 µg/kg) was observed in the sample of Starter ration from Mansehra whereas the least mean value (2.19 µg/kg) for AFB2 were noted in the samples from the same area. The Grower feeds were found more contaminated with AFB2 as compared to starter ration. The minimum contents of AFB2 (3.00 µg/kg) were determined in Starter rations from Swat and the maximum contents (22.10 µg/kg) were found in the sample collected from Charsadda. The least mean value (7.34 µg/kg) for AFB2 was recorded in the samples from Mansehra region while the highest mean value (12.95 µg/kg) was noted for AFB2 in the Grower feeds samples collected from Mardan regions.

Effect of Environmental Zones on Aflatoxin G1 Production (AFG1)

The ANOVA (Table 4a) for AFG1 revealed that regions were significantly (Pd"0.01) varied for

Table 3b. AFB2 levels in poultry feeds collected from local poultry farms of KPK districts

Districts	Starter feed (n=33) Aflatoxin B2 (µg/kg)				95% LC	
	No of sample +ve	Minimum	Maximum	Mean ± SEM	Lower	Upper
Charsadda	33	1.15	8.85	3.14de ± 0.28	2.534	3.729
Chitral	33	1.50	7.00	3.87cd ± 0.31	3.227	4.527
Mansehra	20	0.00	18.98	2.19e ± 0.60	0.956	3.432
Mardan	33	1.79	17.81	7.28b ± 0.58	6.095	8.476
Sawabi	33	1.38	14.91	9.25a ± 0.55	8.124	10.392
Sawat	28	0.00	13.21	4.76c ± 0.605	3.534	6.002
Districts	Grower Poultry Feed (n=33) Aflatoxin B2 (µg/kg)				95% LC	
	No of sample +ve	Minimum	Maximum	Mean ± SEM	Lower	Upper
Charsadda	33	4.50	22.10	10.11bc± 0.60	8.889	11.346
Chitral	33	4.40	19.70	11.11ab± 0.84	9.396	12.840
Mansehra	33	2.50	15.30	7.34d ± 0.65	6.017	8.679
Mardan	33	6.50	21.40	12.95a ± 0.83	11.257	14.645
Sawabi	33	5.40	20.10	11.30ab± 0.52	10.237	12.368
Sawat	33	3.00	18.90	8.69cd ± 0.65	7.364	10.017

AFG1 level in both types of poultry rations (Starter and Grower). More difference (F=19.0) were examined in the districts for AFG1 production in Grower feeds.

It is evident (Table 4b) that the AFG1 production were not detected in most of the Starter poultry feed from all the districts while the maximum AFG1 level (13.24 µg/kg) was present in the Starter feed collected from Mansehra district. The lowest mean value of AFG1 (0.77 µg/kg) was noted in the Starter feed samples from Sawat region and the maximum level of AFG1 (18.50 µg/kg) was examined in the Grower feed sample from Mardan.

Table 4a. ANOVA for AFG1 level (µg/kg) in poultry feeds collected from local poultry farms of KPK districts

		Aflatoxin G1 (Starter)		
Source	dfa	MS	Fb	P
Districts	5	115.267	28.0	0.0000
Error	192	4.124	-	-
Total	197	-	-	-
		Aflatoxin G1 (Grower)		
Source	dfa	MS	Fb	P
Districts	5	161.856	19.0	0.0000
Error	192	8.499	-	-
Total	197	-	-	-

a = degree of freedom, b = variance ratio.

The least mean value (2.78 µg/kg) was recorded for AFG1 in the Grower feed samples collected from Mansehra, while the other mean values were found in the range from (4.16 to 8.76 µg/kg).

Effect of Environmental Zones on Aflatoxin G2 Production (AFG2)

The ANOVA for AFG2 showed that the districts were significantly (Pd"0.01) varied in respect of AFG2 production in starter and grower feeds samples (Table 5a). The districts had significant (P<0.01) effect on AFG2 level, while the value F=28.0 was recorded for AFG2 (Grower feeds).

Table 5b data showed that maximum number of Starter and Grower feed samples collected from different districts were negative for AFG2 production; while the maximum AFG2 concentration (3.50µg/kg) was detected in the Starter feed sample from poultry farms of Mansehra district. The highest mean concentration value (0.681µg/kg) was recorded for AFG2 in the samples of Starter poultry feed from poultry farms of Sawabi district and the least mean concentration value (0.76µg/kg) was observed for AFG2 in the Grower feed samples collected from Mansehra district followed by, Swat (1.52 µg/kg), Charsadda (1.67µg/kg), Chitral (1.88µg/kg), Mardan (4.01 µg/kg) and Sawabi (4.36 µg/kg). In the present study, a total of 398 samples collected from selected districts of

Table 4b. AFG1 levels in poultry feeds collected from local poultry farms of KPK districts

Districts	Starter feed (n=33) Aflatoxin G1 (µg/kg)				95% LC	
	No of sample +ve	Minimum	Maximum	Mean ± SEM	Lower	Upper
Charsadda	27	0.00	3.98	1.59c ± 0.19	1.194	1.989
Chitral	21	0.00	3.40	1.37c ± 0.21	0.935	1.805
Mansehra	13	0.00	13.24	0.97c ± 0.41	0.131	1.812
Mardan	31	0.00	12.30	2.79b ± 0.38	2.010	3.384
Sawabi	32	0.00	11.65	5.73a ± 0.55	4.602	6.874
Sawat	13	0.00	3.75	0.77c ± 0.18	0.395	1.162
Districts	Grower Poultry Feed (n=33) Aflatoxin G1 (µg/kg)				95% LC	
	No of sample +ve	Minimum	Maximum	Mean ± SEM	Lower	Upper
Charsadda	33	1.90	14.20	5.26b ± 0.40	4.440	6.080
Chitral	33	1.770	9.90	4.97b ± 0.50	3.944	6.001
Mansehra	31	0.00	6.40	2.78c ± 0.29	2.179	3.384
Mardan	33	3.00	18.50	8.76a ± 0.81	7.100	10.421
Sawabi	33	3.00	16.50	7.60a ± 0.45	6.678	8.527
Sawat	33	1.00	11.20	4.16bc ± 0.41	3.230	5.001

KPK, Pakistan were investigated for mycobiota and occurrence of aflatoxins. Initially all the collected samples were free from aflatoxins and mycobiota. However the collections at day 7th and 40th showed the contamination of aflatoxins and fungi in the feed. This was due to unhygienic conditions of the form. The degree of contamination of the feed was related to the geographical locations of the farms. The total fungal viable count in both types of poultry feeds was significantly different amongst geographical zones. Feed samples from tropical and humid regions were found to be more affected by fungal contamination. These findings were similar to the results of[22-24] who investigated that fungus can grow well on high carbohydrate and protein containing substrates in tropical and humid regions with respect to its proper storage, humidity, temperature and time of exposure. The presence of different fungal genera, representing both field and storage fungi and the incidence of *Aspergillus*, *Penicillium* and *Fusarium* in higher percentage, was particularly important, because these are known to be toxin producers[25, 26]. Our findings are in close conformity with[27, 28], who previously recognized that aflatoxins producing genera are predominant over other genera in tropical environments. Fungi contaminating agricultural commodities can produce toxic metabolites which are often unavoidable and a point of worldwide concern. Pathogenic microorganism and their secondary metabolites (Aflatoxin) in general chain of nutrition represent the most important risk to animal and human health[11].

Table 5a. ANOVA for AFG2 level (µg/kg) in poultry feeds collected from local poultry farms of KPK districts

		Aflatoxin G2 (Starter)		
Source	dfa	MS	Fb	P
Districts	5	1.697	5.35	0.0000
Error	192	0.316	-	-
Total	197	-	-	-
		Aflatoxin G2 (Grower)		
Source	dfa	MS	Fb	P
Districts	5	70.53	28.0	0.0000
Error	192	2.522	-	-
Total	197	-	-	-

a = degree of freedom, b = variance ratio.

According to Food and Drug Administration (FDA) of United State, the maximum permissible limits of AFB1 should not exceed 10µg/kg for chicks less than 4 weeks of age[29] Our study revealed that in both the starter and grower feed samples the mean values for AFB1 were above the permissible limits (>20 µg/kg) for chicken older than 4 weeks. It is presumed that the presence of such high amount of aflatoxins in the poultry ration under

Table 5b. AFG2 levels in poultry feeds collected from local poultry farms of KPK districts

Districts	Starter feed (n=33) Aflatoxin G2 (µg/kg)				95% LC	
	No of sample +ve	Minimum	Maximum	Mean ± SEM	Lower	Upper
Charsadda	9	0.00	1.30	0.200bc± 0.07	0.0465	0.353
Chitral	6	0.00	1.50	0.209c ± 0.08	0.0410	0.372
Mansehra	11	0.00	3.50	0.106c ± 0.10	-0.110	0.322
Mardan	11	0.00	3.20	0.398b ± 0.13	0.130	0.667
Sawabi	25	0.00	2.00	0.681a ± 0.11	0.441	0.922
Sawat	1	0.00	1.39	0.080c ± 0.05	-0.033	0.193
Districts	Grower Poultry Feed (n=33) Aflatoxin G2 (µg/kg)				95% LC	
	No of Sample +ve	Minimum	Maximum	Mean ± SEM	Lower	Upper
Charsadda	29	0.00	4.80	1.67b ± 0.16	1.330	2.021
Chitral	22	0.00	4.80	1.88b ± 0.29	1.283	2.480
Mansehra	11	0.00	3.00	0.76c ± 0.20	0.355	1.184
Mardan	31	0.00	11.30	4.01a ± 0.39	3.199	4.824
Sawabi	33	1.20	8.50	4.36a ± 0.29	3.777	4.961
Sawat	23	0.00	5.90	1.52bc ± 0.24	1.035	2.019

bad management and unhygienic conditions is the cause of frequent outbreaks of aflatoxicoses and associated mortality in poultry industry[30]. The maximum AFB1 concentration (99.505µg/kg) were noted in grower poultry ration collected from Charsadda district, while the minimum AFB1 contents (5 µg/kg) were found in the starter feed from Swat region. But aflatoxins even in lower doses can not only cause obvious mycotoxicoses but also lead to the impairment of immune resistance to infections causing health problems resulting in economic losses in the form of decreased productivity[31]. Interestingly our results also revealed that the all aflatoxins negative samples collected from different location of KPK Pakistan were found contaminated with AFB2, AFG1 and AFG2. The difference in the prevalence and levels of contamination of aflatoxins in poultry feeds depends on geographical area, relative humidity, temperature, storage conditions and especially feed handling procedures[32]. Mean aflatoxins levels in both Starter and Grower feeds samples were above the safe limit of 20 µg/Kg as recommended by World Health Organization so there is a dire need for constant monitoring of fungal and aflatoxins contaminations in poultry feeds.

CONCLUSION

The results showed that even aflatoxins negative feed can be contaminated by mycotoxins in local open shed farm of KPK Pakistan due to unhygienic environment which directly affect the poultry ration present in the feeding pan. Even in cold and hot areas a very little difference were seen in aflatoxins concentration. Training of poultry farmers by the government authorities, non-government bodies on potential health consequences of fungi and aflatoxins is necessary. Stringent regulations regarding reduction of aflatoxins in poultry feeds are recommended.

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