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Assessment of Heavy Metals in Different Tissues of Broilers and Domestic Layers

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Abstract: Five poultry farms were selected to collect the feed, body tissues and litter samples of broiler and domestic layers. Poultry feeds were the combination of foodstuff and Pre-mixers while Pre-mixers were the combination of different heavy metals. Samples of muscles, liver, skin and litters of both birds were analyzed to determine the translocations of heavy metals from feed to birds and their excretion through litters. The highest levels of cupper (340±3.44 mg kg^{-1}) and manganese (102±6.11 mg kg^{-1}) in muscles; manganese (340±6.48 mg kg^{-1}) and iron (4008±6.4 mg kg^{-1}) in liver; manganese (210±3.55 mg kg^{-1}) and iron (2683±3.8 mg kg^{-1}) in skin; copper (252±1.05 mg kg^{-1}), manganese (233±2.47 mg kg^{-1}) and iron (2307±6.1 mg kg^{-1}) in litters were determined at farm-D. The maximum contents of iron (11009±10 mg kg^{-1}) in muscles and copper (313±2.87 mg kg^{-1}) determined at farm-A while the highest manganese concentration (127±3.65 mg kg^{-1}) in litters of broilers was assessed at farm-B. It was concluded from the results that maximum contents of heavy metals accumulated in the body tissues of broilers whereas lesser of their quantities excreted through litters. The levels of all heavy metals were much higher than permissible levels determined by World Health Organizations. Precocious steps must be taken to avoid use of such health hazardous concentrations of heavy meals in poultry feeds.

Key words: Assessment · Poultry · Heavy Metals · Tissues · Feed

INTRODUCTION

A complete and balanced diet is necessary for human health and vitality. Protein is an essential element to form a perfect diet. Protein is usually produced by two kinds of resources that are plants and animals. Pakistan is deficient in production of proteins from animals. Currently 66% Pakistanis are lacked in protein in their daily food. A human demand 103.6 g protein on daily basis but available protein is only 70 g. The difference in demand is 33.4 on daily basis. Protein is mainly produced by animals on a large scale in Pakistan. Due to rapid growth of human being with the passage of time short fall of protein is also increasing day by day, protein short fall seems more serious and painful in poor developing countries as compared to the developed countries. People of developed countries take 80-96 g protein daily [1]. Many researchers have been researched to find out the comparison between the profit and deficit related to the poultry industry and then values in the market. It is the need of the time that people should have the capacity to buy there daily intake diet by safe resources [2, 4].

To overcome the problem of deficiency these heavy metals are being used as supplementary diet. Mostly these are being used in poultry industry to meet the protein requirement and they are normally being used as mineral supplements [5]. Many studies are conducted to assess the heavy metals from poultry feed and results showed the mixing of high concentration of heavy metals [6]. But in poultry industry these heavy metals are being used in excess amount. When these metals are added in feed more than the required level, these can accumulate in body tissues of broiler and in human being on its consumption and can be released in litter to cause environmental impacts [7]. Heavy metals can enter in food chain on consumption of broiler. There are many studies carried out to detect the heavy metals accumulation in chicken meat. Khan *et al.* [8] assessed the risk of polluted

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and excessive amount of various ingredients used in animal feed. These toxic elements present in feed pose serious health hazards to consumers and secondary consumers due to biomagnifications. Many studies were about the dispersion of toxic metals among various soil samples, especially 25-year poultry waste-amended soil. Copper and Zinc normally accumulate close to the soil surface where the total amounts of Cu and Zn in waste-amended soils were significantly higher than in no amended soils [9].

Toxic metals are possible environmental pollutants with the capacity to cause health problems in human beings [10]. Many studies showed that elements used in feed deposit in body and cause harmful impacts. In case of poultry industry deposition of heavy metals in body of broiler were result of their excessive use in poultry feed. Concentration of these toxic metals were also higher in litter depending on composition of poultry feed. Different experiments under the parameters of different metals like cadmium, zinc, copper, Lead, Chromium, Nickel, Barium, Cobalt, Strontium, Titanium, Mercury and Silver were conducted. Results revealed that most of the samples were with high concentrations of these heavy metals than the National Hygienic Standards. In poultry litter samples amount of aluminum and arsenic concentrations were detected very high. Their impacts on soil and crops were further analyzed on using the litter as fertilizer [11]. For marketing of poultry industry, poultry litter is very important and can be used for crops as fertilizer, because this contains high nitrogen, Phosphorus and Potassium concentrations but it also contains concentration of heavy metals [29, 30]. The present study is sketched to detect the heavy metals in broiler feed and their accumulation in body tissues and litter of broiler. A comparison of heavy metals in muscles, liver, skin and litter samples of broiler and domestic layer is also planned.

MATERIALS AND METHODS

Five poultry farms were selected from District Faisalabad, Pakistan to collect feed, body tissues and litter samples of broiler and domestic fowels. Two poultry farms were selected from 20km away from city at Jaranwala Road, designated as Farm-A and Farm-B. Two poultry farms were 15km from city at Samundari road named as Farm-C and Farm-D and remaining eighteen kilometer away at jhang road named Farm-E. One sample of domestic layer was collected from nearest village of each selected poultry farm. Different ingredients were used for making poultry feed such as small grains (wheat, rice, maize, millet, sorghum barley, dried sea food, blood of animals, broken pulses and different vitamins) and heavy metals. Poultry feed is the combination of foodstuff and Pre-mixers (Table 1). Farm-A, B, C and D used locally manufactured foodstuff available in market while used by Farm-E was of Hi-tech. Pre-mixers are combination of different heavy metals manufactured locally on demand and their concentrations were not known to the poultry farmers. Samples of broiler feed (foodstuff + Pre-mixers) and tissue of muscles, liver and skin were collected from each poultry farm (Table 1). Similarly litter samples were collected from each poultry farm. The samples were grinded with pestle mortar and sieved to attain the size of 0.3-0.5mm. All values were compared with the limits set by World Health Organization (FAO) and Food and Agriculture Organizations (FAO) of United Nations (UN). For analysis of these samples slides were prepared by using the method described by Nisar *et al*. [12]. Samples were analyzed at proton induced X-ray emission (PIXE) established at Center of Advance Studies of Physics (CASP) at Government College Lahore, Pakistan. Statistically the data was subjected to Analysis of Variance (ANOVA) to find out the variance of heavy

Table 1: Heavy metal concentrations (mean±S.E.) in food stuff and feed of selected poultry farms and WHO/FAO permissible limits

Heavy Metals	*Concentration in mg* kg^{-1} Farm A (Foodstuff + P.M=Feed A)	Farm B (Foodstuff + P.M=Feed B)	Farm C (Foodstuff + P.M=Feed C)	Farm D (Foodstuff + P.M=Feed D)	Farm E (Foodstuff + P.M=Feed E)	(Mean ±S.E)	P.L.
Cu	200+2800= 3000	150+2500=2650	300+2800=3100	325+2850=3175	250+2000=2250	2835±172	8
Mn	400+4750=5150	375+4400=4775	367+4800=5167	525+4900=5425	259+3400=3659	4836±312	60
Fe	1054+30000=31054	1000+28500=29500	1254+30000=31254	1260+30600=31860	1200+28000=29200	30574±519	80
Ni	0+28000=28000	0+27000=27000	0+33000=33000	0+33600=33600	0+26000=26000	29520±1578	0
Zn	89+1500=1589	132+1299=1431	165+1557=1722	178+1600=1778	49+1367=1416	1587±74	40

P.L, Permissible Limit; P.M, Pre-Mixers

metals in broiler muscles, liver, skin and litter samples of all poultry farms. Least Significant Difference (LSD) was applied to make multiple comparisons of heavy metal concentrations in broiler muscles, liver, skin and litter samples [14].

RESULTS

The concentrations of copper, manganese and iron were high in broiler feed of selected poultry farms. Feed was the combination of food stuff and pre-mixers. Metals that were added as pre-mixers were copper, manganese and Iron. Highest concentrations of copper (3175 mg kg^{-1}mg kg^{-1}), manganese (5425 mg kg^{-1}) and were detected in broiler feed of Farm-D and lowest concentrations of these metals 2250, 3659, 29200, 26000 and 2416 mg kg^{-1}, respectively were detected in broiler feed of Farm-E (Table 2). There was highly significant (P<0.01) difference in all detected metal concentrations in broiler muscle samples of different poultry farms. copper (340±3.44 mg kg^{-1}) and manganese (102±6.11 mg kg^{-1}) concentrations were high in muscle samples of Farm-D while iron (11009±10 mg kg^{-1}) concentration was high in muscle samples of Farm-C. Lowest copper (167±1.96 mg kg^{-1}) and iron (808±5.47 mg kg^{-1}) concentrations were detected in muscle samples of Farm E while manganese was not detected in muscle samples of Farm-B and E. All detected heavy metals in broiler muscle samples of different poultry farms were greater than the set permissible limits, copper, 8; manganese, 60; iron, 80 and zinc, 40 mg-kg^{-1}. Copper, manganese and iron concentrations, which detected were 84-170, 2-20, 2-24 times higher than their permissible limits respectively (Table 2).

Iron levels in muscles were very low in Significant (P<0.01) differences in metal concentrations of liver samples of five poultry farms were recorded. Among all the poultry farms manganese (340±6.48 mg kg^{-1}) and iron (4008±6.4 mg kg^{-1}) concentrations were high in liver samples of Farm-D while lowest concentrations of these heavy metals were found to be in liver samples of Farm-E (Table 2). Comparison of heavy metals detected in broiler liver samples of five selected poultry farms with their permissible limits. Manganese concentration in liver samples was 31-68 times greater than permissible limit while Iron concentration was 5-9 times higher than the permissible limit. Heavy metal concentrations in broiler skin samples of five poultry farms significantly (P<0.01) differs. Copper was not detected in skin samples of Farm-B and C. Manganese and was not detected in skin samples of Farm-A, B and E. Concentrations of iron (2683±3.8 mg kg^{-1}) and manganese (210±6.14 mg kg^{-1}) were high in skin samples of Farm-D while copper concentration (313±2.87 mg kg^{-1}) was found to be high in skin sample of Farm-A (Table 2).

Comparison of heavy metals in broiler skin among five selected poultry farms with their permissible limits were carried out. There was significant (P<0.05) correlation of copper concentration of broiler feed with muscles (r = 0.907) and litter (r = 0.964). Manganese concentration showed significant (P<0.05) relation with litter (r = 0.988). Copper, manganese and iron were found in all samples of broiler while in domestic layer manganese and iron were detected in all samples and copper was found to be only in liver. Manganese concentration was significantly (P<0.05) higher in liver samples of broiler while iron was significantly (P<0.05) higher in liver samples of domestic layer and in litter samples of broiler.

Table 2: Heavy metal concentrations (mean±SE) in different body tissues and litters of broilers nourished at different farms selected for experimentation

		farms				
Sample types	*Heavy metals*	A	B	C	D	E
muscles	Cu	237±3.58C	193±2.58D	3054.87±B	340±3.44A	167±1.96E
	Mn	9±1.0500C	-	84±3.54B	102±6.11A	-
	Fe	11009±10A	993±3.87D	1445±3.1C	1505±3.4B	808±5.47E
Liver	Cu	-	-	-	-	-
	Mn	224±3.55C	198±3.96D	301±3.97B	340±6.48A	156±6.47E
	Fe	3213±40.0C	2827±20D	3942±5.4B	4008±6.4A	2320±4.5E
Skin	Cu	313±2.87A	-	-	313±6.14B	112±C
	Mn	-	-	147±4.65B	210±3.55A	-
	Fe	1956±12.4C	158±6.58E	1983±6.7B	2683±3.8A	930±6.7D
Litter	Cu	150±3.88C	101±1.87D	202±1.87B	252±1.05A	51±0.14E
	Mn	168±1.87C	127±3.65A	192±2.65B	233±2.47A	92±1.66E
	Fe	1641±2.84C	1204±8.4D	1906±8.7B	2307±6.1A	848±4.2E

Rows sharing similar alphabets followed by figures do not differ significantly (P>0.01)

Table 3: Heavy metal concentrations (mean±SE) in different body tissues and litters of domestic layers

	Tissue types			
Elements	muscles	liver	skin	litter
Cu	-	200±05.210	-	-
Mn	32±03.580	10±010.510	24±001.00	53±02.64
Fe	2035±30.5	26339±20.6	2420±10.5	829±7.65

Manganese concentration was high in broiler muscles, liver, skin and litter samples but statistically it showed significant ($P<0.05$) difference in liver and litter samples only. Iron accumulation was higher in liver and skin samples of domestic layer but it showed significant difference in liver only while its concentration was found to be higher in muscles and liver samples of broiler but it was significantly ($P<0.05$) higher only in litter samples of broiler. Iron, manganese and copper levels in all body tissues of domestic layers were very lower than limits of heavy metals in broilers tissues recorded at all poultry farms under study (Table 3).

DISCUSSION

In order to improve poultry production, different heavy metals are being added in poultry feed to enhance the growth rate of broiler. Excessive use of these heavy metals may cause accumulation in different body parts of the broiler and excretion in litter. Concentration of heavy metals was determined skin, muscles, liver and litter of broilers and domestic layers. This study was conducted to determine the heavy metals in broiler feed and their accumulation in muscles, liver and skin. Broiler litter was also analyzed to assess the residues of these metals. Heavy metals in broiler feed of selected poultry farms were only copper, manganese and iron. Concentrations of these heavy metals were already high than the WHO/FAO [27, 28] permissible limits for broiler feed in foodstuff and pre-mixers were added to enhance the growth rate, which make concentrations of these heavy metals 100 times greater than permissible limits. Highest concentrations of copper, manganese and iron were detected in broiler feed of Farm-D and lowest concentrations of these metals were detected in broiler feed of Farm-E. Detected concentrations of copper in skin samples were 56-157 times greater than the set permissible limit of WHO/FAO [27, 28]. The levels of heavy metals in feeds must be used depending the age of animals [13]. These heavy metals are also essential elements for growth but in excess amount may cause many health problems not only to broiler itself but also to its consumers [13]. Many studies showed that heavy metals i.e. manganese, nickel, copper, zinc, cadmium, lead, arsenic, mercury, selenium and chromium into poultry feed were 100 times more than the required value [15, 16]. Heavy metals in broiler feed are a serious problem because of their toxicity, bioaccumulation and biomagnifications in the food chain. These contaminants often have direct physiological toxic impacts because they can be accumulated in body tissues and released in litter [9, 11, 17-20]. The current study indicated that manganese and iron accumulated in body tissues of broiler but their accumulation in muscles and skin was depending upon their concentrations in broiler feed. Copper was mainly accumulated in muscles of broiler. In our results the highest copper levels in litters at farm-D found much lowered than determined by Cang *et al*. [11]. Manganese concentration was found to be 29-42 times higher than the permissible limit. Iron concentration was below the permissible limit in skin samples of Farm-B. In skin samples of broiler iron and manganese concentrations were much high at farm-D. Concentration of iron was 2-6 times greater than the permissible limit.

It was determined in many studies that concentration of the heavy metals i.e. Copper, manganese, iron, zinc and Nickel were higher in muscles, liver, skin and other organs. Current study also showed the trend of heavy metal accumulation was, mostly, higher in metabolic organs. These heavy metals perform many functions in body but their presence in excess amount may cause many toxic impacts [19, 21]. Results indicated that concentrations of heavy metals in broiler feed showed positive correlation with muscles, liver, skin and litter. If, concentrations of iron and manganese were high in broiler feed than their concentrations were also high in broiler muscles, skin, liver and litter while copper showed significant relationship of broiler feed with muscles and litter. In litter samples of broiler all selected heavy metals were detected. Present results of iron in liver tissues were in the line of Ghita *et al*. [22] who also determined the higher concentrations of copper, iron, chromium, zinc and nickel in liver and intestine than in muscles. Many studies reported that high concentration of heavy metals in broiler feed results high concentration of those metals in broiler litter. Use of excessive amount of heavy metals in poultry feed not only accumulate in various body tissues of the

broiler but also pollute the soil and water on careless desecrating the poultry litter. copper and zinc accumulated in close to soil surface and could be useful for plant growth but use in excess amount of heavy metals can increased toxicity of soil. On using broiler litter as fertilizer with excessive amount of these heavy metals could enter in human body by food chain [11, 20, 23, 24].

Comparison of these heavy metals in body tissues and litter samples of broiler and domestic layer showed that manganese was mainly accumulated in broiler liver. Nickel was accumulated only in body tissues of broiler and excreted in its litter while zinc was only detected in muscles and litter samples of broiler. Iron concentration was high in litter and muscle samples of broiler and in liver and skin samples of domestic layer while copper was accumulated in liver of domestic layer and in muscles, skin and litter of broiler. Accumulation of copper occurred in liver due to slow metabolism for long period as all domestic layers were selected of average age of 72 weeks, while broilers were selected only in the age of 6 weeks. This was reported in many studies that copper mainly stored in liver, which also perform function as an oxidizer and perform the function to send minerals to the whole body. Han *et al*. [9].

found that about 90% of metals (arsenic, copper, lead and mercury) fed to broilers excreted through litter. Heavy metal contents in chicken meat mainly accumulated in metabolic organs as muscles, liver and kidney [25, 26]. This study provides information about accumulation of heavy metals in broiler meat and can be helpful in risk assessment of consumers. Results showed that daily intake of broiler meat as per WHO [27, 28] limits ranges from 5-219 g day^{-1} depending upon the detected concentrations of different heavy metals. Average intake of broiler meat for all heavy metals is 58 g day^{-1} or 1.7 kg^{-1} month. High concentration of heavy metals in broiler feed resulted in their bioaccumulation in body tissues of broiler and excretion in litter. That is not only harmful for broiler itself but can also be harmful for consumers on excessive consumption and for environment on careless desecrating of litter.

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