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Heavy Metals and Aflatoxin Contaminants in Commercial and Desi Poultry Eggs Consumed in District Peshawar-Pakistan

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Abstract:
The present study was designed to determine heavy metals and aflatoxin contaminants in commercial and desi poultry eggs consumed in District Peshawar-Pakistan. A total of 650 egg samples was collected from commercial and desi poultry from three different sale points. Among them, 216 eggs were selected for analysis. All egg samples were tested for incidence of heavy metals, iron (Fe), cupper (Cu) & lead (Pb) and aflatoxin B1 (AFB1). The average concentration of Fe in commercial eggs was recorded 6.16±0.17ppm, Cu 0.37±.05ppm and Pb 0.32±0.01ppm, respectively. The average concentration of Fe, Cu and Pb in desi poultry eggs was 3.69±0.08, 0.21±0.008 and 0.15±0.02 ppm, respectively. It was concluded that the level of heavy metals is higher in commercial, while both commercial and desi eggs contain heavy metal concentrations below the permissible limited. There was no significant effect of cooking and freezing on the level of heavy metals. The significant difference (P<0.05) was observed between commercial and desi egg yolk. Aflatoxin B1 and heavy metals concentration in chicken eggs were analyzed through TLC and atomic absorption. The results showed that AFB1 contamination levels in commercial eggs were more than desi eggs. The average aflatoxin B1 level was 2.77±0.00 ppb in commercial eggs. Whereas, in desi eggs no aflatoxin B1 was detected. The significant difference (P<0.05) was observed between the commercial and desi egg for aflatoxin B1. The permissible level of aflatoxin B1in eggs was 3µg/g. In the present study, the level of aflatoxin B1 residue was in range, from 2-3 ppb, which was not more than the permissible limit. Our present study reveals that eggs consumed in District Peshawar-Pakistan are safe for human consumption and there is no risk of heavy metals and aflatoxin contamination.

Keywords: *contaminants, commercial eggs, desi eggs, aflatoxin B1, heavy metals*

1. Introduction

The agriculture sector in Pakistan plays an important role in the economy. According to Pakistan economic survey 2011-2012, it contributes 21% to the gross domestic product (GDP). In agriculture, the livestock share was 55.1% in 2008, which grew by 4% in 2011-12. Poultry sector contributes 25.8% to the total meat production in the country. Its contribution in livestock and agriculture is 11.5% and 6.4% respectively. The poultry sector has revealed a vigorous growth of 8 to 10% annually, which was natural potential. Currently, the desi poultry eggs production is 3809.00 million, while commercial poultry eggs are 13114.00 million in numbers.

Hygienic practices for eggs and egg products such as egg yolk, egg albumin and egg shell taken into consideration. However, in different countries production systems and processing procedures are used to protect eggs and egg products. The contamination of eggs and egg products is mainly by two common routes i.e. internally during the egg formation and externally during laying of egg on contaminated area. The hygienic rules are important to control both internal and external factors because if the egg is not placed in hygienic condition, there will be attack of microbes, among which *E.coli* and *Salmonella* are very harmful (Romo, 2004, Brown and Stringer, 2002, Alloui and Ayachi, 2012).

Trace minerals play important role in making of the basic component of control mechanism and act as enzyme activator. Essential elements include Iron (Fe) manganese (Mn), zinc (Zn), cobalt (Co) and copper (Cu) while non-essential elements are cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg). The term heavy metal is defined as the element, which has specific gravity greater than five is called heavy metal. The heavy metals, which are potentially toxic include Pb, Cd, As, and Hg. Other mineral elements which are nutritionally important and also fit this category include cobalt, vanadium, iron, contamination is serious threads because of their toxicity, bio magnifications and bioaccumulation in the food chain (Demirezen and Uruç, 2006).

The deficiency of metals in body may lead to impairment of vital biological process, but when the level of these metals increases from normal, it produces a toxic effect such as cadmium exposure causes bone and kidney damage. Pb exposure may lead to developmental and neurobehavioral effect on foetus and children. Excessive level of Cu damages the liver, brain and kidney. Poultry eggs are considered a highly nutritious food that is of great importance for human health (Surai and Sparks, 2001).

Mycotoxin has undesirable effect on both health and productivity of poultry birds. Generally, mycotoxicosis results in reduced feed intake, diminished FCR (food consumption ratio), decrease in egg production and susceptibility to various infections depending upon the types of toxins ingested. Contamination of feed with mycotoxin is a worldwide problem. Among mycotoxin aflatoxin, B1 (AFB1) is the most important mycotoxin encountered in feeds worldwide. Aflatoxin B1 reduces the egg production and egg weight, when ingested by poultry birds. Aflatoxin residues have been found in animal tissues, eggs and poultry following the experimental ingestion of aflatoxin-contaminated feed (Williams et al., 2004).

Eggs might contain elevated levels of heavy metals that originate mainly from food and water. Study on the mineral content of eggs is becoming increasingly important for many reasons that are related to health and nutritional value of eggs, the consequences of egg metals on its embryonic development and the use of eggs as bio-indicators for environmental metal pollution (Surai and Sparks, 2001, Sparks, 2006, Pappas et al., 2006). Monitoring heavy metal levels in chicken eggs is of great importance, from nutritional, toxicological and environmental point of view (Abdulkhaliq et al., 2012).

2. Materials and Methods

2.1. Study Area

The major part of the present study was conducted at Department of Poultry Science, Faculty of Animal Husbandry and Veterinary Sciences, while some part of the research was completed at the Departments of Soil Sciences, Animal Nutrition and Microbiology, The University of Agriculture, Peshawar.

2.2. Sample Collection, Size and Experimental Design

A total of 650 eggs were collected randomly from both commercial and desi poultry sale points. Among them, 216 egg samples were selected from commercial and desi poultry for analysis. The commercial and desi egg samples were splited into egg yolk, egg albumin and eggshell. A total of 216 samples both from commercial and desi poultry eggs were processed for heavy metals (Fe, Cu & Pb) and mycotoxin (AFB1). Samples showing positive results were cooked and frozen with 50:50 ratios to assess the effect of cooking and freezing on the hygienic status of eggs from commercial & desi eggs. Heavy metals (Fe, Cu & Pb) concentrations and aflatoxin (B1) in chicken eggs were detected through an atomic absorption spectrophotometer and thin layer chromatography, respectively. The data collected on different parameters was analyzed by using completely randomized design (CRD).

2.3. Determination of Heavy metals in Poultry eggs by Atomic Absorption Spectrophotometer

For heavy metal the atomic absorption spectrophotometer was conducted through the procedure of (Haswell, 1991, Varma, 1984). Eggs were cut in the air cell end using pointed forceps and dissecting scissor that was cleaned with soap and rinsed with distilled water and the contents of egg were placed in a chemically cleaned glass jar. Take egg white and egg yolk and dried at 60º C to get constant weight.

2.4. Determination of aflatoxin B1 Residues in Poultry eggs by Thin Layer (TLC) Chromatography

Thin layer chromatography was conducted according to the procedure of (Kaitaranta and Bessman, 1981). A 25 gram egg yolk and egg albumin sample was taken and 100 ml of acetone: distilled water (81 ml: 19ml) solution were added and was shake for 40 min and then the sample was filtered. 85 ml of 0.2 N NaOH, 15 ml of 6.6% $FeCl_3$ solution, 2.5-gram cellite powder and 1.5 gram $CuCO_3$ were added to the filtrate and shake for 20 min. The sample was filtered and added with 75 ml of 0.03% H_2SO_4 and 10 ml of 35% chloroform was shake vigorously for 5 min and was suspended in a separating funnel for 30 min. Then the lower layer in flask was collected and 50 ml of KOH: KCl solution in the ratio 1:10 were added and suspended for 30 min. Again, the lower layer in china dish was collected, dried, dissolved in 1 ml chloroform, and spotted on TLC plates with micro syringe as long with standard spots. Then the plates were developed in developing tank containing chloroform: acetone (90: 10) solution and was air-dried, observed under ultra violet lamp.

2.5. Wet Digestion of Egg Samples

A 1 gm sample was taken and transferred into 50 ml digestion tube and added with 20 ml nitric acid. Then the sample was heated for 30-45 min in fume hood. Then sample was cooled down and added with 10ml of 70% per chloric acid and heated again until it becomes colorless. After cooling, it was transferred into volumetric flask by making volume of the sample 100 ml by adding distill water. Then the sample was filtered through whatman filter paper -1 and used for mineral analysis in atomic absorption.

2.6. Statistical Analysis

Microsoft excel worksheet was used to finalize the data for sorting and basic statistics and the data was analyzed by analysis of variance (ANOVA), using general linear model formula of the statistical analysis system (SAS, 2007). T- Tests (LSD) for variable were applied to separate the means.

3. Results

In the present research contaminants in commercial and desi poultry eggs consumed in District Peshawar was investigated. This chapter consists of results on incidence level of heavy metals (Pb, Fe and Cu) and aflatoxin B1 residues poultry eggs. The important results are presented in the following.

3.1. Concentration of Fe, Cu and Pb in commercial and desi egg yolk

The table 05 shows the concentration (Mean± SE ppm) of Fe in commercial egg yolk from three different sales point in District Peshawar. The average concentration of Fe in egg yolk was 6.27±0.19 ppm and from desi egg yolk was recorded as 4.54±0.16 ppm as shown in Table 1. The significant difference ($P<0.05$) was observed between commercial and desi egg yolk in fresh samples. The results showed that there was no significant difference in Fe concentration in commercial and desi egg yolk post-cooking and freezing. The concentration (Mean± SE; ppm) of Cu in the commercial egg yolk from three different sales points is given in Table 2. The concentration of Cu in the egg yolk from commercial eggs before cooking from I, II and III sales points was 0.38±0.04, 0.36±0.03 and 0.38±0.08 ppm respectively. The average concentration of copper in commercial egg yolk was 0.37±0.05 ppm. The concentration of Cu post-cooking was 0.31±0.02 ppm. Whereas a freezing the (Mean± SE; ppm) Cu level in commercial egg yolk was recorded 0.35±0.04 as shown in Table 2. Significantly, no difference was found post-cooking and freezing. The Concentration of (Mean± SE; ppm) Cu in fresh samples of desi eggs yolk was 0.21±0.00 ppm. Cooking and freezing have no significant effect on the level of copper in the egg yolk. The results show that the commercial egg yolk significantly higher Cu level ($P<0.05$) as compared to desi egg yolk. The Pb concentration (Mean± SE ppm) present in commercial egg yolk of fresh samples at different sale points is depicted in Table 3. The overall concentration of Pb in commercial egg yolks was recorded 0.32±0.01 ppm. No significant effect of cooking and freezing was observed on Pb concentration in egg yolk from commercial eggs. The Mean± SE concentration (ppm) of Pb in fresh sample of desi egg yolk was observed 0.15±0.02 ppm. Significantly no difference ($P>0.05$) was found post-cooking and freezing. While significant difference was observed between commercial and desi egg yolk for Pb in all sale points of District Peshawar.

	Fresh samples		**Post- cooking**		**Post- freezing**	
Target area	Commercial eggs	desi eggs	commercial eggs	desi eggs	commercial eggs	desi eggs
Sale point I	$6.39±0.00^a$	$4.88±0.08^b$	$6.32±0.17^a$	$4.37±0.08^b$	$6.34±0.00^a$	$4.41±0.08^b$
Sale point II	$6.27±0.10^a$	$5.04±0.33^b$	$6.19±0.10^a$	$4.92±0.33^b$	$6.21±0.10^a$	$4.98±0.33^b$
Sale point III	$6.16±0.17^a$	$3.69±0.08^b$	$6.11±0.11^a$	$3.56±0.08^b$	$6.13±0.17^a$	$3.60±0.08^b$
Over all	$6.27±0.09^a$	$4.54±0.16^b$	$6.21±0.12^a$	$4.29±0.16^b$	$6.23±0.09^a$	$4.31±0.16^b$

Table 1: Pre and post treatment level of iron (ppm) (Mean± SE) in egg yolk from commercial and desi layers.
Mean within rows with different superscripts are significantly different (α=0.05)

Target area	**Fresh samples**		**Post-cooking**		**Post-freezing**	
	Commercial eggs	Desi eggs	Commercial eggs	Desi eggs	Commercial eggs	Desi eggs
Sale point I	$0.38±0.04^a$	$0.22±0.005^b$	$0.31±0.01^a$	$0.19±0.004^b$	$0.35±0.02^a$	$0.20±0.005^b$
Sale point II	$0.36±0.03^a$	$0.19±0.02^b$	$0.29±0.01^a$	$0.15±0.02^b$	$0.32±0.02^a$	$0.17±0.026^b$
Sale point III	$0.38±0.08^a$	$0.23±0.00^b$	$0.35±0.06^a$	$0.21±0.00^b$	$0.37±0.07^a$	$0.21±0.00^b$
Over all	$0.37±.05^a$	$0.21±0.008^b$	$0.31±0.02^a$	$0.18±0.0^b$	$0.35±0.04^a$	$0.19±0.01^b$

Table 2: Pre and post treatment level of copper (ppm) (Mean± SE) in egg yolk from commercial and desi layers.
Mean within rows with different superscripts are significantly different (α=0.05)

	Fresh samples		**Post-cooking**		**Post-freezing**	
Target area	Commercial eggs	Desi eggs	Commercial eggs	Desi eggs	Commercial eggs	Desi eggs
Sale point I	$0.34±0.05^a$	$0.13±0.03^b$	$0.31±0.01^a$	$0.10±0.08^b$	$0.33±0.04^a$	$0.12±0.00^b$
Sale point II	$0.36±0.00^a$	$0.19±0.02^b$	$0.33±0.00^a$	$0.12±0.01^b$	$0.35±0.00^a$	$0.16±0.00^b$
Sale point III	$0.28±0.00^a$	$0.15±0.01^b$	$0.24±0.00^a$	$0.11±0.02^b$	$0.27±0.00^a$	$0.13±0.00^b$
Over all	$0.32±0.01^a$	$0.15±0.02^b$	$0.29±0.003^a$	$0.11±0.03^b$	$0.31±0.01^a$	$0.13±0.00^b$

Table 3: Pre and post treatment level of lead (ppm) (Mean± SE) in egg yolk from commercial and desi layers.
Mean within row with different superscripts are significantly different (α=0.05)

3.2. The concentration of Fe, Cu and Pb in commercial and desi egg albumin

The concentration of Fe (Mean± SE ppm) in egg albumin from commercial in fresh samples was 3.34±0.03 while post cooking and freezing was recorded as3.20±0.04 and3.23±0.04 ppm, respectively. The results showed no significant effect of cooking and freezing on the level of Fe in commercial egg albumin. However, the average concentration of Fe in desi egg albumin was observed as 3.20±0.04ppm in fresh samples but post cooking and freezing the level was observed as 2.70±0.04 and 2.72±0.05 ppm, respectively as shown (Table 4). The results showed significantly different ($P<0.05$) in Fe level in commercial and desi egg albumen. The overall concentration of Cu in commercial egg albumin in fresh samples (Mean± SE ppm) was observed0.23±0.02 (Table 5). No significant difference in Cu level was found in three sale points. Cooking and freezing did not change Cu level. While in fresh samples of desi, egg albumen the concentration of Cu was recorded as 0.14±0.00 ppm. Post cooking and freezing have no significant effect on the level of Cu in desi egg albumin. The significant difference ($P<0.05$) was observed between commercial and desi egg albumen. The Mean± SE concentrations (ppm) of Pb in commercial egg albumen at different sale points are given in (table 6). The overall concentration of Pb was recorded as 0.16±0.02ppm, in fresh samples of commercial egg albumen. After proper cooking the level of Pb was 0.13±0.02 ppm. Cooking and freezing have no significant effect on Pb the level of egg albumen. The overall concentration of Pb (Mean± SE ppm) in fresh samples of desi egg albumin was as 0.12±0.00 ppm. The results showed that there was no significant effect of cooking and freezing on Pb level in egg albumen. The results showed significant difference ($P<0.05$) Pb level in commercial and desi eggs.

Target area	**Fresh samples**		**Post-cooking**		**Post- freezing**	
	Commercial eggs	desi eggs	Commercial eggs	desi eggs	commercial eggs	desi eggs
Sale point I	$3.41±0.02^a$	$2.88±0.08^b$	$3.21±0.01^a$	$2.75±0.04^b$	$3.24±0.03^a$	$2.79±0.05^b$
Sale point II	$3.47±0.01^a$	$2.65±0.05^b$	$3.31±0.06^a$	$2.58±0.01^b$	$3.35±0.03^a$	$2.61±0.08^b$
Sale point III	$3.14±0.07^a$	$3.10±0.07^b$	$3.09±0.07^a$	$2.77±0.05^b$	$3.10±0.07^a$	$2.76±0.02^b$
Over all	$3.34±0.03^a$	$2.87±0.06^b$	$3.20±0.04^a$	$2.70±0.03^b$	$3.23±0.04^a$	$2.72±0.05^b$

Table 4: Pre and post treatment level of iron (ppm) (Mean± SE) in egg albumin from commercial and desi layers. Mean within rows with different superscripts are significantly different (α=0.05)

	Fresh samples		**Post-cooking**		**Post- freezing**	
Target area	commercial eggs	desi eggs	commercial eggs	desi eggs	commercial eggs	desi eggs
Sale point I	$0.23±0.03^a$	$0.12±0.00^b$	$0.19±0.01^a$	$0.10±0.00^b$	$0.21±0.00^a$	$0.12±0.00^b$
Sale point II	$0.25±.03^a$	$0.11±0.00^b$	$0.21±.03^a$	$0.09±0.00^b$	$0.23±.03^a$	$0.10±0.00^b$
Sale point III	$0.22±0.02^a$	$0.21±0.01^b$	$0.18±0.01^a$	$0.17±0.01^b$	$0.19±0.01^a$	$0.21±0.01^b$
Over all	$0.23±0.02^a$	$0.14±0.00^b$	$0.19±0.01^a$	$0.12±0.00^b$	$0.21±0.01^a$	$0.14±0.00^b$

Table 5: Pre and post treatment level of copper (ppm) (Mean± SE) in egg albumin from commercial and desi layers. Mean within rows with different superscripts are significantly different (α=0.05)

Target area	**Fresh samples**		**Post-cooking**		**Post- freezing**	
	commercial eggs	desi eggs	commercial eggs	desi eggs	commercial eggs	desi eggs
Sale point I	$0.14±0.04^a$	$0.13±0.00^b$	$0.11±0.03^a$	$0.10 ±0.00^b$	$0.13±0.01^a$	$0.11±0.00^b$
Sale point II	$0.16±0.03^a$	$0.11 ±0.00^b$	$0.13±0.01^a$	$0.09 ±0.00^b$	$0.14±0.02^a$	$0.10± 0.00^b$
Sale point III	$0.18±0.01^a$	$0.11 ±0.00^b$	$0.14±0.02^a$	$0.08 ±0.00^b$	$0.16±0.01^a$	$0.11± 0.00^b$
Overall	$0.16±0.02^a$	$0.12±0.00^b$	0.13±0.02	$0.09±0.00^b$	0.15±0.01	$0.11±0.00^b$

Table 6: Pre and post treatment level of lead (ppm) (Mean± SE) in egg albumin of commercial and desi layers. Mean within rows with different superscripts are significantly different (α=0.05)

Target area	**Fresh samples**		**post-cooking**		**post – freezing**	
	commercial eggs	desi eggs	commercial eggs	desi eggs	commercial eggs	desi eggs
Sale point I	$3.0±0.00^a$	0.00^b	$3.0±0.00^a$	0.00^b	$3.0±0.00^a$	0.00^b
Sale point II	$2.0±0.00^a$	0.00^b	$2.0±0.00^a$	0.00^b	$2.0±0.00^a$	0.00^b
Sale point III	$3.0±0.00^a$	0.00^b	$3.0±0.00^a$	0.00^b	$3.0±0.00^a$	0.00^b
Over all	$2.77±0.00^a$	0.00^b	$2.77±0.00^a$	0.00^b	$2.77±0.00^a$	0.00^b

Table 7: Pre and post treatment of aflatoxin B1 (ppb)(Means± SE)in the yolk+albumin of commercial and desi layers. Mean with in rows having different superscripts are significantly different (α=0.05).

3.3. Level of aflatoxin B1 in commercial and desi egg yolk+albumin

The results shows presence of aflatoxin B1 (AFB1) (Means± SE ppb) in the egg contents from both commercial and desi, eggs sampled from different location of District Peshawar are present in (table 7). The mean concentration of AFB1before cooking of eggs was recorded as 2.77±0.01 ppb and after cooking the level of aflatoxin B1 2.77±0.01 ppb. The data revealed that pre or post treatment of egg contents has no significant effect on aflatoxin B1 level in the contents. The concentration of aflatoxin B1 in desi egg yolk and albumen was not detected. The significant difference ($P<0.05$) was observed in commercial and desi eggs.

4. Discussion

The commercial and desi poultry eggs are the major source of protein, vitamins and iron in human diet. The contents of the eggs play an important role in body development, growth, and tissue repairing. However on the other hand, the contamination of the eggs with toxic metals and mycotoxin cannot be ignored by presenting serious public health hazard (Fakayode and Olu-Owolabi, 2003). Heavy metals were entering from parents to offspring and from the hens to eggs through food chain. Furthermore, Heavy metals contaminate the environment, which causes a serious environmental pollution (Ayar et al., 2009).

4.1. The concentration of Fe, Cu and Pb in commercial and desi poultry egg yolk

Results of the present study indicated that commercial and desi egg yolk contained significantly higher level of Fe, Cu and Pb than egg albumin. The average concentration of Fe in commercial eggs yolk was reported as 6.27±0.09 ppm. Whereas the concentration of Fe in desi egg yolk was observed as 4.54±0.16 ppm. The present findings agreed with research of (Holt et al., 2011) who reported that commercial eggs consisted of higher concentration of the Fe (7.82±0.02 ppm) as that of desi egg. Fe is an essential element and important part of hemoglobin and myoglobin. The recommended daily allowance of iron was 10-15 mg/day (Chowdhury et al., 2011). (Shang and Wang, 1997) reported the Fe level in the egg yolk was ranged 2.7-20 ppm from four district of china. Whereas (Chowdhury et al., 2011) also reported the maximum Fe concentration in egg yolk (10.27±0.003). Cooking and freezing significantly no effect on the concentration of Fe in both types of eggs. The present study showed that there is significant difference ($P<0.05$) between commercial and desi eggs.

<u>4.1.1. Cu (copper)</u>

The average concentration of Cu in commercial eggs was studied (0.37±.05 ppm), whereas in desi eggs the Cu concentration (0.21±0.008) from three different sale points. After cooking of commercial egg and desi egg the level of Cu was observed as (0.31±0.02) and desi eggs (0.18±0.0 ppm). Cooking and freezing had significantly no effect on the level of copper in the commercial and desi eggs. The finding of the present study was comparable to (Abdulkhaliq et al., 2012). The recommended daily allowance of copper was 1.5-2.3 mg/day (Chowdhury et al., 2011). The maximum acceptable level of Cu reported as 0.11 ppm (Danks, 1988), copper has very important role in erythrocyte and hemoglobin production and the absorption of iron in blood. (Chowdhury et al., 2011) reported that Cu is essential element which produce a toxic effect when exceeds from normal concentration in egg yolk and responsible for the hyperactivity in autistic children. Reduced level of Cu in the body may cause different pathological conditions such as anemia, osteoporosis and depigmentation in animals while excess intake of Cu causes cirrhosis (Uauy et al., 1998).

<u>4.1.2. Pb (lead)</u>

The presence of Pb above permissible level may cause toxicity by influencing different enzymatic disturbance (Khurshid and Qureshi, 1984). The maximum permissible limit of Pb in foodstuffs is 1-5 ppm (Underwood, 1977). In the present study, the level of Pb in commercial and desi eggs was recorded 0.32±0.01 and 0.15±0.02 ppm, respectively, pretreatment. Post treatment and freezing have no significant effect on the level of Pb. The significant difference ($P<0.05$) was observed between the commercial and desi egg yolk. The present findings of our study are comparable to the finding of (Chowdhury et al., 2011). Who found that commercial egg yolk contained Fe, Cu and Pb as (10.27±0.003, 0.16±0.003 and 0.05±0.003ppm). The present findings agreed with results of (Holt et al., 2011) reported that commercial eggs consisted of higher concentration of the Cu (0.42±0.03 ppm) and Pb (0.35±0.01 ppm) than desi eggs. This could be due to mineral supplementation in diet and water for commercial poultry and less concentration of above elements in desi eggs attributed to feeding on natural feedstuffs without any mineral supplement in their diets (Leeson, 2008). The recommended daily allowance Pb was 0.3 mg per day for adults (Chowdhury et al., 2011). The higher level of heavy metals in commercial poultry eggs in the present study could be due to higher contamination of water and diets (Surai, 2002) that the hen should a lot of deposition of metals in the egg yolk. Surai, 2000 reported that egg prevents the deposition of toxic levels of the metals in order to protect the embryo.

4.2. The concentration of Fe, Cu and Pb in commercial and desi egg albumen

<u>4.2.1. Fe (Iron)</u>

Red blood cells contains iron and hemoglobin which carry oxygen from the lungs to the whole body and serves as a transport medium for electrons in the cell and tissue (Camara et al., 2005, Aisen and Listowsky, 1980). Results of the present study revealed that commercial egg albumen (Fe, Cu and Pb) contained significantly higher concentration whereas significantly lower concentration of these metals in desi egg albumin (Table 4, 5, and 6).The egg collected from three different sale points of District Peshawar were studied for heavy metals in commercial and desi egg albumen. The concentration of Fe (Mean± SE; ppm) in egg albumin from commercial before cooking was 3.34±0.03 while after cooking and freezing was 3.20±0.04 and3.23±0.04 ppm, respectively. The results showed no significant effect of cooking and freezing on the level of Fe in commercial egg albumin. However, the average concentration of Fe desi egg was observed as 3.20±0.04before cooking but after cooking and freezing the level was 2.70±0.04 and 2.72±0.05 ppm, respectively. The results showed significantly different ($P<0.05$) in Fe level in commercial and desi egg albumen from three sale points in District Peshawar. The finding of our results is comparable to the results of (Camara et al., 2005) who also reported the higher level of Fe in commercial egg than desi egg. The results correlate with (Chowdhury et al., 2011), who also found the concentration of Fe in commercial egg white to be 4.48±008 and local egg white 2.96±0005. In contrast to our study the highest concentration of iron was detected in the egg yolk (27.27±003) and egg white (9.48±005) (Chowdhury et al., 2011). The Fe is the most fundamental nutrient for the living organism and part of hemoglobin binding and carrying capacity of oxygen to whole body. In excess iron can be detrimental to health (Peuranen et al., 1994, Dalzell and Macfarlane, 1999). On the base of body weight, WHO / FAO (1999) set the permissible limit of heavy metal. The tolerable level of iron is 48 mg per day in an adult person.

4.2.2. Cu (copper)

The findings of the present study revealed the copper concentration in commercial and desi egg albumin was significantly different ($P<0.05$) in all three sale points, respectively. The overall concentration of Cu in commercial egg albumin before cooking (Mean± SE ppm) was observed0.23±0.02. Cooking and freezing did not change Cu level. While in desi egg albumen the concentration of Cu was recorded as 0.14±0.00 ppm. Heating and freezing have no significant effect on the level of Cu in desi egg albumin. The significant difference ($P<0.05$) was observed between commercial and desi egg albumen. The finding of the present study was correlated with (Uluozlu et al., 2009, Uauy et al., 1998, Rooke et al., 2010) who reported that level of Cu in albumen of commercial egg was greater than that of the desi. (Shang and Wang, 1997) found the Cu concentration in commercial egg 0.1-1.5 ppm and desi egg 0.2-1 ppm. The highest concentration of Cu (0.6-6.09) was reported by (Uluozlu et al., 2009), which was different to our research work. The Cu is co-factor for different type of proteins and plays an essential role in cellular respiration (Lanno et al., 1985). However, increase level of copper is toxic by producing free radicals in the different tissues of body. The copper toxicity may affect the gut and digestive enzymes, which reduce the gut motility (Woodward et al., 1995).

4.2.3. Pb (lead)

The Mean± SE concentrations (ppm) of Pb in commercial egg albumen at different sale points are given in table 6. The overall concentration of Pb was recorded as 0.16±0.02ppm, before cooking of commercial egg albumen. After proper cooking the level of Pb was 0.13±0.02 ppm. Cooking and freezing have no significant effect on Pb level egg albumen. The overall concentration of Pb (Mean± SE ppm) in desi egg albumin was 0.12±0.00 ppm as shown in table 6. The results showed that there was no significant effect of cooking and freezing on Pb level in egg albumen. The results showed significant difference ($P<0.05$) Pb level in commercial and desi eggs. Cooking and freezing have no significant effect on the concentration of Pb in the egg. The present study resemble with the study of (Vincevica-Gaile et al., 2013) who reported that eggs of commercial poultry contained Pb higher than local eggs. According to (Abdulkhaliq et al., 2012) the Pb level in egg albumen was 0.59 ppm. In contrast to our study, the average Pb concentration was 0.15 ppm. The Pb is toxic metal when increases from permissible limits may lead to mental development in children and in adults it cause high blood pressure and heart disease (Demayo et al., 1982). The present results on heavy metals suggest that the concentration of the heavy metals in the commercial and desi eggs are in the range of human necessities based on their recommended daily allowance. Concerned authority should take necessary steps for reducing the toxic metal contamination into the food chain.

4.3. Level of aflatoxin B1in the commercial and desi egg yolk

The present study revealed that the average concentration of AFB1 in commercial egg yolk+albumen 2.77±0.01 ppb and was not detected in the desi poultry egg yolk from three different locations in District Peshawar. Cooking and freezing have no effect on level of aflatoxin B1 for complete destroying of aflatoxin B1 need high temperature as (Luter et al., 1982) reported that 150°C or higher temperature require to reduce aflatoxin B1 up to 95%. The current study resembles with the study of (Pandey and Chauhan, 2007) who reported that the level of aflatoxin B1in commercial eggs was ranged from 3.7-4.0 ppb, while trace amount of aflatoxin was detected in desi eggs. (Jacobson and Wiseman, 1974) reported average amounts of 22 and 36 ppb aflatoxin 'B1 in the albumen and yolk of poultry eggs. The reason for the presence of aflatoxin B1 in commercial egg yolk could be due to feeding of aflatoxin B1 contaminated diet to commercial layer (Tchana et al., 2010). In the poultry diet cereal grains are the most important ingredients which are frequently contaminated with mycotoxin (Gowda et al., 2008). According to (Del Bianchi et al., 2005) aflatoxin B1 deposited in the reproductive organs and then transferred to egg yolk and albumin. However, (Herzallah, 2013) found the levels of aflatoxin B1 residues 0.03 ppb in commercial egg. The maximum acceptable concentration, which was approved by (European Committee Regulations, 2001) in cereals, peanuts and dried fruits for human consumption, is 4ng/g of the total aflatoxin and for aflatoxin B1 2ng/g alone (Ricci et al., 2007). Fungal species such as *Aspergillus flavus* produce aflatoxin B1 by affecting foodstuffs and cause immune suppression and liver cancer in human and animals (Radu-Rusu et al., 2013, Loveland et al., 1988, Cardwell et al., 2005). Among the aflatoxins, aflatoxin B1 (AFB1) is the most potent and causes hydroxylated derivatives (Pandey and Chauhan, 2007, Shephard, 2003), which are a big threat to the health of humans and cause cancer and malnutrition. These derivatives also provide a favorable medium for the growth of viruses like hepatitis B and HIV (Sun et al., 1999, Kirk et al., 2006).

5. Conclusions

The level of Fe, Cu, and Pb in the commercial and desi eggs was in the range of WHO permissible limit. The aflatoxin B1 (AFB1) level in commercial eggs (2.77 ppb) was significantly different from desi eggs (0.00 ppb). The reason of aflatoxin B1 residues in commercial poultry, eggs was due to feeding contaminated diets. Which were collected from different location of District Peshawar-Pakistan.

6. Recommendations

The eggs should be collected in clean cardboards and stored in cool room, delivered to the market in sanitized baskets to prevent contamination. The dirty, broken and undercooked eggs should be avoided to use for human consumption. Proper cooking of egg contents reduces the salmonella & E.coli load. Freezing the eggs, contents just stop the activities of microbes. Proper cooking and freezing reduce the incidence of microbes in egg contents. The concentration of the heavy metals in the commercial and desi eggs are in the range of human necessities based on their recommended daily allowance and the people should not worry about them except in the case where their deficiency produces disorder or diseases. The concerned authority should take necessary steps for reducing the aflatoxin B1 contamination, microbes and heavy metals in water and food chain. All those poultry eggs, which exceed the maximum permissible limits, may be declared as unhygienic for human consumption.

7. Author's Contribution

Muhammad Ishaq, and Kamal Niaz, carried out the whole experiment, and laboratory work, Muhammad Mobashar designed the experiment and guided during the experiment. Muhammad Ishaq, and Kamal Niaz also helped in analysis of data, drafting, and revision of the manuscript. All authors interpreted the data, revised manuscript for important intellectual contents, and approved the final version.

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9. Conflict of interest

The authors declare no conflict of interest.

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