Qualitative Analysis And Assessment Of Heavy Metals In Some Poultry Feeds From Chattogram Division, Bangladesh.

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Abstract: The current study was carried out to determine the quality as well as the concentration of heavy metals in 12 poultry feed samples. Six branded of poultry feeds including starter and grower types were used in this experiment. The qualitative analysis of all samples was revealed that in both starter and grower samples, the protein was obtained in the range of (16.95 – 18.61)% & (16.67 – 18.97)% followed by (88.32 – 90.19)% & (85.00 – 91.32)% for dry matters, (6.55 – 8.09)% & (5.50 – 7.10)% for total ash, (5.50 – 8.09)% for total ash, (2.10 – 3.01)% & (2.03 – 3.32)% for fat, (60.41 – 62.68)% & (58.23 – 64.53)% for carbohydrates and (0.15 – 0.24)% & (0.20 – 0.26)% for acid insoluble ash. The concentration of heavy metals was obtained in various ranges for two types of samples. Starter type of samples were ranges as (<DL – 1.14) ppm, (0.88 – 150.32) ppm, (2.01 – 3.40) ppm, (67.87 – 141.11) ppm, <DL and (7.99 – 18.69) ppm for chromium (Cr), Lead (Pb), Nickel (Ni), Manganese (Mn), Cadmium (Cd) and Copper (Cu) respectively. On the other side, the concentration of heavy metals like Cr, Pb, Ni, Mn, Cd and Cu in grower samples were found in the range of (<DL – 1.08) ppm, (<DL – 67.31) ppm, (1.49 – 4.09) ppm, (86.30 – 156.30) ppm, (<DL – 0.83) ppm and (10.44 – 22.47) ppm respectively.

Key words: Qualitative, assessment, heavy metals, poultry feeds, Chattogram.

1. INTRODUCTION

Poultry is a very important sector which is produced more than 30% of poultry products especially meat over the world as second meat resource [1]. In Bangladesh, poultry industries fulfill about 30% of total meat demand and also play an important role to supply about 27% of animal protein [2]. Huge amount of poultry feed is consumed to produce poultry finish product such as chicken, egg etc. The main sources of the raw materials for poultry feed formulation is cotton seed, corn, barley, sunflower seeds, bone meal, oil seeds and wheat [3]. Poultry feed is considered as an excellent food for chicken, duck and other birds that is enriched with protein, energy, minerals, vitamins and nutrients. It is more essential to control the quality of this feed in production period. At present, some of the companies do not follow the perfect method to produce poultry feed. Moreover, they also use contaminated raw materials during feed manufacture. However, raw materials may be contaminated with toxic metals come from different sources like metal containing pesticides, herbicides, fertilizer, food processing industries, fuels from fossil, smoke emission from vehicles, mining industries, municipal wastes etc [4, 5]. Toxic metals are main culprits that occur various side effects in human body and are not biodegradable [6, 7, 8]. It is mention that some elements like Fe, Mn, Ni, Zn, Cu etc. are used in human diet as minerals when it is not exceeded to acceptable concentration [9]. But if the concentration of these metals is crossed the permissible limit then problem must be arisen in the environment and in also living organisms [10]. Heart disease, cancer, hypertension, kidney disease are occurred in human body due to adverse effect of toxic metals [11]. In addition, Arsenic, Lead, Cadmium, Nickel and Chromium are more responsible to the neurological and biochemical problem [12]. The opinion of the current study is the assessment of both quality and heavy metals in some poultry feed in Chattogram division of Banglad

1. MATERIALS AND METHODS

1.1 Sample Collection:

A total of 12 poultry feed samples inclusive starter and grower of 6 branded were collected from different areas of Chattogram division.

1.2 Qualitative analysis

This study is related to some factors such as moisture content, percentage of protein, dry matter content, total ash, acid insoluble ash and carbohydrate analyzed in accordance with AOAC standard method.

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2.3.1 Determination of protein: Protein was estimated using Kjeldahl method divided into three steps like digestion, distillation and titration. Weighed 2g of dried sample and kept into a Kjeldahl digestion flask. A catalyst known as digestion mixture was made by combination of mercury (II) oxide (0.04g), sodium sulfate (0.7g) and copper sulfate (0.06g). About 1.0g of catalyst and 10ml of concentrated sulfuric acid were contacted with the sample in the Kjeldahl digestion flask. The mixed materials were heated on the heating mantle until the clear solution was obtained in the Kjeldahl digestion flask. Then the solution was allowed to cool and added about 50ml of distilled water. The digested sample was transformed to alkaline solution with addition 50ml of 40% sodium hydroxide solution. The solution was distilled and produced ammonia gas collected in a beaker containing 100ml of 2% boric acid solution. Then added 2-3 drops of methyl red indicator with the solution and titrated

with standard sulfuric acid. The reactions were occurred

Protein + H_2SO_4 = $(NH_4)_2SO_4$ (aq) + CO_2 (g) + SO_2 (g) + H_2O (l)

$(NH_4)_2SO_4$ (aq) + 2NaOH = Na_2SO_4 (aq) + $2H_2O$ (l) + $2NH_3$ (g)

Where,

V = Volume of used sulfuric acid (as a titrant)
S = Strength of used Sulfuric acid
M = Molecular weight of nitrogen
W = Weight taken of sample.

2.3.2 Determination of dry matter content: In this purpose, it was necessary to measure moisture content in the samples initially. For this reason, moisture content in all the samples was measured directly by the moisture analyzer machine [Brand: RADWAG, Model: MAC 50, RADWAG Wagi Electronics, Made in Poland]. The following equation was applied to estimate dry matter content:

% Dry matter = 100 – % Moisture.

2.3.3 Determination of total ash: After washing, an evaporating dish was dried at (100 ± 2) °C in an oven for about 5 hrs. Accurately weighed certain amount of samples was transferred to evaporating dish and then the dish was placed in a muffle furnace for ignition at 550 °C temperature. The sample was kept in the mentioned temperature until it becomes whitish color. The ash was cooled in desiccators and taken the final weight.

$$\% \text{ Total Ash} = \frac{W_A}{W_S} \times 100$$

W_A = Weight of total ash and W_S = Weight taken of sample.

2.3.4 Determination of fat: Weighed accurately 20g of absolutely dried sample in a cellulose thimble. Then fat was extracted by n-hexane using Soxhlet apparatus.

$$\% \text{ Fat} = \frac{W_F}{W_S} \times 100$$

Where,
W_F = Weight of fat and W_S = Weight taken of sample.

during experiment as follows:

NH_3 (g) + H_3BO_3 = $NH4^+$ + $H_2BO_3^-$

$$\% \text{ Protein} = \frac{V \times S \times M}{W} \times 100 \times 6.25$$

ula was used to calculate carbohydrate in the poultry feed samples [13]:

% Carbohydrate = % Dry matter – [% Protein + % Crude Fat + % Total Ash]

2.3.6 Determination of acid insoluble ash: The total ash was contacted with 100 ml of 2N hydrochloric acid and heated for 5 minutes. Then the solution was cooled and filtered through ashless filter paper. The filter paper was washed with hot distilled water completely. After that the filter paper was transferred to a previously dried as well as weighed sintered crucible and heated at 600 °C for 6 hours. Then the crucible was cooled in a desiccators and weighed accurately [14].

$$\% \text{ Acid insoluble ash} = \frac{(W_A + W_C) - W_C}{W_S} \times 100$$

W_C = Weight of sintered crucible
W_S = Weight taken of sample

Heavy metals analysis: Estimation of heavy metals in selected poultry feed was done using Atomic Absorption Spectrometer (Thermo Scientific, iCE 3300). Weighed around 20g of all samples in an evaporating dish and contacted with 1ml of concentrated nitric acid. The samples were kept in muffle furnace and heated at (100 ± 2) °C for about 1 hour. Then the samples were ignited at 550°C for 4 hours. The samples were changed in whitish color and allowed to cool. After this, 10ml of concentrated hydrochloric acid (30%) was added to the samples and heated on a hot plate for about 10 minutes. Then the clear sample was cooled at ambient temperature and filtered in a 100ml of volumetric flask by using Whatman no 42 filter paper. Finally, added deionized water up to the mark [15]. Heavy metals viz. chromium, lead, nickel, manganese, cadmium and copper were quantified by running prepared poultry feed samples through AAS machine.

2. RESULT AND DISCUSSION

The proximate composition values of poultry feed including starter and grower samples were presented in Table-1 and Table-2 respectively. In starter feed samples, it was observed that low amount of moisture was recorded in P6 where as the sample P3 was given high amount of moisture. Samples P4 and P3 have consecutively shown the maximum as well as minimum protein values. However, the highest quantity of dry matter was found in P6 and the lowest one was found in P3. Total ash was measured in P2 and P6 as the lowest and the highest values respectively. The poor amount of fat was recorded in P3 and the high amount of fat was also recorded in sample P1. Carbohydrate was estimated as upper limit in P6 and as lower limit in P5. Low quantity of acid insoluble ash was found in P4 where as sample P6 was shown as high quantity. Among the starter feed samples, P3 was shown the highest quantity of moisture and protein. Reversely, the fat and dry matter was found in the same sample as the

lowest quantity. The poor amount of protein and acid insoluble ash was observed in P4. In addition, P6 was shown the high quantity of dry matter, total ash, carbohydrate and acid insoluble ash [Table-1 and Figure- 1, 2, 3, 4, 5 & 6]. The mean and standard deviation values of moisture, protein, dry matter, total ash, fat, carbohydrate and acid insoluble ash were calculated as 11.05 ± 0.65, 18.03 ± 0.64, 88.92 ± 0.65, 6.94 ± 0.58, 2.58 ± 0.35, 61.56 ± 1.03, 0.20 ± 0.03 respectively [Table-3]. On the other hand, in grower feed samples, maximum amount of moisture were obtained from P3 where P6 was also shown minimum amount. It was observed that both starter and grower feed of P6 sample were shown low moisture content [Table-1 & 2]. However, in grower feed, the lowest and highest values of protein were found in sample P4 and P1 respectively. Total ash was quantified in grower feed P1 as high value and also P4 was found as poor level. It was recorded that the lowest amount of fat was measured in P3 and the highest one also measured in P5. Nevertheless, carbohydrates content in grower sample P4 considered as high quantity where the grower sample P3 was found in low quantity. The maximum quantity of acid insoluble ash was recorded in P6 as well as the minimum quantity was also recorded in grower sample P4. It was noted that P1 was shown high amount of protein and total ash where P3 was contained low quantity of dry matter, fat and carbohydrate. Besides, three parameters such as protein, total ash and acid insoluble ash were obtained as poor amount in grower feed P4 but sample P6 was contained the highest value of dry matter and acid insoluble ash [Table-2 and Figure- 1, 2, 3, 4, 5, 6 & 7]. The mean and standard deviation of seven parameters such as moisture, protein, dry matter, total ash, fat, carbohydrate and acid insoluble ash in grower feed were consecutively calculated as 11.22 ± 2.16, 17.93 ± 0.76, 88.78 ± 2.17, 6.33 ± 0.68, 2.66 ± 0.52, 61.86 ± 2.38 and 0.23 ± 0.02 [Table-3].

The heavy metals viz. Cr, Pb, Ni, Mn, Cd and Cu were estimated in both starter and grower feeds [Table- 4 & 5]. The experiment was revealed that in starter feeds, the quantity of Cr was found in less than detection limit (<DL) for sample P6 where the high amount of Cr was measured in P2. According to European Union guideline (EU 2003) five samples like P1, P2, P3, P4 and P5 were exceeded the permissible limit (0.00 $mgkg^{-1}$), but National Research Council (NRC 2006) was suggested to consider the acceptable range of Cr as 0.3 $mgkg^{-1}$ [15]. In accordance with World Health Organization (WHO) two samples e. g. P1 and P3 were crossed extremely the permissible concentration of Pb. The highest concentration of Pb was found in P3 [Table-4]. The concentrations of Ni for all starter samples were obtained within standard level [16]. Further information was that according to Standard Organization of Nigeria the concentration of Mn for all starter samples were exceeded the acceptable range [16]. The maximum and minimum amounts of Mn were measured in starter samples P2 and P5 correspondingly. However, Cd was found in all starter samples as <DL where the five starter samples like P1, P2, P3, P4 and P6 were crossed the permissible limit of Cu [17]. The lowest concentration of Cu was found in P5 as well as the highest one was obtained in P1 [Table-4 and Figure- 8, 9, 10, 11, 12 & 13]. Conversely, the study was presented that two grower samples such as P3 and P5 were contained 0.63 $mgkg^{-1}$ and 1.08 $mgkg^{-1}$ of Cr respectively that were crossed permissible level. But another grower feeds were shown the concentration of Cr as <DL. Pb was detected as <DL in P6 and the high concentration of Pb was also detected in P3. According to World Health Organization (WHO) guidelines, three samples e. g. P1, P2 and P3 were exceeded permissible limit (5$mgkg^{-1}$) of Pb [Table-5]. Nickel was found in P6 as high concentration which was exceeded the standard level [16]. Besides, the poor amount of manganese was estimated in grower sample P5 and the high amount of manganese was measured in P3. It was noticed that the entire grower samples were crossed the acceptable range of Mn [16]. Cadmium was found in only two samples named P1 and P2, but according to WHO guidelines these samples did not cross standard limit (1.00 $mgkg^{-1}$). Moreover, the concentration of Cu in all grower samples were crossed the tolerable level. Low concentration of Cu was detected in P5 and high concentration was also detected in P3 [Table-5 and Figure- 8, 9, 10, 11, 12 & 13]. In addition, the mean values of heavy metals in starter were calculated as 0.85±0.32 for Cr followed by 51.88±88.25 for Pb, 2.68± 0.55 for Ni, 103.43±31.19 for Mn and 13.23±4.41 for Cu. However, for grower feed the mean value of Cr, Pb, Ni, Mn, Cd and Cu were obtained in 0.85±0.31, 17.84±27.90, 2.63±1.02, 129.63±25.67, 0.73±0.14 and 14.81± 5.30 respectively [Table-6]

TABLE 1: Qualitative analysis of poultry feeds (Starter)

Sample ID	Moisture (%)	Protein (%)	Dry Matter (%)	Total Ash (%)	Fat (%)	Carbohydrates (%)	Acid Insoluble Ash (%)
P 1	11.11	18.5	88.89	6.81	3.01	60.57	0.22
P 2	11.33	17.78	88.67	6.55	2.25	62.09	0.21
P 3	11.68	18.61	88.32	6.62	2.1	60.99	0.19
P 4	11.31	16.95	88.69	6.61	2.49	62.64	0.15
P 5	11.26	18.5	88.74	6.98	2.85	60.41	0.18
P 6	9.81	17.87	90.19	8.09	2.75	62.68	0.24

TABLE 2: Qualitative analysis of poultry feeds (Grower)

TABLE 4: Heavy metal concentration in poultry feeds (Starter)

Sample ID	Metal concentration ($mgkg^{-1}$)					
	Chromium	Lead	Ni	Mn	Cadmium	Copper
P 1	1	81.43	2.65	99.36	<DL	18.69
P 2	1.14	4.28	2.11	141.11	<DL	11.48
P 3	0.88	150.32	3.14	107.6	<DL	11.03
P 4	0.3	0.88	2.76	135.07	<DL	18.66
P 5	0.95	1.5	2.01	67.87	<DL	7.99
P 6	<DL	2.88	3.4	69.57	<DL	11.53
Acceptable Limit	0.3 $mgkg^{-1}$ (Act No. 21, NRC 2006) (0.00$mgkg^{-1}$ EU 2003)	5.0 $mgkg^{-1}$ (WHO/FAO/ EU) 1.0 $mgkg^{-1}$ (UK)	4.05 $mgkg^{-1}$ (Act No. 21, NRC 2006)	55-60 $mg\ kg^{-1}$ (SON 2003)	1.0 $mgkg^{-1}$ (WHO/ FAO/ EU)	10 $mgkg^{-1}$ (SON 2003)

*<DL – Less than Detection Limit

Sample ID	Moisture (%)	Protein (%)	Dry Matter (%)	Total Ash (%)	Fat (%)	Carbohydrates (%)	Acid Insoluble Ash (%)
P 1	11.47	18.97	88.53	7.1	2.65	59.81	0.24
P 2	11.87	17.69	88.13	5.89	2.05	62.5	0.25
P 3	15	18.18	85	6.56	2.03	58.23	0.21
P 4	10.3	16.67	89.7	5.5	3	64.53	0.2
P 5	10.01	18.3	89.99	5.85	3.32	62.52	0.25
P 6	8.68	17.76	91.32	7.08	2.89	63.59	0.26

TABLE 3: Mean value of qualitative parameters in poultry feeds

	Mean ± SD						
Sample Category	Moisture (%)	Protein (%)	Dry Matter (%)	Total Ash (%)	Fat (%)	Carbohydrate (%)	Acid Insoluble Ash (%)
Starter	11.05 ± 0.65	18.03 ± 0.64	88.92 ± 0.65	6.94 ± 0.58	2.58 ± 0.35	61.56 ± 1.03	0.20 ± 0.03
Grower	11.22 ± 2.16	17.93 ± 0.76	88.78 ± 2.17	6.33 ± 0.68	2.66 ±0.52	61.86 ± 2.38	0.23 ± 0.02

TABLE 6: Mean value of the concentration of heavy metals in poultry feeds.

	Mean ± SD					
Sample Category	Chromium	Lead	Ni	Mn	Cadmium	Copper
Starter	0.85±0.32	51.88±88.25	2.68± 0.55	103.43± 31.19	-	13.23±4.41
Grower	0.85±0.31	17.84±27.90	2.63±1.02	129.63± 25.67	0.73±0.14	14.81± 5.30

TABLEL 5: Heavy metal concentration in poultry feeds (Grower)

	Metal concentration ($mgkg^{-1}$)					
Sample ID	Chromium	Lead	Ni	Mn	Cadmium	Copper
P 1	<DL	8.36	2.39	137.78	0.63	19.29
P 2	<DL	9.81	2.06	148.28	0.83	12.23
P 3	0.63	67.31	3.66	156.3	<DL	22.47
P 4	<DL	0.68	1.49	113.6	<DL	13.98
P 5	1.08	3.06	2.11	86.3	<DL	10.44
P 6	<DL	<DL	4.09	135.53	<DL	10.48

*Acceptable limit of manganese: 30-40 $mgkg^{-1}$ for grower feed (SON 2003).

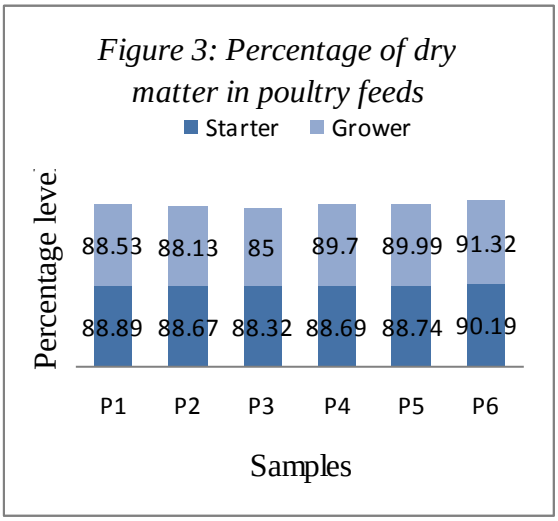


Figure 3: Percentage of dry matter in poultry feeds

Figure 1: Percentange of moisture in poultry feeds.

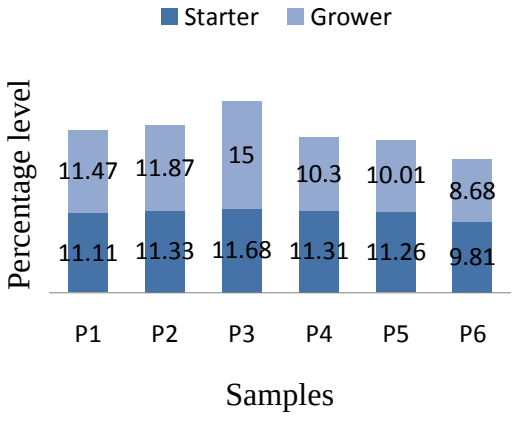


Figure 2: Percentage of protein in poultry feeds.

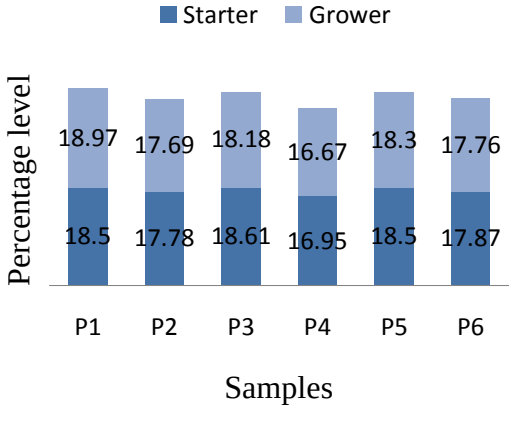


Figure 4: Percentage of total ash in poultry feeds

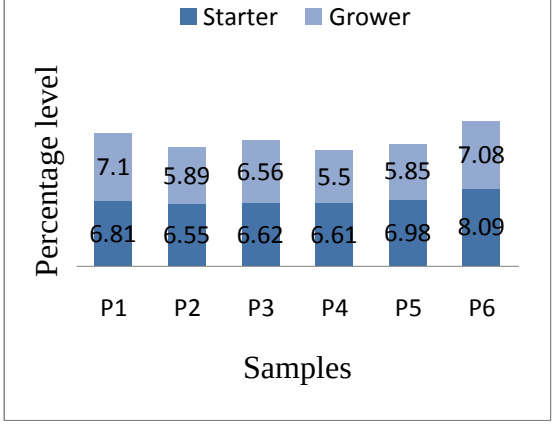


Figure 5: Percentage of fat in poultry feeds.

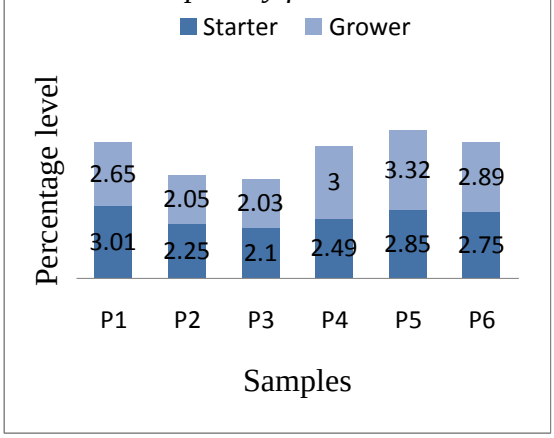


Figure 6: Percentage of carbohydrate in poultry feeds.

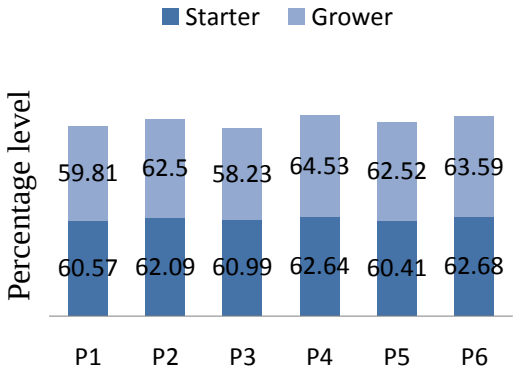


Figure 7: Percentage of acid insoluble ash in poultry feeds.

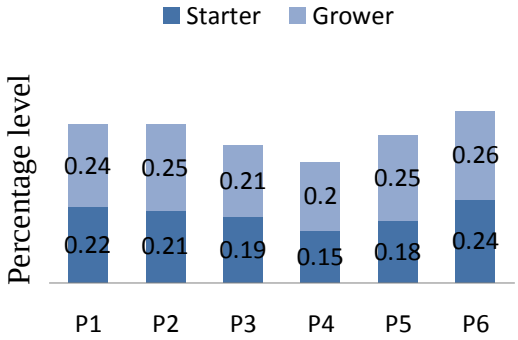


Figure 9: Concentrations of lead in poultry feeds.

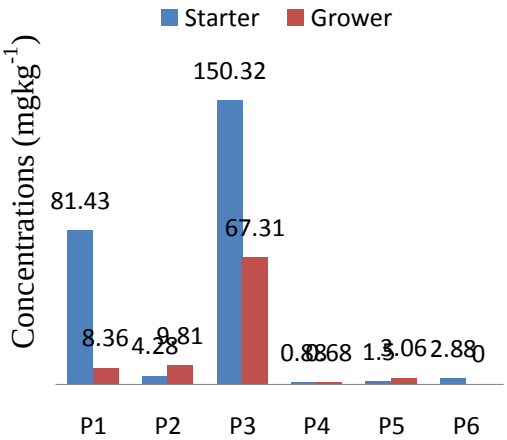


Figure 10: Concentrations of nickel in poultry feeds.

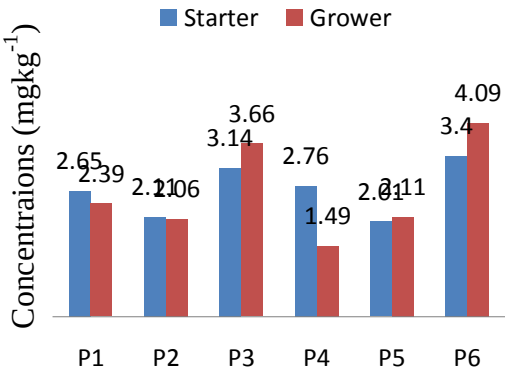


Figure 8: Concentrations of chromium in poultry feeds.

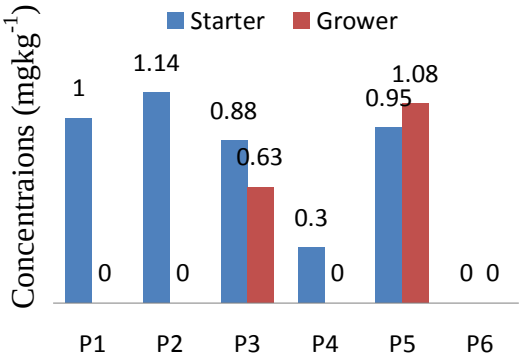


Figure 11: Concentrations of manganese in poultry feeds.

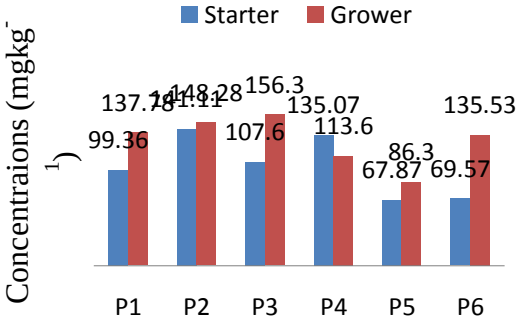


Figure 12: Concentrations of cadmium in poultry feeds.

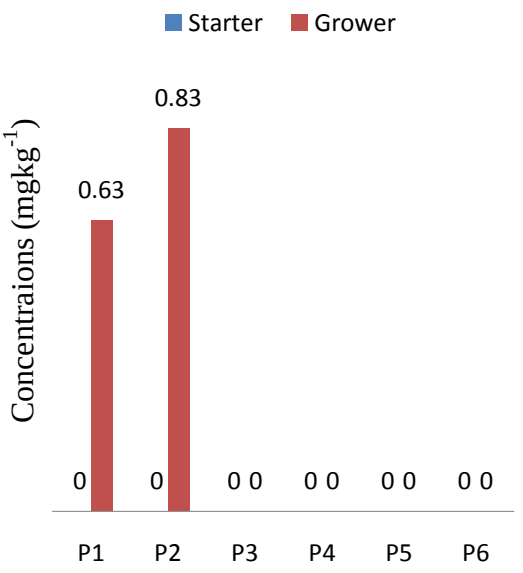
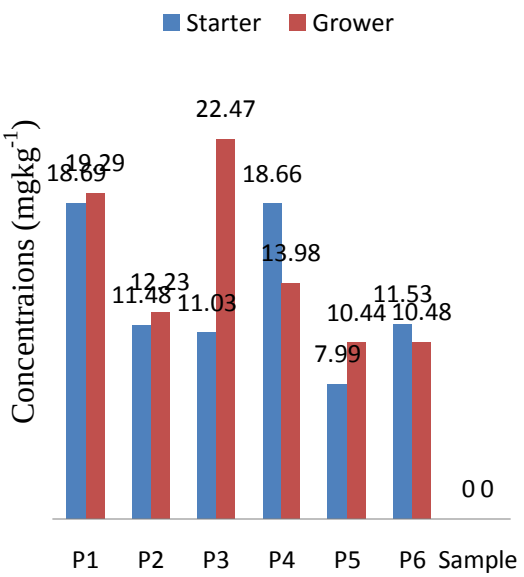


Figure 13: Concentrations of copper in poultry feeds.



3. CONCLUSION

The present experiment was presented that among the poultry feed samples the values of some qualitative parameters viz. moisture, protein, total ash, dry matter, fat, carbohydrate and acid insoluble ash were not significantly high. In all the samples, P6 for starter feed was contained maximum amount of dry matter, total ash, carbohydrate and acid insoluble ash. Furthermore, heavy metals were obtained in poultry feeds including starter and grower in various concentrations. Most of the samples were shown in high concentration. Lead was found as the highest concentration among the heavy metals. Moreover, in both starter and grower feed, maximum samples were crossed permissible limit of chromium, lead, manganese and copper. The demand of poultry products is increasing day by day in the world. It is more essential to control the quality of feed during manufacture. Besides, the industrialist should be avoid contaminated raw materials for manufacturing of

poultry feed. Finally, it is very necessary to make public awareness for preventing environmental pollution.

4. ACKNOWLEDGEMENT

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