Effect of ACES on Chemical Constituents of Feathers of Pigeon (Columba livia)

Gupal, K.K. Bhardwaj* and Nisha Rathor

Department of Chemistry, K. R. (P.G) College, Mathura, Uttar Pradesh, India.

*Corresponding Author: kkbhardwajkr@rediffmail.com

Abstract: **- The present study is carried out to analyze sodium, calcium, magnesium, phosphorus, iron, copper, zinc, manganese, by atomic absorption spectrometer (AAS) crude protein by Kjeldahl method and amino acids by reverse phase high performance liquid chromatography (RP-HPLC) from feathers of pigeon (Columba livia) before the administration of four ACES antioxidant vitamins A, C,E and selenium. There after these four ACES will be mixed with the pigeon feed and the pigeon will be kept on blended feed for a period of one month. The feathers of vitamin administered pigeon (Columba livia) were analyzed for the same mineral, protein and amino acid constituents.**

Key world: Pigeon's Feather, Mineral, Protein, ACES, RP-HPLC, AAS.

I. INTRODUCTION

The rock dove (*Columba livia*) [1] or rock pigeon is a member of the bird family Columbidae (doves and pigeons) [2]. The rock dove was first described by Gmelin in 1789 [3]. The genus name *Columba* is the Latinized form of the Ancient Greek word *kolumbos*, [4]. In common usage, this bird is often simply referred to as the "pigeon". Wild rock doves are pale grey with two black bars on each wing while domestic and feral pigeons are very variable in colour and pattern. There are few visible differences between males and females [5].

Pigeons (*C. livia*) are live in cities, squares and locations which contain abundant feed. They are considered extremely interesting bioindicators due to some biological and ecological features, such as: limited mobility throughout the year, higher metabolic and respiration rates than humans, as well as ingestion of seeds and feed exposed to the deposition of particles in the environment [6]. Pigeons change their feathers once a year before the winter season. This natural process takes place between April and June during which time the feathers are completely substituted and fully developed. This period is important to assess how long they have been exposed to the environment, in view of the fact that metals such as Cd and Pb are directly influenced by exposure time [7].

Feathers have been used for metal biomonitoring since the 1960s [8]. Blood and tissues such as brain, lung, liver, stomach, muscles, bones and even eggs and feathers may be used to evaluate metal levels in feathers [6,9-10]. Unwashed feathers adequately show metal concentrations especially when different collection sites are investigated (rural, urban, and industrial areas), because air particulate material is deposited into bird feathers during flight [11]. Washed feathers show metal concentrations absorbed by birds especially through respiration and feeding, which are positively correlated with metal levels found in other tissues, such as liver and muscles [12].

In the present paper, an extensive study on the mineral elements and amino acid compositions of pigeon (*C. livia*) feathers from various locations of Uttar Pradesh (India) has been reported. Twenty two local pigeon samples, collected at different times and from different places, were analyzed for 6 elements and amino acids using Perkin–Elmer A- Analyst 800 atomic absorption spectrometer by suitable hollow cathode lamps after the digestion. The objective of the present study was to provide a comprehensive account of the mineral elements and amino acid values in pigeon (*C. livia*) feathers and find out their pattern of occurrence in pigeon (*C. livia*) feathers.

II. MATERIAL AND METHOD

In the present paper feathers of rock dove (Columba livia) were taken and analyzed for sodium, calcium, magnesium, phosphorus, iron, copper, zinc, manganese, protein and amino acids before the administration of four ACES antioxidant vitamins A, C,E and selenium. There after these four ACES will be mixed with the pigeon feed and the pigeon will be kept on blended feed for a period of one month. The blended feed contains vitamin and mixture provides the following (per kg of diet): 25,000 IU of vitamin A; 100 mg of vitamin E; 6 mg of vitamin C; Se was 0.3 ppm in each diet, provided as sodium selenite, organic Se [1]. The feathers of vitamin administered pigeon (Columba livia) were analyzed for the same mineral, protein and amino acid constituents.

A. Reagents and samples:

All the solvents (Analytical Grade) were purchased from Rankem (India). HNO_3 and $HClO_4$ were also purchased from Rankem (India). Amino acids standard were purchased from Himedia (India). Samples of adult

pigeon feathers (C. livia) were collected from three different cities of west Uttar Pradesh in India and specimens were preserved.. Feathers of pigeon (*C. livia*), were thoroughly washed with water and dried in an air oven at 40 ^{o}C for 72 hrs for further use. For HPLC analysis, millipore water was used throughout the studies. The stock and standard solution were prepared in mobile phases.

Moisture content of pigeon feathers (C. livia) was determined according to an air-oven method. Ash content was determined by incinerating at 410-440 °C until the constant weight was achieved.

B. Instrumentation:

Mineral nutrients in feathers of pigeon (*C. livia*) were analyzed using a Perkin‑Elmer A Analyst 800 atomic absorption spectrometer by suitable hollow cathode lamp after the digestion of ash of leaves using HNO_3, H_2SO_4 and $HClO_4$ acid and diluting with double distilled water to a specific volume.

Amino acids were analyzed using reverse phase high performance liquid chromatography using waters HPLC system. The HPLC system consists of water 1525 binary HPLC pump and 717 plus auto sampler (waters®). The system was operated at ambient temperature. The chromatographic peaks of amino acids were identified and quantified by Breeze^{TM} software (Version 3.2). Amino acids were analyzed AccQ Tag^{TM} reverse phase (3.9×150 mm) 4 μ m analytical column equipped with 2475 multifluorescence detector (emission and excitation wavelength 395 and 250nm). Cystine and Methionine were analyzed from the same method of acid hydrolysis after treatment using performic acid oxidation.

C. Sample preparation for analysis of trace elements:

A 50.0 g of feathers of pigeon (*C. livia*) were crushed, grinded in a mortar. Dry ashing method
was adopted by placing the properly dried sample into the versatile crucible overnight in an electric muffle furnace maintaining the temperature between 400-440 °C. This ashing will destroy all the organic material from the sample. The ash was removed from crucible and dried in desiccators. The yield of ash was appox. 6.2 g/ 100g. One gm of ash was taken and digested using conc. HNO_3, H_2SO_4 and $HClO_4$ in the ratio of 10:6:3. Digested ash was stored in sterilized bottles and used for the determination of Na, Ca, Mg, Zn, Mg, Fe, Cu and P by flame atomic absorption spectroscopy. Phosphorus was analyzed with colorimeter using ammonium vanadate-molybdate method [14]. Three replicates were prepared for each sample.

D. Sample preparation for analysis of amino acids:

Total nitrogen and the protein content were determined based on the Kjeldahl method using the conversion factor of 6.25. All the above determination were based on the method of AOAC (1990) [15].
The sample was hydrolyzed in triplet using 6N HCl at 110 ^{o}C for 24 hrs and derivatized using AccQ reagent (6’ Aminoquinol-Nhydroxysuccinimdylcarbamite) [16]

III. RESULT AND DISCUSSION:

A. Minerals:

In the present research the trace minerals such as Na, Ca, Mg, Zn, Fe, Mn and Cu were
determined by using atomic absorption spectroscopy in mg/100g. The moisture and protein contain in feathers of pigeon (C. livia) was found 19% and 68 gm/100gm respectively.

Results in Table-1 and Figure 1 show the presence of variable amount of metals before the administration of four ACES antioxidant vitamins A, C, E and selenium in these samples. In general, the order of concentration of metals has been found as Ca > P > Na > Fe > Mg > Zn > Mn > Cu. The concentration of phosphorus was found in the range between 309 mg/100g to 322 mg/100g. The high phosphorus concentration was found at site-I (b) Agra, while site-III(b), Hathras show low phosphorus concentration. The balance of phosphorus and calcium is regulated by parathyroid hormone, which increases urinary excretion of phosphate under conditions of high phosphate and low calcium intake [17]. Recommended dietary allowances have been set at 460‑1250 mg of phosphorus per day for different age groups by the United States Institute of Medicine [18].Calcium uptake in pigeon (C. livia) was higher i.e 427 mg/100g at site-I(c), Agra while minimum 408 mg/100g at site-III(a), Hathras. It controls the membrane structure, membrane permeability and provides the stability to cell [19]. Calcium is essential for healthy bones, teeth and blood [20]. The health of the muscles and nerves depends on calcium. The recommended daily allowance of Ca for children is between 500mg and 1000 mg and for adults 800 mg [21]. The concentration of Manganese was found in the range between 5.6 mg/100g to 3.8 mg/100g. The high Manganese concentration was found at site-I(C)Agra, while site-III(C), Hathras show low Manganese concentration.

Sodium content in feathers of pigeon (C. livia) ranged from 106.4 mg/100g to 108.5 mg/100g. Maximum sodium content was found at site -I(b), Agra and the minimum was noted at site- III(b), Hathras. Sodium as an essential macroelement has physiological effect in human and
animal cellular and metabolic mechanism. The increased level of sodium content has direct link
to the high blood pressure [22] . The daily recommended range of Na in developing countries

is between 2400-5175 mg/day [23]. Magnesium maximum uptake was found at site-III(c), Agra about 28.6 mg/100g while lower uptake was found at site-III(C), Hathras about 26.3 mg/100gIron content in pigeon (C. livia) ranged from 93.2mg/100g to 95.4 mg/100g. Maximum iron content was found at site-I(b), Agra and the minimum was reported at site-III(b),Hathras.

Samples of pigeon (C. livia) collected from site-III(c), Agra contain comparatively higher amount of zinc (18.6 mg/100g),whereas site-II(b), Hathras show low concentration of zinc (17.1 mg/100g). Copper content in pigeon (C. livia) feathers ranged from 1.3 mg/100g to 1.1mg/100g. Maximum Copper content was found at site-I, Agra and the minimum was noted at site-C, Hathras.

Results in Table-2 and Figure 2 show the presence of variable amount of metals after the administered of four ACES antioxidant vitamins A, C, E and selenium in these samples. On comparison of table-1 and table-2, it is concluded that the concentration of sodium, calcium, magnesium, phosphorus, copper, zinc, manganese was decreased and concentration of iron increased on administration of four ACES antioxidant vitamins A, C,E and selenium.

B. Amino acids:

The investigated material was uniform, and has been collected from different sampling sites of west Uttar Pradesh, in order to estimate the effect of various factors on the chemical composition of the raw material. The protein content in feathers of pigeon (C. livia) before the administration of four ACES antioxidant vitamins A, C,E and selenium was 64.34 g/100gm and after the administration of four ACES antioxidant vitamins A, C,E and selenium was found 67.24 g/100gm. The present method determines the seventeen amino acids namely Leucine, Valine, Lysine, Threonine, Phenylalanine, Isoleucine, Methionine, Histidine, Alanine, Arginine, Aspartic acid, Cystine, Glutamic acid, Glycine, Proline, Serine and Tyrosine. Glutamine and asparagines was expressed as glutamic acid and aspartic acid respectively. In which first eight amino acids are essentials amino acids where as last nine were non essentials amino acids.Essential amino acids constituted before and after the administered of four ACES antioxidant vitamins A, C,E and selenium was 35.36% and 35.61% of total amino acids as reported in table-3, figure-3 and table-4, figure-4 respectively.

On analyses of obtained results it was found that the concentration of all amino acids before and after the administered of four ACES antioxidant vitamins A, C,E and selenium was found in the order Serine > Glutamic acid > Aspartic acid > Proline > Leucine > Glycine > Valine > Arginine > Alanine > Phenylalanine > Isoleucine > Threonine > Cystine > Tyrosine > Lysine > , Histidine >Methionine. The concentration of Serine, Proline, Leucine,Glycine, Valine, Alanine, Phenylalanine, Isoleucine, Threonine, Cystine, Tyrosine, Histidine and Methionine was increased on administered of four ACES antioxidant vitamins A, C,E and selenium while the concentration of aspartic acid, glutamic acid, arginine and lysine get decreased.

Serine was found highest average values before and after the administration of four ACES antioxidant vitamins A, C,E and selenium 9959.7 mg/100g and 10150.58 mg/100g followed by glutamic acid 9667.04 mg/100g and 9494.42, aspartic acid 9530 mg/100gm and 9339.17 mg/100g, proline 8678.60 mg/100g and 8915.04 mg/100g respectively.

Methionine, histidine and lysine were found lowest concentration among all the amino acids present in feathers of pigeon (C. livia). The average value methionine before and after the administration of four ACES antioxidant vitamins A, C,E and selenium was 1325.78 mg/100g and 1382.05 mg/100g, histidine 1374.07 mg/100g and 1448.79 mg/100g, lysine 2160.56 mg/100g and 2176.99 mg/100g.

IV. CONCLUSION

The results obtained in this study show that feather of pigeon (C. livia) are a good source of minerals and amino acids. The composition of minerals and amino acids was alter after the administered of four ACES antioxidant vitamins A, C,E and selenium, blended feed for a period of one month. The concentration of iron increased at all the sites, while the concentration of remaining minerals gets decreased on administered of four ACES. The concentration of amino acids viz. aspartic acid, glutamic acid, arginine and lysine decreased while the concentration of remaining amino acids gets increased on administered of four ACES.

REFERENCES

[1] “English name updates” - IOC Version 2.9, IOC World Bird List, 2010.
[2] Gibbs, David; Eustace Barnes, John Cox. *Pigeons and Doves: A Guide to the Pigeons and Doves of the World*. United Kingdom: Pica Press. p. 624. ISBN 1-873403-60-7.
[3] In J.F. Gmelin's edition of Linné's *Systema Naturae* appeared in Leipzig, 1788-93.
[4] Liddell, Henry George and Robert Scott *“A Greek-English Lexicon (Abridged Edition)”*. United Kingdom: Oxford University Press. ISBN 0-19-910207-4, 1980.
[5] "Rock Pigeon". *All About Birds*. Cornell Laboratory of Ornithology. Retrieved 2008-02-19.
[6] Nam, D.-H., & Lee, D.-P. “Monitoring for Pb and Cd pollution using feral pigeons in rural, urban and industrial environments of Korea”. *Science of the Total Environment,* (2006). *357*, 288–295.
[7] Bianchi, N., Ancora, S., Di Fazio, N., & Leonzio, C. “Cadmium, lead, and mercury levels in feathers of small passerine birds: Noninvasive sampling strategy”. *Environmental Toxicology and Chemistry* (2008) *27*(10), 2064–2070.

[8] Burger, J., & Gochfeld, M. "Lead and cadmium accumulation in eggs and fledgling seabirds in the New York bight". *Environmental Toxicology and Chemistry*, (1993) *12*, 261–267.
[9] Swaileh, K. M., & Sansur, R. "Monitoring urban heavy metal pollution using the House Sparrow (*Passer domesticus*)". *Journal of Environmental Monitoring*, (2006) *8*, 209–213.
[10] Almansour, M. I. "Using feathers as a biological indicator of lead environmental pollution". *Pakistan Journal of Biological Sciences*, (2004) *7*(11), 1884–1887.
[11] Nam, D.-H., Lee, D.-P., & Koo, T.-H. "Factors causing variations of lead and cadmium accumulation of feral pigeons (*Columba livia*)". *Environmental Monitoring and Assessment*, (2004) *95*, 13–22.
[12] Dauwe, T., Bervoets, L., Blust, R., & Eens, M. "Tissue levels of lead in experimentally exposed Zebra Finches (*Taeniopygia guttata*) with particular attention on the use of feathers as biomonitors". *Archives of Environmental Contamination and Toxicology*, (2002) *42*, 88–92.
[13] L. Peric, N. Milosevic D, Z. Kanacki, N. Dzinic, L. Nollet and P. Spring "Effect of selenium sources on performance and meat characteristics of broiler chickens" J. Appl. Poult. Res. (2009) 18 :403–409.
[14] Sharma D.K, Rai S, Arora S.S, Gupta P.M, Sharma R, Chopra A.K "Study of the trace elements in Aloe vera L. (Aloe barbandensis Miller) viz. Liliaceae and its biological and environmental Importance" J. Chem. Pharm. Res., (2011) 3:64-68.
[15] Association of Official Analytical Chemists. "Official Methods of Analysis", published by the A.O.A.C, Washington, D.C., USA, 14 th Ed 1990.
[16] Kawana S, Nakagawa K, Hasegawa Y. and Yamaguchi S. "Simple and rapid analytical method for detection of amino acids in blood using blood spot on filter paper, fast-GC/MS and isotope dilution technique". J. Chromatogr. B Analyt Technol. Biomed.Life Sci. (2010) 878: 3113-8.
[17] Sharma P.V., DravyaGuna Vijnana "Chaukhambha Bharti Academy,Varanasi, India", (2006) 1: 162-166.
[18] Chunekar K.C.Editor Bhavpraakash Nighantu of BhavaMisra. Chaukhambha Bharti Academy, Varanasi: 110 (2010).
[19] Goud V.K., Polasa K. and Krishnaswamy K "Effect of turmeric on xenobiotic metabolizing Enzymes" Plant Foods Hum Nutr. (1993) 44:87‑92.
[20] Cohly, H.H.P., et al. "Effect of antioxidant (turmeric, turmerin and curcumin) on humanimmunodeficiency virus" International Journal of Molecular Sciences. (2003) 4 :22-33.
[21] Irving, G.R.B., et al. "Curcumin: The potentialfor efficacy in gastrointestinal diseases. Best Practice and Research" Clinical Gastroenterology. (2011) 25 : 519-34.
[22] Ansari T.M., Ikram N., Najam-ul-haq M.,Fayyaz I., Ghafoor I. and Khalid N. "Essential trace metal (Zinc, Manganese, Copper andIron) levels in plants of medicinal importance." Journal of biological sciences. (2004) 4 : 95-99.
[23] Trinidad P. Trinidad, Rosario S. Sagum, Marco P. de Leon, Aida C. Mallillin, Melissa P. Borlagdan. "Zingiber Officinale and Curcuma Longa as Potential Functional Foods/Ingredients". Food and Public Health (2012) 2 : 1-4.

Table -1: Concentration of trace elements (mg/100g) in Pigeon (C. livia) Feather at different sampling sites before administered ACES.

Pigeon (C. livia) Feather				**Total Ash: 6.15 g/100g**					
	Site-I (Agra)			**Site-II (Mathura)**			**Site-III (Hathras)**		
Mineral	**A**	**B**	**C**	**a**	**b**	**c**	**a**	**b**	**c**
Na	**108.4**	**108.5**	**108.3**	**107.7**	**107.5**	**107.4**	**106.9**	**106.4**	**106.7**
Mg	**28.3**	**28.5**	**28.6**	**27.6**	**27.8**	**27.4**	**26.8**	**26.6**	**26.3**
Ca	**422**	**423**	**427**	**415**	**416**	**419**	**408**	**409**	**411**
Fe	**95.4**	**95**	**95.2**	**94.6**	**94.3**	**94.7**	**93.6**	**93.2**	**93.7**
Cu	**1.3**	**1.2**	**1.3**	**1.2**	**1.1**	**1.3**	**1.1**	**1.1**	**1.2**
Zn	**18.2**	**18.5**	**18.6**	**17.8**	**17.5**	**17.7**	**17.4**	**17.1**	**17.3**
Mn	**5.2**	**5.1**	**5.6**	**4.2**	**4.6**	**4.7**	**3.9**	**4.1**	**3.8**
P	**318**	**322**	**319**	**312**	**316**	**311**	**310**	**309**	**312**

Table -2: Concentration of trace elements (mg/100g) in Pigeon (C. livia) feather at different sampling sites after administered ACES.

Pigeon (C. livia) Feather							Total Ash: 6.12 g/100g		
	Site-I (Agra)			Site-II (Mathura)			Site-III (Hathras)		
Mineral	A	B	C	a	b	c	a	b	c
Na	107.3	107.4	107.6	106.5	106.2	106.7	105.8	106.2	105.6
Mg	26.2	26.3	26.3	25.3	25.6	25.1	24.5	24.7	24.9
Ca	412	411	414	409	410	407	406	405	403
Fe	98.3	98.6	98.5	96.3	97.6	97.2	95.4	95.3	95.6
Cu	1.2	1.2	1.3	1.2	1.1	1.2	1.1	0.98	0.99
Zn	16.1	16.1	16.3	15.3	15.7	15.4	15.2	15.1	15.6
Mn	4.8	4.7	4.8	4.1	4.3	4.2	3.9	4.1	4.1
P	308	309	306	302	306	305	299	298	301

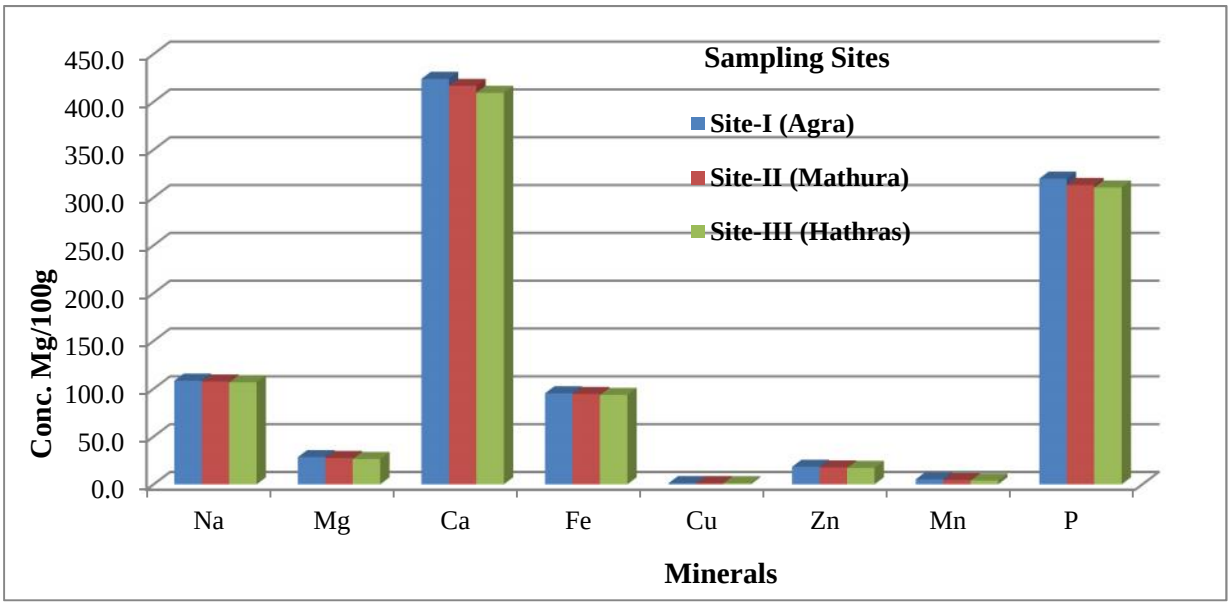

Figure -1: Concentration of trace elements (mg/100g) in Pigeon (C. livia) Feather at different sampling sites before administered ACES.

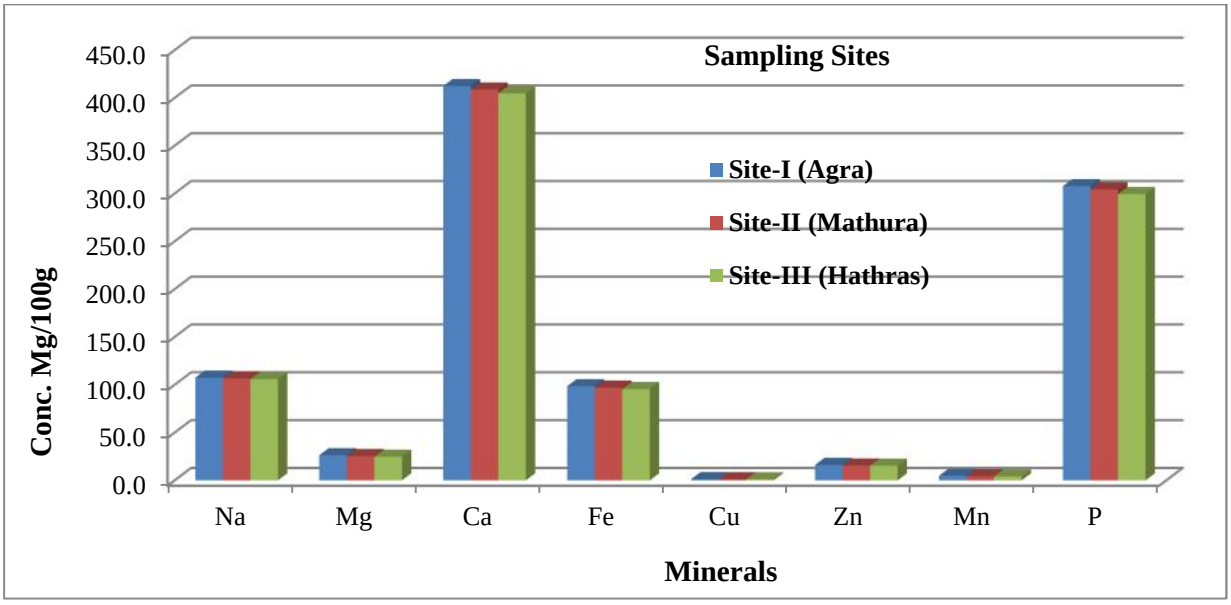

Figure -2: Concentration of trace elements (mg/100g) in Pigeon (C. livia) Feather at different sampling sites after administered ACES.

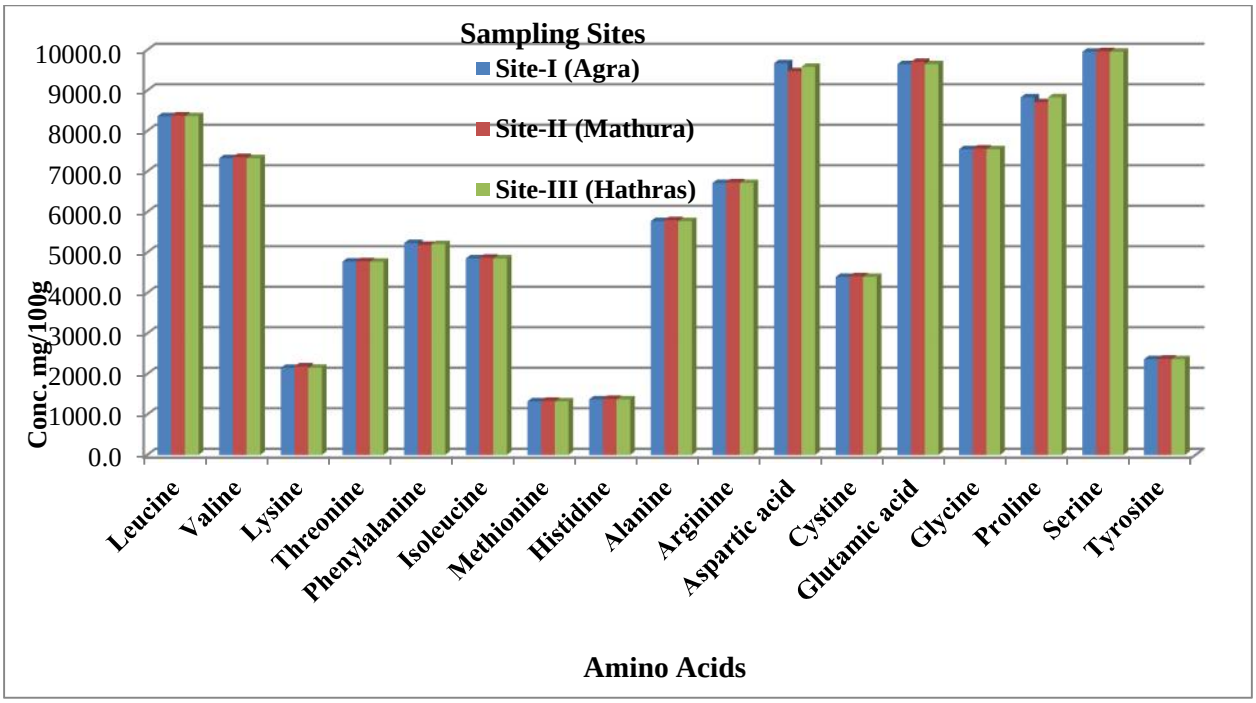

Figure -3: Concentration of amino acids (mg/100g) in Pigeon (C. livia) Feather at different sampling sites before administered ACES.

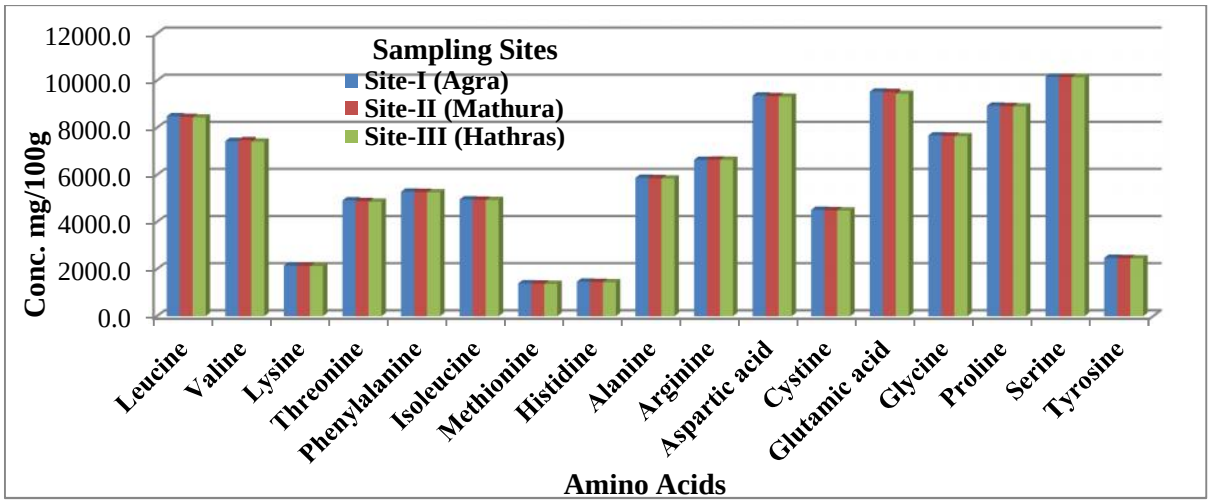

Figure -4: Concentration of trace amino acids (mg/100g) in Pigeon (C. livia) Feather at different sampling sites after administered ACES.

Essential Amino Acids	Site-I (Agra)			Site-II (Mathura)			Site-III (Hathras)		
	a	**b**	**C**	**a**	**b**	**c**	**a**	**b**	**c**
Leucine	8418	8420	8423	8382	8379	8383	8365	8362	8362
Valine	7389	7387	7386	7354	7352	7356	7324	7322	7326
Lysine	2208	2205	2207	2187	2182	2185	2153	2148	2146
Threonine	4822	4819	4824	4782	4779	4783	4763	4765	4781
Phenylalanine	5206	5202	5207	5187	5182	5183	5165	5162	5285
Isoleucine	4895	4892	4891	4872	4869	4867	4852	4848	4851
Methionine	1352	1349	1351	1338	1334	1333	1322	1319	1322
Histidine	1402	1404	1398	1384	1381	1379	1372	1368	1371
Non-essential Amino Acids									
Alanine	5820	5821	5224	5798	5795	5797	5776	5771	5773
Arginine	6758	6752	6751	6733	6728	6731	6714	6712	6711
Aspartic acid	9498	9496	9493	9475	9471	9472	9451	9448	9447
Cystine	4435	4432	4431	4411	4409	4406	4395	4397	4393
Glutamic acid	9698	9693	9694	9675	9672	9766	9651	9648	9647
Glycine	7588	7583	7582	7566	7563	7562	7549	7547	7546
Proline	8878	8874	8873	8845	8841	8439	8831	8832	8829
Serine	9989	9986	9981	9972	9969	9968	9958	9954	9951
Tyrosine	2398	2386	2383	2377	2375	2372	2362	2359	2364

Table -3: Concentration of amino acids (mg/100g) in Pigeon (C. livia) Feather at different sampling sites before administered ACES.

Essential Amino Acids	Site-I (Agra)			Site-II (Mathura)			Site-III (Hathras)		
	a	**b**	**c**	**a**	**b**	**c**	**a**	**b**	**c**
Leucine	8487	8482	8485	8441	8439	8442	8422	8423	8425
Valine	7435	7432	7431	7492	7489	7494	7376	7372	7373
Lysine	2145	2146	2143	2142	2143	2139	2112	2114	2113
Threonine	4915	4911	4913	4871	4869	4868	4843	4843	4841
Phenylalanine	5286	5282	5281	5262	5265	5263	5242	5245	5244
Isoleucine	4955	4951	4953	4933	4936	4935	4923	4921	4924
Methionine	1392	1394	1391	1379	1381	1379	1364	1361	1364
Histidine	1462	1458	1461	1441	1442	1439	1432	1435	1432
Non-essential Amino Acids									
Alanine	5872	5873	5872	5851	5854	5852	5828	5829	5827
Arginine	6634	6631	6633	6651	6649	6652	6632	6632	6631
Aspartic acid	9365	9362	9367	9324	9325	9321	9303	9304	9301
Cystine	4512	4511	4514	4489	4485	4483	4476	4474	4472
Glutamic acid	9534	9531	9529	9508	9507	9506	9398	9396	9397
Glycine	7675	7672	7674	7652	7653	7651	7625	7623	7621
Proline	8936	8931	8935	8904	8902	8901	8889	8891	8887
Serine	10163	10158	10161	10148	10146	10143	10135	10132	10131
Tyrosine	2476	2472	2473	2454	2452	5451	2439	2440	2438

Table -4: Concentration of amino acids (mg/100g) in Pigeon (C. livia) Feather at different sampling sites after administered ACES.