

Effect of ACES on Chemical Constituents of Feathers of Cock (*Gallus domesticus*)

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

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

I. INTRODUCTION

The cock (*Gallus domesticus*) is a domesticated fowl, a subspecies of the red Junglefowl. As one of the most common and widespread domestic animals with a population of more than 24 billion in 2003 [1]. Social groups typically consist of one male with upto four females and their chicks, though larger groups of upto 20 birds can form in more open environments. Other males are either solitary or form unisexual groups of two or three birds. Family groups have a well-defined home range and a regular roosting location. Cock originally referred to chicks, not the species itself. The species as a whole was then called domestic fowl or just fowl. In the UK and Ireland adult male cocks over the age of 12 months are primarily known as cocks, whereas in America, Australia and Canada they are more commonly called roosters. Males less than a year old are cockerels [2].

Feathers on cocks serve an important role in providing protection and insulation for the body. Too much feather loss makes it more likely that injuries will occur to the exposed flesh resulting in infection or bruising of the tissues. An excessive feather loss can result in higher energy utilization requirements to maintain the body temperature. Birds with excessive feather loss often require more feed to produce the energy necessary to compensate for the heat lost from the exposed areas. Good feather growth and maintenance requires adequate amounts of proteins, amino acids, vitamins and minerals in their diets. It is not unusual to trace a feathering problem in a backyard flock to inadequate feeding. A well balanced poultry feed formulated for appropriate age and type of bird will assure that the flock is receiving the necessary nutrients to maintain feather growth and maintenance.

Cock feathers have unique structure and properties not found in any natural or synthetic fibers. Although feathers as such cannot be processed as the protein fibers wool and silk due to the complex structure of the feathers. The secondary structures of feathers i.e. the barbs have the structure and properties that make them suitable for use as natural protein fibers. The low density, excellent compressibility and resiliency, ability to dampen sound, warmth retention and distinctive morphological structure of feather barbs make them unique fibers. For example, the density of cock feathers is about 0.8 g/cm³ compared to about 1.5 g/cm³ for cellulose fibers and about 1.3 g/cm³ for wool [3-4]. Proteins are polymers formed by various amino acids capable of promoting intra- and inter-molecular bonds, allowing the resultant materials to have a large variation in their functional properties. Feathers are bio-resource with high protein content. Poultry slaughterhouses produce large amounts of feathers. Further, burning feathers inspecial installations is economically ineffective. Uncontrolled disposal of feathers is environmentally unacceptable. Five percent of the body weight of poultry is feathers; the slaughterhouse with a capacity of 50,000 birds, can easily produce 2-3 tones of dry feathers per day. The keratin from feather shows an elevated content of the amino acids glycine, alanine, serine, cysteine and valine, but lower amounts of lysine, methionine and tryptophan.

Cock feathers contain approximately 91% protein (keratin), 1% lipids, and 8% water [5]. The amino acid sequence of a cock feather is very similar to that of other feathers and also has a great deal in common with reptilian keratins from claws [6]. The sequence is largely composed of cystine, glycine, proline, serine and contains almost no histidine, lysine, methionine [7]. Feathers are bio-resource with high protein content more than 750 g per kg crude protein. Five percent of the body weight of poultry is feathers. The keratin from feather shows an elevated content of the amino acids glycine, alanine, serine, cysteine and valine, but lower amounts of lysine, methionine and tryptophan. Reducing agents used in this research for reduction of disulfide bonds are thioglycolic acid, potassium cyanide, and sodium sulfide. The reductants act very quickly and without bringing about any chemical alteration or damage to the protein yield. Products prepared from the solutions behave as true proteins, and not as products of hydrolysis. Their solutions are precipitated by ordinary protein precipitants such as sulfosalicylic acid and ammonium sulfate.

A deficiency of certain amino acids (methionine, arginine), minerals (Na, P, Ca, Mg), protein and fibre is a factor known to influence incidence of feather pecking [8-9]. Increasing protein level in the diet had a positive effect on plumage and lowered FP and cannibalism. Low level of protein in diet increases risk of FP and cannibalism, because feathers serve as compensatory source of nutrients deficient in food [10]

II. MATERIAL AND METHOD

In the present study feathers of cock (*Gallus domesticus*) 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 cock feed and the cock 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 [11]. The feathers of vitamin administered cock (*Gallus domesticus*) 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). HNO₃ and HClO₄ were also purchased from Rankem (India). Amino acids standard were purchased from Himedia (India). Samples of adult cock (*Gallus domesticus*) feathers were collected from three different cities of west Uttar Pradesh in India and specimens were preserved. Feathers of cock (*Gallus domesticus*) were thoroughly washed with water and dried in an air oven at 40 °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 in cock (*Gallus domesticus*) feathers 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 cock (*Gallus domesticus*) 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 HNO₃, H₂SO₄ and HClO₄ acid and diluting with triple 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®). Amino acids were analyzed AccQ Tag™ reverse phase (3.9×150 mm) 4 μm analytical column equipped with 2475 multifluorescence detector (emission at 395 nm and excitation 250nm wavelengths). Cystine and Methionine were analyzed from the same method of acid hydrolysis after treatment using performic acid oxidation. The system was operated at ambient temperature. The chromatographic peaks of amino acids were identified and quantified by Breeze™ software (Version 3.2) [12].

C. Sample preparation for analysis of trace elements:

A 50.0 g of feathers of cock (*Gallus domesticus*) 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 approx. 6.21 g/ 100g. One gm of ash was taken and digested using conc. HNO₃, H₂SO₄ and HClO₄ in the ratio of 10:6:3. Digested ash was stored in sterilized bottles and used for the determination of Na, Ca, Mg, Zn, Mn, Fe and Cu by flame atomic absorption

spectroscopy. Phosphorus was analyzed with colorimeter using ammonium vanadate-molybdate method [13]. 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) [14]. The sample was hydrolyzed in triplet using 6N HCl at 110 °C for 24 hrs and derivatized using AccQ reagent (6-Aminoquinol-N-hydroxysuccinimide carbamate) [15].

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 content in feathers of cock (*Gallus domesticus*) was found 19% and 80.48 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 $Ca > P > Na > Mg > Fe > Zn > Cu > Mn$. The concentration of phosphorus was found in the range between 324 mg/100g to 331 mg/100g. The high phosphorus concentration was found at site-I (a) Agra, while site-III(c), 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 [16]. 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 [17]. Calcium uptake in cock (*Gallus domesticus*) was higher i.e 532.7 mg/100g at site-I(b), Agra while minimum 527.1 mg/100g at site-III(a), Hathras. It controls the membrane structure, membrane permeability and provides the stability to cell [18]. Calcium is essential for healthy bones, teeth and blood [19]. 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 [20]. The concentration of Manganese was found in the range between 0.72 mg/100g to 0.66 mg/100g. The high Manganese concentration was found at site-I(a & c)Agra, while site-III(c), Hathras show low Manganese concentration.

Samples of cock (*Gallus domesticus*) collected from site-III(a & b), Agra contain comparatively higher amount of zinc 8.4 mg/100g, whereas site-II(c), Hathras show low concentration of zinc 7.8 mg/100g. Copper content in cock (*Gallus domesticus*) feathers ranged from 1.11 mg/100g to 1.01 mg/100g. Maximum Copper content was found at site-I(b), Agra and the minimum was noted at site-III (a), Hathras.

Sodium content in feathers of cock (*Gallus domesticus*) ranged from 108.7 mg/100g to 105.6 mg/100g. Maximum sodium content was found at site -I(b), Agra and the minimum was noted at site- III(a), Hathras. Sodium as an essential macro-element 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 [21]. The daily recommended range of Na in developing countries is between 2400-5175 mg/day [22]. Magnesium maximum uptake was found at site-III(a & b), Agra about 28.3 mg/100g while lower uptake was found at site-III(C), Hathras about 26.9 mg/100g. Iron content in cock (*Gallus domesticus*) ranged from 21.7 mg/100g to 19.9 mg/100g. Maximum iron content was found at site-I(a & b), Agra and the minimum was reported at site-III(b),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.

Table -1: Concentration of trace elements (mg/100g) in cock (*Gallus domesticus*) feather at different sampling sites before administered ACES.

| Cock (<i>Gallus domesticus</i>) feather | | | | Total Ash: 6.21 g/100g | | | | | |
|---|---------------|-------|-------|------------------------|-------|-------|--------------------|-------|-------|
| Mineral | Site-I (Agra) | | | Site-II (Mathura) | | | Site-III (Hathras) | | |
| | A | B | C | A | b | c | a | b | c |
| Na | 108.2 | 108.7 | 108.4 | 106.8 | 106.3 | 106.5 | 105.6 | 105.9 | 105.7 |
| Mg | 28.3 | 28.3 | 28.1 | 27.4 | 27.2 | 27.3 | 27.2 | 27.1 | 26.9 |
| Ca | 532.5 | 532.7 | 531.8 | 528.3 | 528.6 | 528.7 | 527.1 | 527.4 | 527.2 |
| Fe | 21.7 | 21.7 | 21.6 | 20.6 | 20.4 | 20.7 | 20.2 | 19.9 | 20.1 |
| Cu | 1.1 | 1.11 | 1.1 | 1.02 | 1.02 | 1.1 | 1.01 | 1.06 | 1.08 |
| Zn | 8.4 | 8.4 | 8.3 | 8.1 | 8.2 | 8.1 | 7.9 | 7.9 | 7.8 |
| Mn | 0.72 | 0.71 | 0.72 | 0.69 | 0.68 | 0.67 | 0.68 | 0.67 | 0.66 |
| P | 331 | 329 | 330 | 328 | 327 | 329 | 327 | 326 | 324 |

Table -2: Concentration of trace elements (mg/100g) in cock (*Gallus domesticus*) feather at different sampling sites after administered ACES.

| Cock (<i>Gallus domesticus</i>) feather | | | | Total Ash: 6.15 g/100g | | | | | |
|---|---------------|-------|-------|------------------------|-------|-------|--------------------|-------|-------|
| Mineral | Site-I (Agra) | | | Site-II (Mathura) | | | Site-III (Hathras) | | |
| | A | B | C | A | b | c | a | b | c |
| Na | 107.3 | 107.6 | 107.5 | 105.5 | 105.2 | 105.4 | 104.6 | 104.7 | 104.5 |
| Mg | 26.2 | 26.1 | 26.2 | 25.3 | 25.2 | 25.1 | 25.1 | 25.1 | 24.9 |
| Ca | 507.3 | 507.4 | 506.8 | 505.7 | 505.4 | 505.3 | 504.6 | 504.7 | 504.5 |
| Fe | 23.3 | 23.6 | 23.4 | 21.8 | 21.4 | 21.9 | 21.2 | 20.9 | 21.1 |
| Cu | 1.04 | 1.06 | 1.03 | 1.00 | 1.00 | 1.04 | 0.98 | 0.99 | 0.99 |
| Zn | 8.2 | 8.1 | 8.00 | 7.97 | 7.98 | 7.97 | 7.67 | 7.67 | 7.63 |
| Mn | 0.69 | 0.69 | 0.68 | 0.68 | 0.67 | 0.67 | 0.66 | 0.66 | 0.65 |
| P | 328 | 327 | 327.5 | 325 | 524 | 525.4 | 324 | 324 | 323.9 |

Figure -1: Concentration of trace elements (mg/100g) in cock (*Gallus domesticus*) feather at different sampling sites before administered ACES.

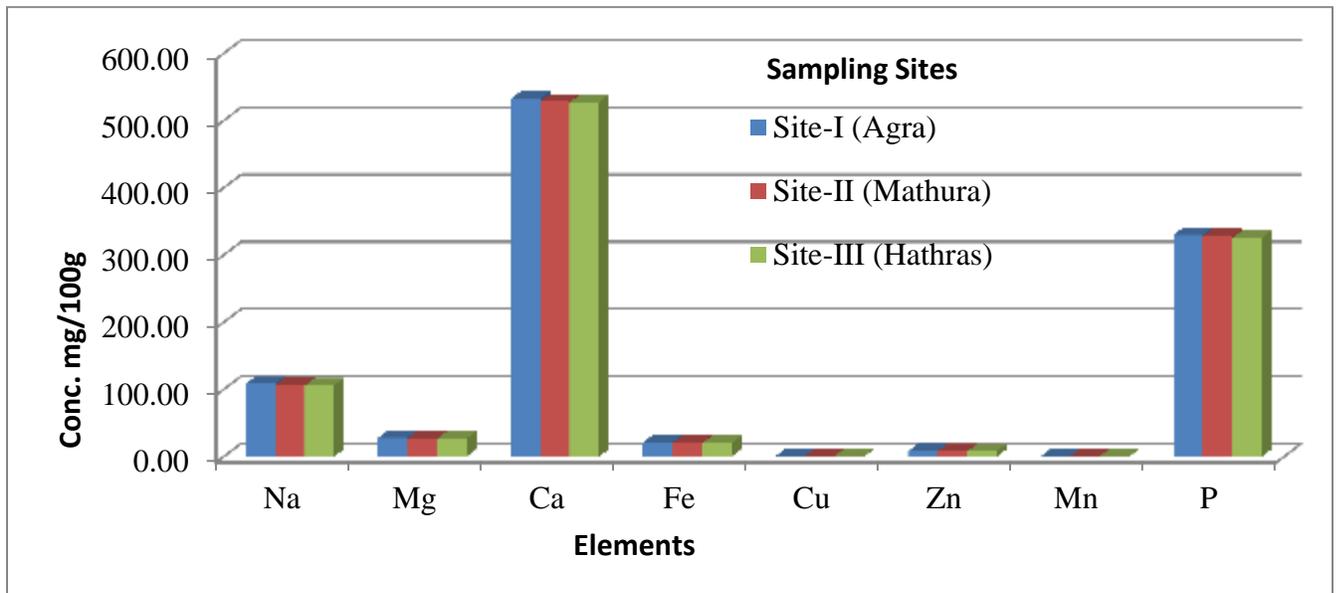
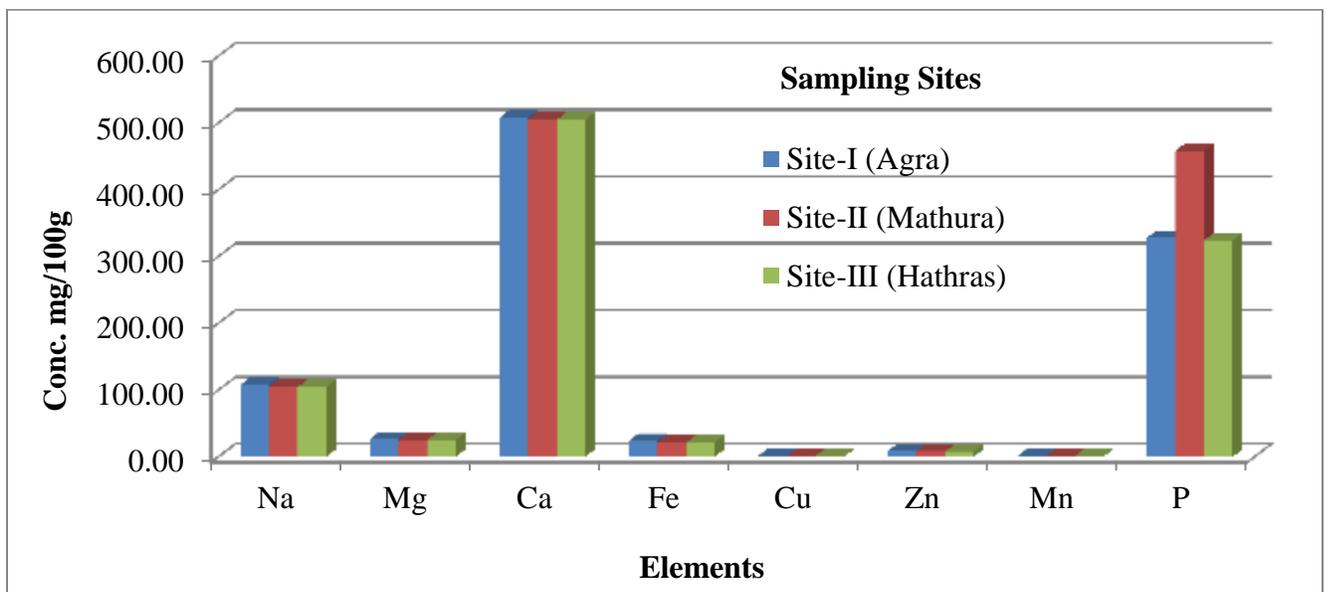


Figure -2: Concentration of trace elements (mg/100g) in cock (*Gallus domesticus*) feather at different sampling sites after administered ACES.



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 cock (*Gallus domesticus*) before the administration of four ACES antioxidant vitamins A, C, E and selenium was 80.48 g/100gm and after the administration of four ACES antioxidant vitamins A, C,E and selenium was found 82.24 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 35.97% and 36.12% 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 > Valine > Glycine > Arginine > Alanine > Phenylalanine > Isoleucine > Threonine > Cystine > Tyrosine > Lysine > Methionine > Histidine. The concentration of Leucine, Valine, Threonine, Phenylalanine, Isoleucine, Methionine, Histidine, Alanine, Cystine, Glycine, Proline, Serine and Tyrosine 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, 9914.33 mg/100g and 11020.22 mg/100g followed by glutamic acid 9667.04 mg/100g and 9880.00, aspartic acid 9714.78 mg/100gm and 9656.78 mg/100g, proline 9112.11 mg/100g and 9170.00 mg/100g respectively.

Lysine, methionine and histidine were found lowest concentration among all the amino acids present in feathers of cock (*Gallus domesticus*). The average value lysine before and after the administration of four ACES antioxidant vitamins A, C,E and selenium was 2608.56 mg/100g and 2574.22 mg/100g, Histidine 1566.89 mg/100g and 1604.67 mg/100g, methionine 1452.78 mg/100g and 1505.88 mg/100g respectively.

Table -3: Concentration of trace elements (mg/100g) in cock (*Gallus domesticus*) 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 | 9015 | 9012 | 9013 | 8975 | 8972 | 8974 | 8945 | 8941 | 8942 |
| Valine | 7885 | 7883 | 7881 | 7844 | 7843 | 7841 | 7824 | 7823 | 7823 |
| Lysine | 2624 | 2643 | 2642 | 2602 | 2601 | 2598 | 2591 | 2589 | 2587 |
| Threonine | 5121 | 5119 | 5117 | 5105 | 5102 | 5098 | 5095 | 5093 | 5092 |
| Phenylalanine | 5643 | 5641 | 5638 | 5632 | 5631 | 5629 | 5621 | 5621 | 5619 |
| Isoleucine | 5287 | 5284 | 5283 | 5275 | 5271 | 5273 | 5267 | 5265 | 5264 |
| Methionine | 1456 | 1453 | 1552 | 1443 | 1442 | 1441 | 1431 | 1429 | 1428 |
| Histidine | 1578 | 1576 | 1575 | 1568 | 1567 | 1567 | 1558 | 1557 | 1556 |
| Non-essential Amino Acids | | | | | | | | | |
| Alanine | 6315 | 6312 | 6314 | 6302 | 6298 | 6297 | 6294 | 6291 | 6293 |
| Arginine | 7234 | 7232 | 7231 | 7213 | 7212 | 7209 | 7205 | 7203 | 7201 |
| Aspartic acid | 9725 | 9723 | 9722 | 9716 | 9714 | 9713 | 9708 | 9707 | 9705 |
| Cystine | 4756 | 4753 | 4751 | 4749 | 4747 | 4746 | 4743 | 4743 | 4741 |
| Glutamic acid | 9923 | 9921 | 9918 | 9917 | 9916 | 9915 | 9908 | 9906 | 9905 |
| Glycine | 7842 | 7841 | 7838 | 7836 | 7832 | 7829 | 7827 | 7825 | 7826 |
| Proline | 9123 | 9121 | 9118 | 9113 | 9112 | 9109 | 9106 | 9104 | 9103 |
| Serine | 10987 | 10983 | 10981 | 10975 | 10974 | 10972 | 10964 | 10963 | 10964 |
| Tyrosine | 2658 | 2657 | 2654 | 2648 | 2647 | 2745 | 2641 | 2638 | 2637 |

Figure -3: Concentration of trace elements (mg/100g) in cock (*Gallus domesticus*) feather at different sampling sites after administered ACES.

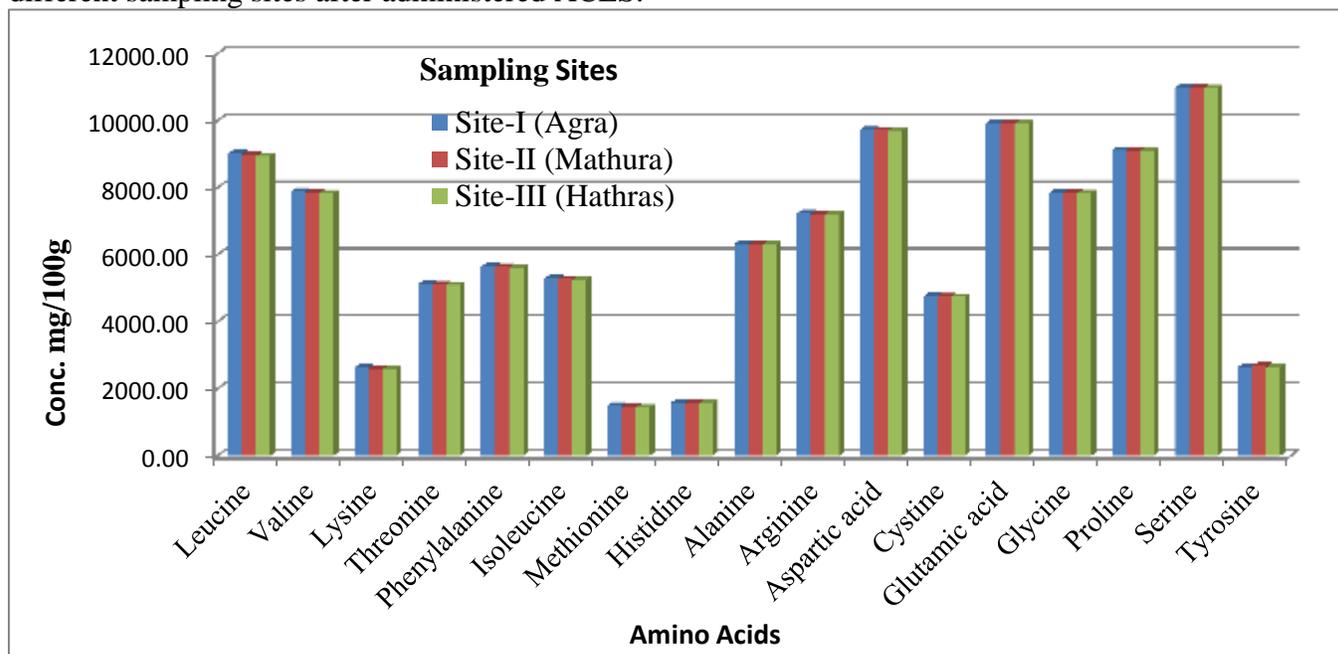
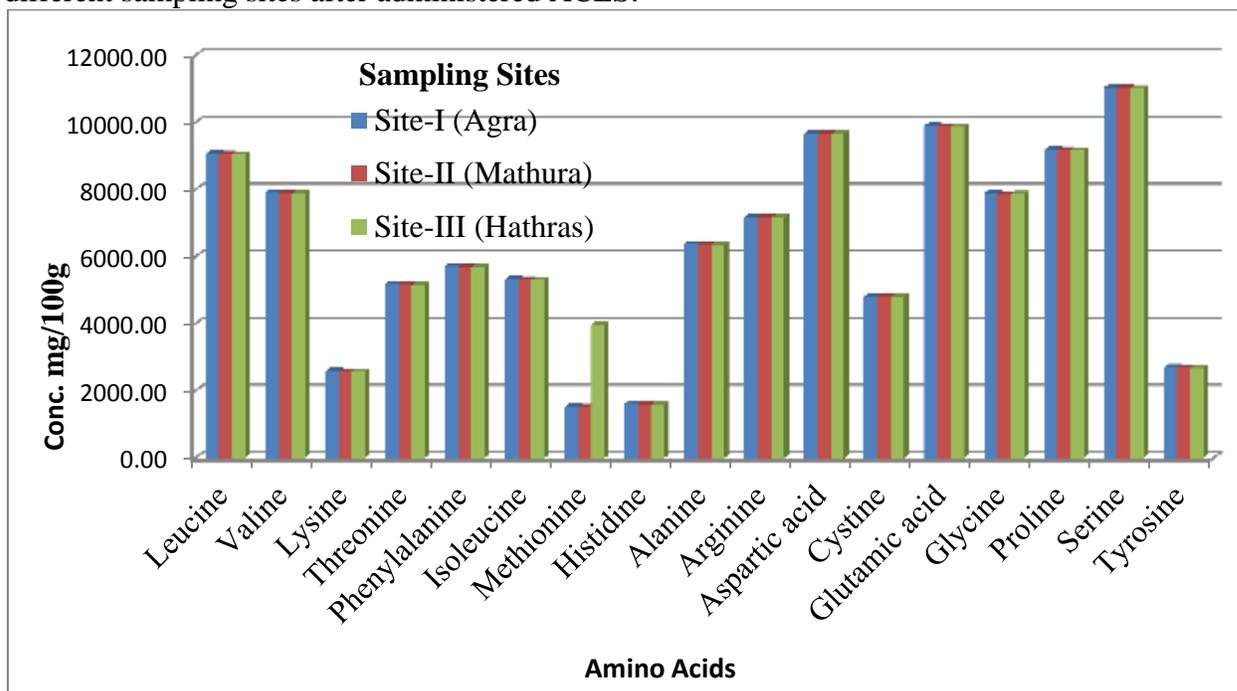


Table -4: Concentration of trace elements (mg/100g) in cock (*Gallus domesticus*) feather at different sampling sites after administered ACES.

| Essential Amino Acids | Site-I (Agra) | | | Site-II (Mathura) | | | Site-III (Hathras) | | |
|----------------------------------|---------------|-------|-------|-------------------|-------|-------|--------------------|-------|-------|
| | a | b | c | a | b | c | a | b | c |
| Leucine | 9065 | 9063 | 9064 | 9054 | 9051 | 9053 | 9043 | 9041 | 9042 |
| Valine | 7911 | 7909 | 7908 | 7906 | 7904 | 7903 | 7896 | 7893 | 7894 |
| Lysine | 2587 | 2585 | 2583 | 2578 | 2576 | 2554 | 2570 | 2568 | 2567 |
| Threonine | 5178 | 5177 | 5176 | 5168 | 5176 | 5175 | 5158 | 5156 | 5154 |
| Phenylalanine | 5712 | 5711 | 5709 | 5702 | 5698 | 5697 | 5694 | 5693 | 5691 |
| Isoleucine | 5316 | 5315 | 5314 | 5308 | 5306 | 5307 | 5302 | 5301 | 5298 |
| Methionine | 1514 | 1512 | 1509 | 1507 | 1506 | 1504 | 1502 | 1501 | 1498 |
| Histidine | 1613 | 1611 | 1609 | 1606 | 1605 | 1604 | 1601 | 1597 | 1596 |
| Non-essential Amino Acids | | | | | | | | | |
| Alanine | 6375 | 6372 | 6373 | 6364 | 6362 | 6361 | 6357 | 6354 | 6356 |
| Arginine | 7187 | 7185 | 7183 | 7178 | 7177 | 7175 | 7171 | 7169 | 7168 |
| Aspartic acid | 9665 | 9663 | 9661 | 9658 | 9657 | 9655 | 9652 | 9651 | 9649 |
| Cystine | 4817 | 4816 | 4814 | 4812 | 4819 | 4817 | 4807 | 4806 | 4804 |
| Glutamic acid | 9889 | 9897 | 9895 | 9878 | 9877 | 9875 | 9871 | 9869 | 9869 |
| Glycine | 7884 | 7883 | 7879 | 7873 | 7841 | 7839 | 7868 | 7867 | 7864 |
| Proline | 9179 | 9178 | 9176 | 9171 | 9169 | 9167 | 9165 | 9163 | 9162 |
| Serine | 11034 | 11031 | 11029 | 11022 | 11019 | 11018 | 11012 | 11009 | 11008 |
| Tyrosine | 2693 | 2691 | 2698 | 2686 | 2684 | 2683 | 2681 | 2678 | 2676 |

Figure -4: Concentration of trace elements (mg/100g) in cock (*Gallus domesticus*) feather at different sampling sites after administered ACES.



IV. CONCLUSION:

The results obtained in this study show that feather of cock (*Gallus domesticus*) are a good source of minerals and amino acids. The composition of minerals and amino acids was altered after the administration 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 administration 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 administration of four ACES.

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