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Effects 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.

Keywords: cock's feather, mineral, protein, ACES, RP-HPLC, AAS

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 up to four females and their chicks, though larger groups of up to 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].

A deficiency of certain amino acids (methionine, arginine), minerals (Na, P, Ca, Mg), protein and fiber 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].

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 0C 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 0C 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, H_2SO4 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 TagTM reverse phase (3.9×150 mm) 4 □m analytical column equipped with 2475 multi-fluorescence 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 TM 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 400-4400C. 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. 6.21g/ 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.

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 HCI at 110 0C for 24 hrs and derivatized using AccQ reagent (6 Aminoquinol-N- hydroxysuccinimdyl carbamite) [15].

Result and Discussion

Minerals

In the present research the trace minerals such as Na, Ca, Mg, Fe, Mn, and Cu were determined by using atomic absorption spectroscopy in mg/100g. The moisture and protein contain 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-1 (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-1(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 500 mg 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-1(a & c) Agra, 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-1(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 -1(b), Agra and the minimum was noted at site- I11(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-111(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-1(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 was increased after administration of four ACES antioxidant *viz*. 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 (Gallus domesticus) 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	528.7	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

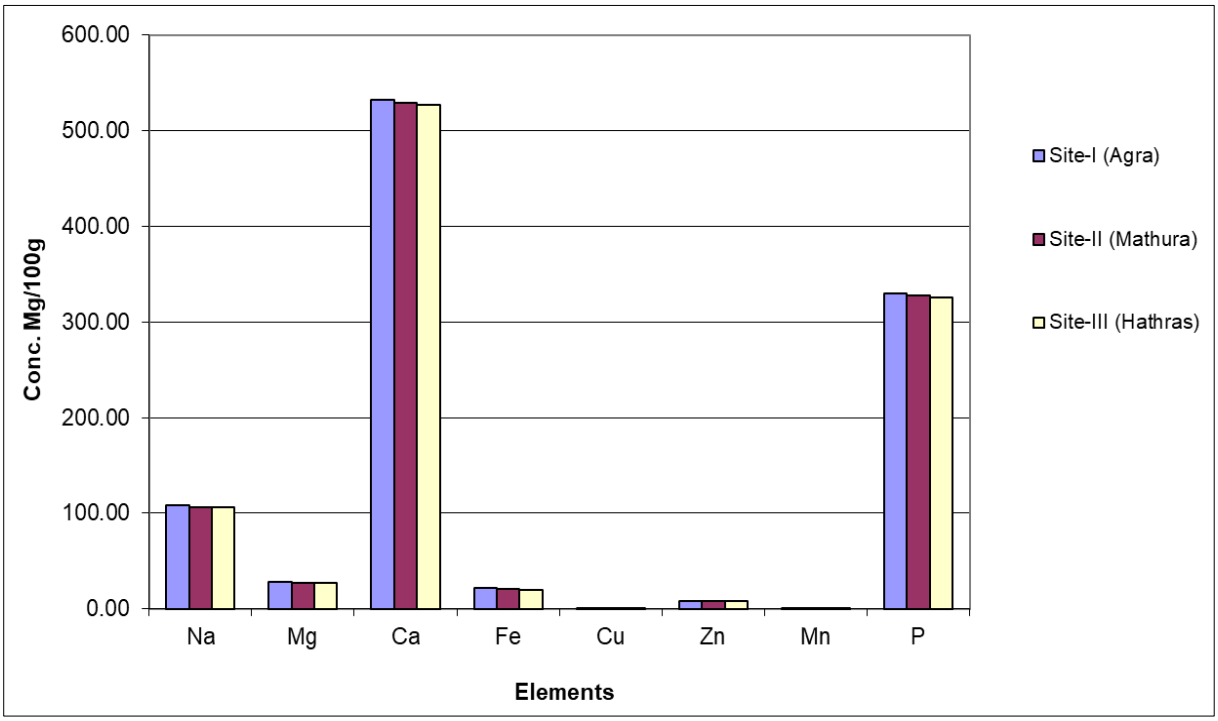

Fig 1: Concentration of trace elements (mg/100g) in cock (*Gallus domesticus*) feather at different sampling sites before administered ACES.

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

Cock (Gallus domesticus) 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	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

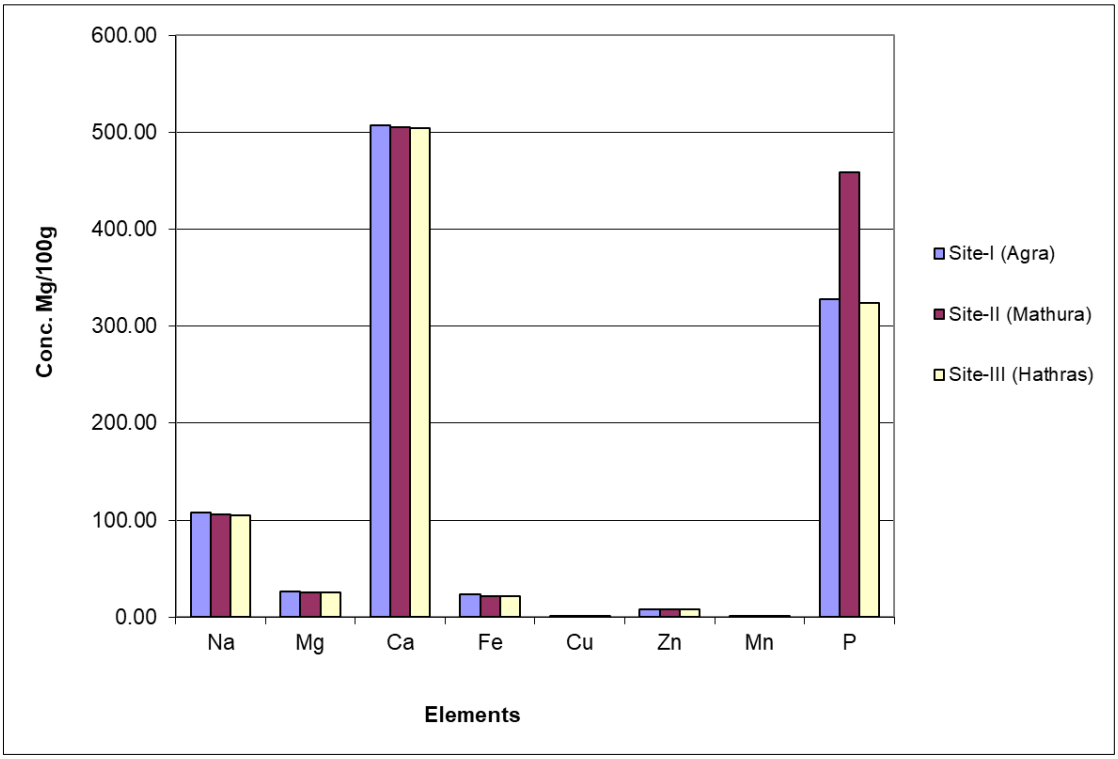

Fig 2: Concentration of trace elements (mg/100g) in cock (*Gallus domesticus*) feather at different sampling sites after administered ACES.

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 analysis 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 amino acids (mg/100g) in cock (*Gallus domesticus*) feather at different sampling sites before administered ACES.

Essential Amino Acid	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	8972
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	1452	1443	1442	1441	1431	1429	1428
Histindine	1578	1576	1575	1567	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

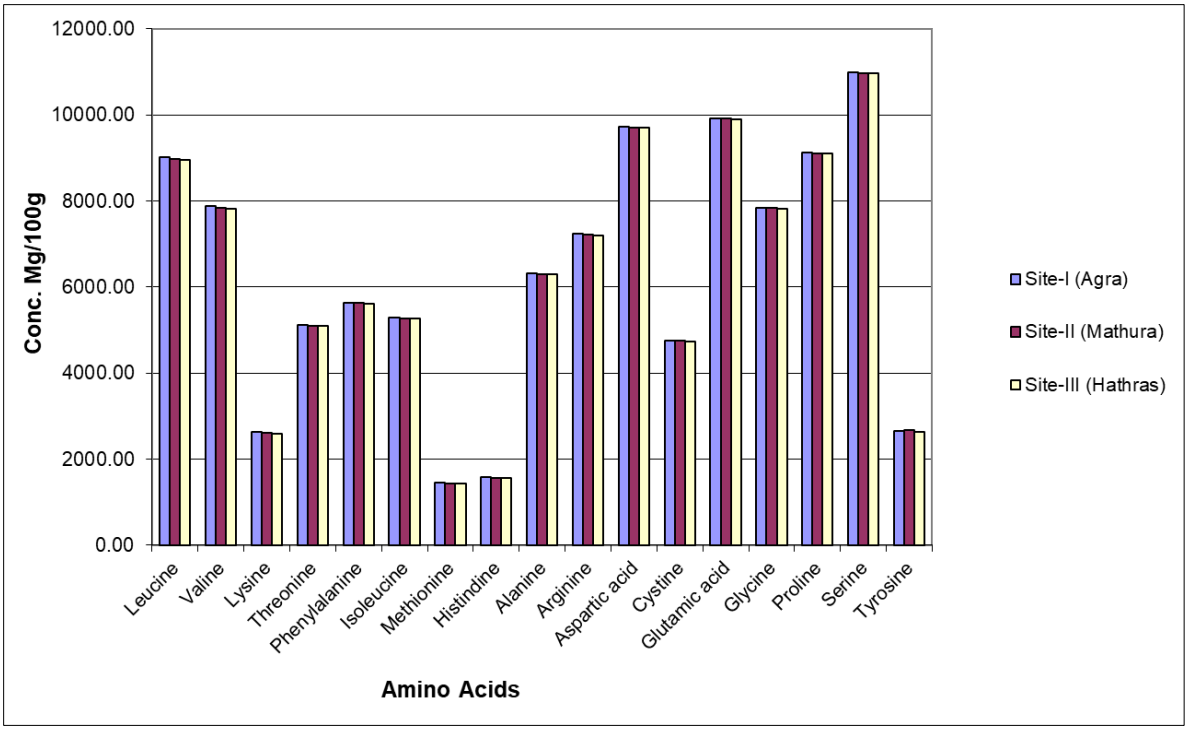

Fig 3: Concentration of amino acids (mg/100g) in cock (*Gallus domesticus*) feather at different sampling sites before administered ACES.

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

Essential Amino Acid	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
Histindine	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

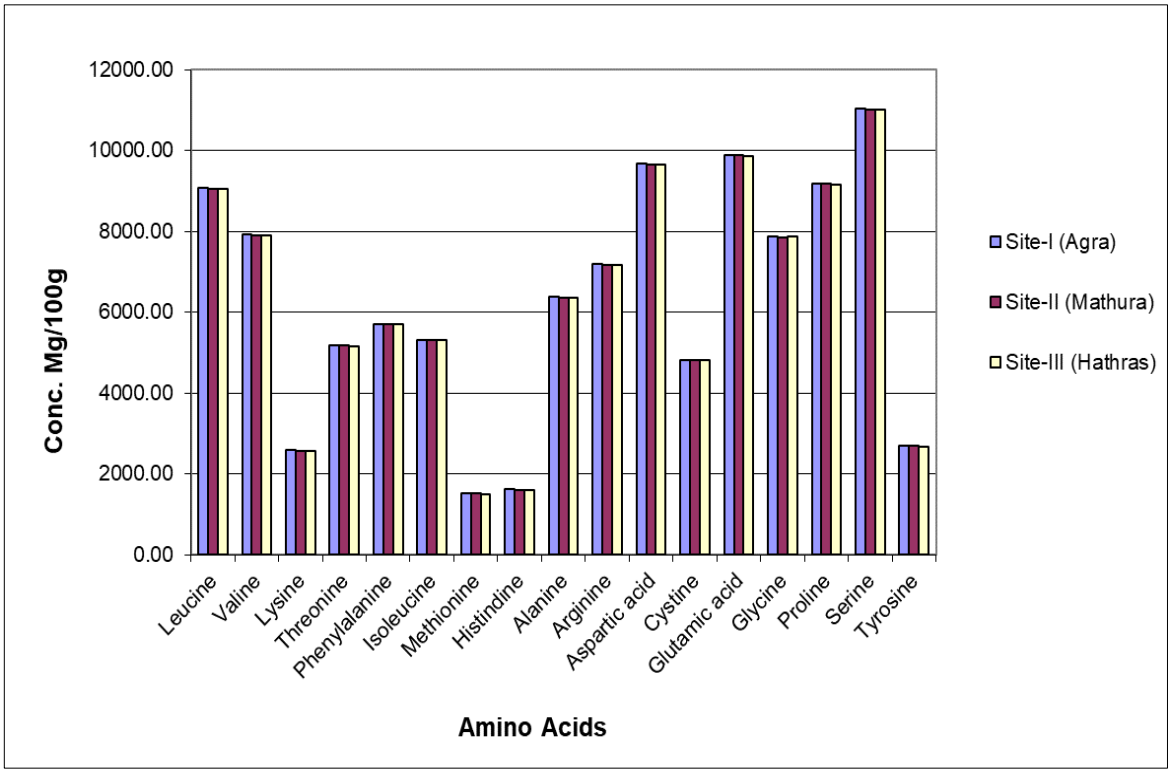

Fig 4: Concentration of amino acids (mg/100g) in cock (*Gallus domesticus*) feather at different sampling sites after administered ACES.

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 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.

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