



Effects of vitamin A, C, E and Selenium on chemical constituents of feathers of quail

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Abstract

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

Keywords: quail feather, trace elements, protein, amino acid, AAS, RP-HPLC

Introduction

The common quail is the smallest European species of the Phasianidae family. The adult is omnivorous, eating of wide variety of vegetation, mainly seeds but also other vegetable matter and invertebrates (molluscs, arthropods and arachnids) at certain times of year. Seasonal variations occur, determined by physiological needs^[1, 2].

The adult male *Coturnix* weighs about 100 to 140 grams. The male birds can be identified readily by the rusty brown coloured feathers on the upper throat and lower breast region. The young birds began to grow at 5 to 6 weeks old. The adult female *Coturnix* are slightly heavier than the male, weighing from 120 to 160 grams. The body colouration of the female bird is similar to the male except that the feathers on the throat and upper breast are long, pointed and much lighter cinnamon. Also the light tan breast feathers are characteristically black stippled.

The nutrients that comprise a quail diet are water, protein, carbohydrate, fat, minerals and vitamins. Quails require at least twice, as much in weight of water as they require in weight of dry feed^[3]. Protein provides the amino acids for tissue growth and egg production. The dietary protein requirement of quail is influenced by metabolizable energy content and the ingredients used to formulate the diets. The earlier investigators raised their quail flocks successfully on turkey starter diets containing about 25-28% crude protein^[4, 5]. It has been investigated that a dietary crude protein level of 24% is needed in starter diet for quail and the protein content may be reduced to 20% by 3rd week of age^[6, 7].

Protein is the most expensive nutrient and must be provided from a high quality source. Protein quality is generally based on the amino acid composition of the feed stuff and the availability of these amino acids from the feedstuff through digestion in the gut of the quail. Amino acids are considered as the building blocks of proteins. Out of 19 total amino acids required by quail, 13 are considered as essential amino acids, because they cannot be produced in the quail's body and must be supplied in the diets and 6 are considered as non-essential because they are synthesized by

the body and need not be supplied in the diet. Quail diets consist mainly of plant products are maize, soyabean meal, sorghum and rice on wheat bran. Methionine and lysine are generally low in plant products. Animal protein products such as fish meal, meat and bone meal etc. are good sources of most of the essential amino acids, but they are usually more expensive than plant protein ingredients. Synthetic lysine and methionine are usually added to the diets to balance the amino acid composition^[8-11]. Beside protein, carbohydrates, fats, vitamins and many other elements form a part of the quail's nutritional requirements.

Minerals are the inorganic elements remaining when a feedstuff is burned. The animal's body consists of approximately 3-5 percent ash on a dry basis. Calcium and phosphorus account for about 75 percent of the total mineral content of ash. Mineral deficiency symptoms in quail include bone disorders, decreased egg production, thin-shelled eggs, reduced growth, poor feather development, and anemia. The essential minerals needed for growth and production are classified as either macro or micro, based on the amount required in the diet. Macro minerals include calcium, phosphorus, sodium, chloride, potassium and magnesium, which are needed in relatively large amounts. The micro minerals include manganese, zinc, iron, copper, iodine, molybdenum and selenium.

Material and Method

In the present paper feathers of Quail (*Coturnix coturnix*) 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 quail feed and the quail 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^[12]. The feathers of vitamin administered quail (*Coturnix coturnix*) were

analyzed for the same mineral, protein and amino acid constituents.

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 feathers of adult quail were collected from three different cities of west Uttar Pradesh in India and specimens were preserved. Feathers of quail 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 of quail feather was determined according to an air oven method. Ash content was determined by incinerating at 410-440 °C until the constant weight was achieved.

Instrumentation

Mineral nutrients in feathers of quail 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 TM Breeze software (Version 3.2). Amino acids were analyzed AccQ Tag TM reverse phase (3.9×150 mm) 4 µm 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^[13].

Sample preparation for analysis of trace elements

A 50.0 g of feathers of quail 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. 6.19 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.

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 °C for 24 hrs and derivatized using AccQ reagent (6'Aminoquinol - N - hydroxy succinimidylcarbamite)^[16].

Result and Discussion

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 quail was found 20% and 74.2 g/100g 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 $\text{Ca} > \text{P} > \text{Na} > \text{Fe} > \text{Mg} > \text{Zn} > \text{Mn} > \text{Cu}$. The concentration of phosphorus was found in the range between 319 mg/100g to 326 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^[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 quail was higher i.e 482 mg/100g at site-I(a), Agra while minimum 456 mg/100g at site III(c), 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 1.5 mg/100g to 1.02 mg/100g. The high Manganese concentration was found at site-I(a)Agra, while site III(C), Hathras show low Manganese concentration. The main function of manganese is to prevent perosis, a condition where the Achilles's tendon slips off its groove behind the hock joint, pulling sideways and backwards. It is also required for normal growth, egg shell deposition, egg production and good hatchability. It is supplemented in the diet in the form of manganese sulphate.

Sodium content in feathers of quail ranged from 108.3 mg/100g to 106.2 mg/100g. Maximum sodium content was found at site-I(a), Agra and the minimum was noted at site- III(c), 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-I(c), Agra about 28.4 mg/100g while lower uptake was found at site-III(C), Hathras about 26.9 mg/100g.

Magnesium is an essential constituent of tissues and body fluids. Its ions serve as activators of important enzymes involved in intermediary metabolism.

When it is absent from the diets, quails grow slowly, exhibit convulsions and may eventually die^[44]. Deficiencies in laying rations produce a rapid drop in egg production. The magnesium requirement was recommended to be 300 mg/kg diet.

It was investigated that magnesium requirement for survival and growth was met by supplementing 150 mg magnesium per kg diet, or 50 mg magnesium per liter drinking water^[25].

Iron content in quail ranged from 35.3 mg/100g to 31.1 mg/100g. Maximum iron content was found at site-I(a), Agra and the minimum was reported at site-III(c), Hathras. Copper content in

quail feathers ranged from 1.2 mg/100g to 1.01mg/100g. Maximum Copper content was found at site I (a), Agra and the minimum was noted at site-III (c), Hathras.

Copper is necessary for iron utilization when haemoglobin is formed. It was reported the iron requirement of growing Japanese quail as 90-120 mg/kg, and of copper as 5 mg/kg diet based on EDTA extracted isolated soybean protein [26].

Samples of quail collected from site-I(a), Agra contain comparatively higher amount of zinc (12.4 mg/100g), whereas site-III(b), Hathras show low concentration of zinc (11.46 mg/100g). Japanese quail are quite sensitive to a dietary deficiency of zinc. Zinc deficiency in quail chicks was characterized by slow growth, abnormal feathering, labored respiration and an in coordinated gait, low tibia ash, and a low concentration of zinc in liver and tibias.

The zinc requirement for normal growth, feathering, tibia length and conformation was 25 mg/kg diet [27]. It was studied the protective effect of a high prior zinc intake for rapidly growing quail to a subsequently fed low zinc diet [28].

The birds fed an initial level of 75 mg zinc/kg grew significantly better than those fed initially 25 mg zinc/kg. Bone might store zinc and it might be mobilized during zinc deprivation. It was reported that reduction in zinc absorption in adult quail by high levels of calcium [29].

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 Quail (*Coturnix coturnix*) feather at different sampling sites before administered ACES.

Quail (<i>Coturnix Coturnix</i>) feather			Total Ash: 6.19 g/100g						
Mineral	Site-I (Agra)			Site-II (Mathura)			Site-III (Hathras)		
	a	b	c	a	b	c	a	b	c
Na	108.3	108.1	107.8	107.4	107.1	106.9	106.8	106.5	106.2
Mg	28.4	28.1	27.8	27.5	27.4	27.4	27.3	27.1	26.9
Ca	482	479	474	470	467	463	461	458	456
Fe	35.3	34.8	34.6	33.8	33.2	32.5	31.6	31.2	31.1
Cu	1.2	1.16	1.12	1.1	1.08	1.06	1.06	1.04	1.01
Zn	12.4	12.3	12.25	12.1	12.07	12.03	11.9	11.67	11.46
Mn	1.5	1.4	1.3	1.2	1.18	1.12	1.08	1.06	1.02
P	326	325	324	324	323	321	322	321	319

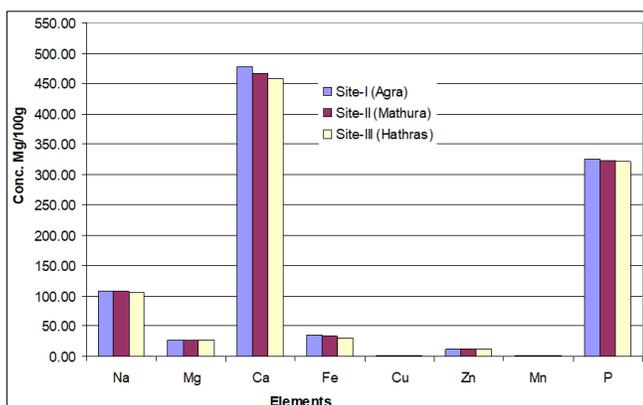


Fig 1: Concentration of trace elements (mg/100g) in Quail (*Coturnix coturnix*) feather at different sampling sites before administered ACES.

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

Quail (<i>Coturnix Coturnix</i>) feather			Total Ash: 6.13 g/100g						
Mineral	Site-I (Agra)			Site-II (Mathura)			Site-III (Hathras)		
	a	b	c	a	b	c	a	b	c
Na	107.8	107.5	107.2	106.2	105.9	105.8	105.7	105.6	105.4
Mg	26.4	26.3	26.1	25.8	25.6	25.3	24.8	24.7	24.6
Ca	476	474	472	471	470	468	468	467	465
Fe	38.3	37.9	37.7	36.1	35.8	35.4	35.2	34.9	34.3
Cu	1.12	1.09	1.07	1.08	1.06	1.03	1.02	1.02	1
Zn	12.1	12.08	12.05	11.9	11.8	12.6	11.5	11.3	11.1
Mn	1.4	1.35	1.32	1.3	1.28	1.21	1.18	1.15	1.12
P	330	328	326	321	320	319	319	317	314

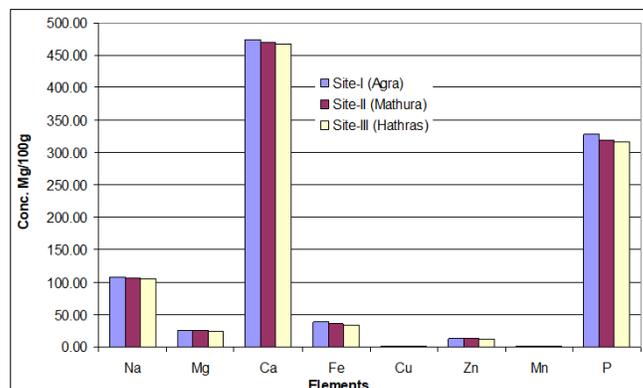


Fig 2: Concentration of trace elements (mg/100g) in Quail (*Coturnix coturnix*) feather at different sampling sites after administered ACES.

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 Quail (*Coturnix coturnix*) before the administration of four ACES antioxidant vitamins A, C, E and selenium was 74.2 g/100g and after the administration of four ACES antioxidant vitamins A, C, E and selenium was found 75.12 g/100g. 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 essential amino acids where as last nine were non essential amino acids. Essential amino acids constituted before and after the administered of four ACES antioxidant vitamins A, C, E and selenium was 35.87% and 35.95% 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, 10220.78 mg/100g and 11016 mg/100g followed by glutamic acid 9760.67 mg/100g and 9669.89, aspartic acid 9517.89 mg/100g and 9465.67 mg/100g, proline 8933 mg/100g and 8978.11 mg/100g respectively.

Methionine, histidine and lysine were found lowest concentration among all the amino acids present in feathers of Quail (*Coturnix coturnix*). The average value methionine before and after the administration of four ACES antioxidant vitamins A, C, E and selenium was 1404.78 mg/100g and 1506.33 mg/100g, histidine 1433.22 mg/100g and 1519.33 mg/100g, lysine 2435.56 mg/100g and 2364.33 mg/100g.

Leucine is a branched chain amino acid along with valine and isoleucine. It is beneficial and functional to protein structure for 60-70% in human body, and blood sugar level regulation which maintains a balance of insulin and glucose [30]. It proposed as a promising pharmaconutrient in the prevention and treatment of sarcopenia and/or type 2 diabetes [31]. Valine is required for muscle metabolism, repair and growth of tissue and maintaining the nitrogen balance in the body. Valine also assists to regulate blood sugar and energy levels [32]. While lysine is required for growth and bone development in children, assists in calcium absorption and assists in maintaining the correct nitrogen balance in the body, as well as maintaining lean body mass. Lysine is also needed to produce antibodies, hormones, enzymes, collagen formation as well as repair of tissue [33].

Aspartic acid is plays a vital role in energy production while alanine plays a key role in maintaining glucose levels in the body by helping the body to convert glucose into energy. Alanine also eliminates excess toxins from the liver [34-35]. They are good health which both NEAA and EAA should be considered in the classic "ideal protein" concept or formulation of balanced diets to maximize protein accretion and optimize health in animals and humans [36].

Iron has many functions in the body and is also important for maintaining a healthy immune system which is essential for blood to work efficiently. Iron functions as haemoglobin in the transport of oxygen. Iron deficiency is not uncommon among athletes, especially long distance runners which can cause of fatigue among these athletes. If the lack of iron in our bodies is severe, we can get iron deficiency anemia. Iron deficiency anemia is probably the most common nutritional disease in the world, affecting at least five hundred million people (Mineral Information Institute) [37-38]. Zinc is involved in well over one hundred different reactions in the body. Some of these reactions help the bodies construct and maintain DNA, the molecule that controls how every single part of our bodies is made and works. It is also needed for the growth and repair of tissues throughout our bodies [39]. This extremely important element is used to form connective tissue like ligaments and tendons. Teeth, bones, nails,

skin and hair could not grow without zinc. The enrichment of zinc would be benefit for reduction of diarrhea and pneumonia mortality in children [40-41]. The previous study presented its biological role in homeostasis, proliferation and apoptosis and its role in immunity and in chronic diseases [42]. Toxicity disease or symptoms of zinc in humans include gastrointestinal irritation, vomiting, decreased immune function and a reduction in high density lipoprotein (HDL) cholesterol. Higher dietary levels of Zn are required in the presence of phytic acid to prevent parakeratosis and allow for normal growth [43]. The optimum dietary level for the individual elements required for humans is very difficult to clarify cause of each variation of physiological response.

Table 3: Concentration of amino acids (mg/100g) in Quail (*Coturnix coturnix*) 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	8716	8712	8714	8705	8702	8697	8695	8692	8689
Valine	7526	7524	7521	7519	7515	7516	7509	7501	7494
Lysine	2448	2443	2441	2438	2434	2436	2432	2427	2421
Threonine	4975	4972	4968	4962	4959	4956	4952	4947	4942
Phenylalanine	5337	5334	5331	5328	5324	5321	5318	5313	5312
Isoleucine	5234	5231	5228	5226	5223	5221	5218	5215	5212
Methionine	1412	1411	1409	1408	1406	1404	1402	1398	1393
Histidine	1488	1486	1483	1481	1397	1394	1392	1387	1391
Non-essential Amino Acids									
Alanine	6128	6121	6124	6116	6113	6108	6103	6098	6094
Arginine	6914	6912	6908	6907	6904	6906	6902	6897	6899
Aspartic acid	9527	9523	9525	9521	9518	9514	9513	9512	9508
Cystine	4586	4583	4581	4578	4576	4573	4572	4569	4563
Glutamic acid	9768	9767	9765	9763	9761	9759	9756	9754	9753
Glycine	7645	7642	7643	7639	7637	7638	7635	7632	7634
Proline	8942	8939	8937	8934	8932	8931	8929	8926	8927
Serine	10233	10231	10229	10221	10218	10219	10217	10213	10206
Tyrosine	2485	2482	2483	2481	2478	2476	2473	2471	2472

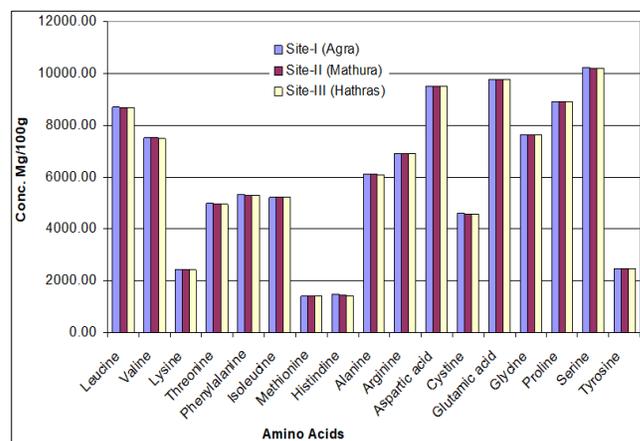


Fig 3: Concentration of amino acids (mg/100g) in Quail (*Coturnix coturnix*) feather at different sampling sites before administered ACES.

Table 4: Concentration of amino acids (mg/100g) in Quail (*Coturnix coturnix*) 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	8769	8764	8762	8761	8763	8759	8756	8753	8751
Valine	7596	7593	7591	7589	7586	7584	7583	7581	7578
Lysine	2372	2369	2368	2365	2363	2364	2361	2358	2359

Threonine	5026	5023	5021	5019	5017	5018	5017	5013	5012
Phenylalanine	5467	5464	5461	5461	5458	5457	5454	5451	5453
Isoleucine	5352	5349	5348	5345	5343	5342	5338	5337	5335
Methionine	1513	1511	1508	1507	1505	1506	1504	1502	1501
Histidine	1527	1523	1524	1521	1519	1517	1514	1516	1513
Non-essential Amino Acids									
Alanine	6178	6176	6173	6172	6168	6171	6167	6163	6164
Arginine	6735	6732	6734	6729	6731	6727	6724	6721	6719
Aspartic acid	9473	9471	9469	9468	9465	9463	9464	9457	9461
Cystine	4688	4685	4683	4682	4679	4676	4672	4669	4671
Glutamic acid	9678	9675	9674	9671	9672	9668	9667	9663	9661
Glycine	7687	7684	7682	7678	7681	7675	7674	7671	7669
Proline	8985	8981	8981	8982	8978	8976	8974	8971	8975
Serine	11023	11021	11018	11017	11019	11014	11012	11009	11011
Tyrosine	2513	2511	2509	2508	2507	2503	2506	2503	2501

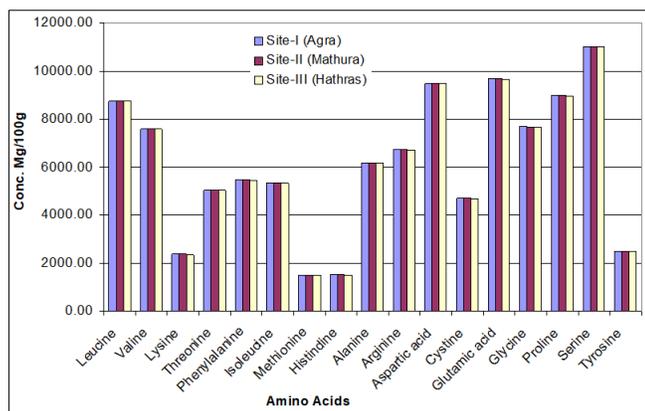


Fig 4: Concentration of amino acids (mg/100g) in Quail (*Coturnix coturnix*) feather at different sampling sites after administered ACES.

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