Amino Acid Analysis of Camel Urine

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Abstract

Recent studies indicated that camel urine has anti-cancer, anti-microbial, anti-parasitic effect, so investigation of camel urine constituent is needed to reveal compounds those stand behind these curative effects. In the current study the claimed amino acid analysis of Sudanese camel’s urine was obtained by hydrolysis using amino acid analyzer. The result obtained indicated the presence of all essential amino acids; three of the non-essential amino acids (proline, asparagine and glutamine) were not detected. Tryptophan concentration was the highest followed by valine as an essential amino acids in most of the age group, while glycine concentration was the highest as non-essential amino acid in all groups.

Keywords: Amino acids; Camel; Urine

Introduction

Amino acids are an organic chemical compound composed of one or more basic amino groups and one or more acidic carboxyl groups. A total of 20 of the more than 100 amino acids that occur in nature are the building blocks of proteins. The essential amino acids are isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, Arginine and histidine. Cysteine and tyrosine are semi essential because they may be synthesized from methionine and phenylalanine, respectively. The main nonessential amino acids are alanine, asparagine, aspartic acid, glutamine, glutamic acid, glycine, proline, and serine [1]. Camel urine found to be rich with amino acids. The objective of this study was to analyze the amino acids of camel urine.

Materials and Methods

Five Urine samples were collected from naturally grazing Sudanese local breed camels. The first sample represent crude urine sample for female camel aged (1-2) year, while the other samples were lyophilized urine for she camels in different ages (3, 5, 7 and more than 7 years) respectively. All chemicals and reagents used in the different stages of urine amino acid dialysis and assay were of pharmaceutical or analytical grades. Amino acids content of urine sample was determined using Saykam amino acids analyzer, S 5200 sample injector, S. 4300 amino acids reaction module and S. 2100 solvent delivery system. Samples were prepared according to the method described by [2].

Sample Preparation

Sample was weighed into glass ampoule. Seven ml of 6M HCl was added, the glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105°C for 22hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered. It should be noted that tryptophan is destroyed by 6M HCl during hydrolysis. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotator evaporator. The residue was dissolved with 5ml to acetate buffer (pH 2.0). The amount loaded was between 10 micro liters, the period of the analysis lasted for 76 minutes.

The net height of each peak produced by the chart recorder (each representing an amino acid) was measured. The half-height of the peak on the chart was found and the width of the peak on the half height was accurately measured and recorded. Approximately area of each peak was then obtained by multiplying the height with the width at half-height nor leucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula. Finally, the amount of each amino acid present in the sample was calculated in g/16gN or g/100g protein using the following formula:

Concentration (g/100g protein) = NH × w at NH/2× std × C
Where: NH = Net height
   W = Width at half height
   nleuc = nor leucine

Results and Discussion

The quantity and peak of each amino acid in urine is shown in table 1, 2, 3, 4 and 5. In (Table 1) leucine concentration was the highest (5.238) followed by histidine (2.264) as essential amino acids. In case of non-essential amino acid glycine concentration was the highest (143.847) followed by aspartic acid (2.6), cysteine and tryptophan were detected by this method. In (Table 2) Tryptophan concentration was the highest (1312) followed by valine (16.1) as essential amino acid while glycine concentration was the highest (44.5) followed by glutamic acid (13.9). (Table 3) tryptophan concentration was the highest (1367) followed by valine (18.5) as an essential amino acids.

| Name of amino acid | Amino acid class | Concentration Umol/l |
|--------------------|------------------|---------------------|
| Aspartic acid      | NS               | 2.6                 |
| Thereonine         | No peak found ES | _                   |
| Serine             | NS               | 0.215               |
| Glutamic acid      | NS               | 1.644               |
| Glycine            | NS               | 143.847             |
| Alanine            | NS               | 1.237               |
| Cystine            | No peak found    | _                   |
| Valine             | ES               | 2.193               |
| Methionine         | No peak found ES | _                   |
| Isoleucine         | ES               | 1.373               |
| Leucine            | ES               | 5.238               |
| Tyrosine           | No peak found NS | _                   |
| Histidine          | ES               | 2.264               |
| Phenyle alanine    | No peak found ES | _                   |
| Lysine             | ES               | 0.784               |
| Arginine           | No peak found ES | _                   |

Table 1: Characteristic of amino acid analyzer of test sample (C0) urine

| Name of amino acid | Amino acid class | Concentration Umol/l |
|--------------------|------------------|---------------------|
| Methionine         | No peak found ES | _                   |
| Leucine            | ES               | 7.646               |
| Isoleucine         | No peak found ES | _                   |
| Phenyl alanine     | ES               | 2.246               |
| Tyrosine           | No peak found NS | _                   |
| Histidine          | No peak found ES | _                   |
| Tryptophan         | ES               | 1312.922            |
| Arginine           | No peak found ES | _                   |

Table 2: Characteristic of amino acid analyzer of test sample (C1) urine

| Name of amino acid | Amino acid class | Concentration Umol/l |
|--------------------|------------------|---------------------|
| Thereonine         | No peak found ES | _                   |
| Glutamic acid      | NS               | 10.335              |
| Serine             | No peak found    | _                   |
| Asparagine         | No peak found NS | _                   |
| Glycine            | NS               | 49.002              |
| Alanine            | NS               | 11.673              |
| Valine             | ES               | 18.46               |
| Cysteine           | NS               | 19.653              |
| Methionine         | No peak found ES | _                   |
| Leucine            | ES               | 9.816               |
| Isoleucine         | No peak found ES | _                   |
| Phenyle alanine    | ES               | 2.502               |
| Tyrosine           | No peak found NS | _                   |
| Histidine          | No peak found ES | _                   |
| Tryptophan         | ES               | 1367.481            |

Table 3: Characteristic of amino acid analyzer of test sample (C2) urine

In case of non-essential amino acid glycine concentration was the highest (49) followed by cysteine (19.7). In (Table 4) tryptophan concentration (222) was the highest followed by leucine (34.96) as an essential amino acid. In case of non-essential amino acid glycine concentration (160) was the highest followed by alanine (154.3). The high concentration of tryptophan, though the documented destruction which occurs during the hydrolysis of the sample, could be justified by the camel urine content of thiol compounds such as cysteine which exert a scavenging role towards oxygen. Oxygen is responsible for the loss of tryptophan during sample preparation not the HCL as it was proposed [3].

| Name of amino acid | Amino acid class | Concentration Umol/l |
|--------------------|------------------|---------------------|
| Aspartic acid      | NS               | 2.222               |
| Thereonine         | ES               | 16.805              |
| Serine             | NS               | 27.428              |
| Asparagine         | No peak found NS | _                   |
| Glutamic acid      | NS               | 114.686             |
Table 4: Characteristic of amino acid analyzer of test sample (C3) urine

The high glycine level which was observed in this study agrees with [4], who performed spectral analysis of PMF (A natural anti-cancer drug derived from camel urine). Camel urine has distinct ability to inhibit the bacterial growth [5], this effect could be attributed to the high level of glutamic acid, which can inhibit growth of some pathogenic bacteria, including *Listeria monocytogenes, Salmonella typhimurium, Staphylococcus aureus, Klebsiella pneumonia* and *Esherichia coli* [6] (Na-Ri Lee et al, 2014).

Leucine content of camel urine reflex its potent ability to control blood sugar level in diabetic patients. In pancreatic β cells, leucine acutely stimulates insulin secretion by serving as both metabolic fuel and allosteric activator of glutamate dehydrogenises to enhance glutaminolysis. Leucine has also been shown to regulate gene transcription and protein synthesis in pancreatic islet β cells via both motor-dependent and -independent pathways at physiological concentrations. Long-term treatment of leucine has been shown to improve insulin secretory dysfunction of human diabetic islets via up regulation of certain key metabolic genes [7].

In (Table 5) tryptophan concentration (160) was the highest followed by valine (16.1) as essential amino acid while glycine concentration (202) was the highest followed by cysteine (4.328) as non-essential amino acid.

| Name of amino acid | Amino acid class | Concentration Umol/l |
|--------------------|------------------|----------------------|
| Aspartic acid      | No peak found NS |                     |
| Thereonine         | No peak found ES |                     |
| Serine             | No peak found NS |                     |
| Asparagine         | No peak found NS |                     |
| Glutamic acid      | No peak found ES |                     |
| Glycine            | NS               | 202.817              |
| Alanine            | No peak found NS |                     |

Table 5: Characteristic of amino acid analyzer of test sample (C4) urine.

The result that obtained from livestock urine showed that camel urine my composed of alkaloid substances, proteins and some essential amino acids which was confirmed by their positive reactions to dragniiff reagent, Mayer’s reagent and potassium iodide vapor, Biuret reagent. This results were in agreement with [8,9], who found alkaloid substance of urine basses in camel and zebu cattle urine by using HPLC technique.

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