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Amino acid profile of chaya and its protein degradation rates in dairy cattle

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ABSTRACT

Chaya (*Cnidoscolus aconitifolius*, family Euphorbeaceae), is a perennial shrub, the leaves of which are eaten by the Mayan people in central America. To explore its potential use as livestock fodder, the amino acid profile and protein degradation rates in fodder chaya containing leaves and succulent stems were analyzed. The amino acid contents (g/100 g protein) in chaya fodder were methionine (0.61), lysine (5.90), arginine (3.62), threonine (2.12), leucine (5.29), isoleucine (3.20), valine (3.73), histidine (1.42) and phenylalanine (4.23). Chaya fodder was rich in lysine, leucine, phenylalanine and valine. The lysine, lucine and valine content in chaya fodder were similar to soybean meal and alfafa hay. Chaya fodder contains 47.7% of rumen undegradable protein which may be useful in high milk producing cattle. In addition, chaya fodder protein solubility in the rumen is 27.95% of CP (Fraction A 5.72% plus Fraction B1 22.23%). Protein Fraction B2, which is intermediately degraded in the rumen, was 33.77% of CP and Fraction B3, most of which escapes degradation was 24.72% of CP. Fraction C, which is unavailable to the animal was 13.56% of protein. This study showed that all the amino acids required to support 10 kg/d of milk production were met by feeding a diet composed solely of chaya fodder.

Key word: Amino acid, Chaya, *Cnidoscolus aconitifolius*, Fodder chaya, Protein

Chaya (*Cnidoscolus aconitifolius*, family Euphorbeaceae), is a perennial shrub of Mesoamerica (Central America). It is also known as ‘tree spinach’ and the leaves of this plant are used a as vegetable by the indigenous people of the Mayan region of Guatemala, Belize, south-east Mexico, the Yucatan peninsula and parts of Honduras (Martin and Ruberte, 1978). Use of chaya as fodder for livestock has not been reported, although occasionally it is fed to the sheep, pig and poultry in the region of its origin. Chaya is a drought tolerant, disease resistant plant which produces copious amount of foliage and has the potential to become an important cultivated fodder in India. In this paper, the amino acid profile of chaya fodder and its protein quality have been presented.

MATERIALS AND METHODS

Forage samples of chaya containing leaves and succulent stems (40–50 day-old growth) were harvested from 2-year-old plants growing at the central research farm of Indian Grassland and Fodder Research Institute, Jhansi. The climate of Jhansi is semi-arid with temperature going up to 42°C in summer and down to 2°C in winter. The average annual rainfall in Jhansi is 900 mm. The harvested forage was chopped and dried in an oven. The dried samples were grounded and analyzed in triplicate for dry matter (DM). crude protein (CP) and acid detergent fiber (ADF) were analyzed according to AOAC (1996). Soluble protein (SP) is defined as protein soluble after 1 hr in borate-phosphate buffer at 39°C. It contains non-protein nitrogen, amino acids and peptides and is instantaneously degraded in the rumen. Soluble crude protein was analyzed following the method of Licitra *et al.* (1996). Neutral detergent fibre (NDF) was analyzed using the procedure described by Mertens (2002). NDF is analyzed without sodium sulphite because it eliminates some of the neutral detergent insoluble protein. Neutral detergent fibre insoluble protein (NDIP) and acid detergent fibre insoluble protein (ADIP) were assessed by micro-Kjeldahl (AOAC, 1995).

The amino acid profile of chaya leaves and fodder were estimated by a Pico-Tag System using the Waters Alliance 2690 and Waters 2487 Dual Wavelength Absorbance Detector. The analysis was done at Advanced Protein Technology Centre of SickKids Research Institute, Toronto, Canada. The process of analysis involved the hydrolysis and pre-column derivatization of the hydrolyzates using phenyisothiocyanate (PITC) followed by reverse phase HPLC. Samples were dried in pyrolyzed borosilicate tubes in a vacuum centrifugal concentrator and subjected to vapour

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phase hydrolysis by 6N HCl with 1% phenol at 110°C for 24 hr under pre-purified nitrogen atmosphere. After hydrolysis, excess HCl was removed by vacuum, hydrolyzates washed with redrying solution and derivatized with PITC to produce phenylthiocarbamyl (PTC) amino acids. The derivatization method was accomplished at room temperature, derivatizes both primary and secondary amino acids (i.e. proline and hydroxyproline) and forms very stable amino acid derivatives detectable at low picomole levels. Derivatized amino acids were redissolved in phosphate buffer and transferred to injection vials which were loaded into the autosampler for automatic injection. The processing of data was done by using Waters Millenium 32 Chromatography Software v. 4.0

The data of the nutrient analysis were input in the software Cornell-Penn-Miner system. CPM Dairy is an applied mathematical nutritional model that computes dairy cattle requirements and supply of energy and nutrients based on characteristics of the animal, the environment and the physicochemical composition of feeds. It predicts nutrient requirements and diet metabolizable energy (ME) and metabolizable protein (MP) supply in lactating dairy cattle at farm level when feed intake and the content of carbohydrate and protein fractions are adequately measured or estimated (Tedeschi *et al.*, 2008).

A diet was formulated for a 450 kg crossbred cattle in second lactation, 120 days into milk giving 10 kg milk/day which received chaya hay only at 2.6% dry matter of the body weight. Milk fat content was estimated to be 4.5% and protein content was at 3.3%. The environmental conditions set within the model were 30°C ambient temperature with 50% relative humidity. The software was run with the above constants and values were obtained for protein degradation rates for Chaya fodder. It also gave an estimate of metabolizable protein (MP) from bacteria and feed, the ratio of rumen degradable protein (RDP) and rumen undegradable protein (RUP). The supply of amino acid and metabolizable amino acids (MAA) were also estimated using the model.

RESULTS AND DISCUSSION

Amino acid content of chaya leaf and fodder chaya

The crude protein content of the dried chaya fodder was 17.96% of DM and the soluble crude protein was 27.93% of the CP (Table 1). The CP content in chaya fodder is similar to most of the leguminous fodder fed to cattle and buffalo like lucerne (alfalfa) (20.0% CP), berseem (17.67% CP) or cowpea (20.24% CP) (Ranjan 1977). The amino acid content in the leaves of chaya fodder shows that it is balanced in all amino acids required for ruminant animals (Table 2). It is particularly rich in lysine, leucine, phenylalanine and valine. The lysine content in chaya fodder (5.90% of protein) was similar (on per cent basis) soybean meal (6.08) and alfafa hay (6.02) etc. Leucine (5.29%), phenylalanine (4.23%), isoleucine (3.20%) and valine (3.73%) in chaya fodder (9.68%) were also similar to the other commonly fed fodder to the livestock. Until now, Amino acid content in chaya fodder has not been reported in the literature. However, the amino acid content in the leaves has been reported by Sarmiento-Franco *et al.* (2003) and Ranhotra *et al.* (1998), who reported similar amino acid profile except that chaya fodder had lower content of methionine in chaya fodder.

Table 1. Protein and fibre content of chaya fodder

Attributes	% of dry matter
Crude protein (N×6.25)	17.96±0.25
Soluble crude protein	5.02±1.34
SP as % of CP	27.93±7.24
NPN %	5.73±1.12
ADIP as % of CP	18.97±3.31
NDIP as % of CP	45.74±2.18

Certain amino acids like methionine, histidine, tryptophan and possibly, leucine may be limiting for milk production, and methionine for wool production (Van Soest 1994). The amino acid composition of milk and their efficiency of utilization indicate that methionine, lysine, histidine, phenylalanine, tryptophan and threonine have high efficiency of utilization (more than 83%) of absorbed amino acid for lactation (O'Connor *et al.* 1993). Bergen *et al*. (1967) compared the amino acids in rumen microbes with egg protein (the nutritional standard for the biological value of protein) and reported that threonine, phenylalanine, and lysine are higher in rumen bacteria, but valine and leucine are lower. In chaya fodder, both the leucine (5.29) and valine (3.73) are present in similar amounts than in most of the other important feeds. Therefore, the chaya protein escaping rumen could provide the amino acids lacking in rumen microbes.

Protein fractions

Feed protein is partitioned into 3 fractions: Fraction A is non-protein nitrogen (NPN), B is true protein and C is bound true protein (Sniffen *et al*. 1992). Fraction A is rapidly degraded in the rumen. Fraction B is subdivided into B1, which is rapidly degraded in the rumen; B2 has intermediate degradation and B3 is slowly degraded within the rumen. A high percentage of B3 escapes degradation in the rumen. Fraction C of protein cannot be degraded by rumen bacteria and does not provide available amino acids post-ruminally (Krishnamoorty *et al*. 1982).

The quality of a feed depends not only on the total protein and other nutrients but also upon how efficiently they are utilized by the animal. Chaya fodder contains 27.95% of protein which is soluble in the rumen and rapidly converted into ammonia (Table 3). The soluble protein (fraction A, 5.72% and B1, 22.23%) in chaya fodder was similar to alfafa hay (30%) but higher than soybean meal (20%) and cottonseed meal (20%) (CPM-Dairy 2008). The fraction B2 which has intermediate degradation in the rumen, was

Table 2. Amino acid content (g/100 g of protein) of chaya fodder and other common feeds

Amino acids	Chaya fodder		Soybean meal	Groundnut meal	Cottonseed meal	Canola meal	Alfafa hay
	Mean	SD					
Isoleucine	3.20	± 0.04	4.25	3.16	3.62	4.94	6.01
Leucine	5.29	± 0.00	6.13	6.06	6.31	7.99	9.26
Lysine	5.90	± 0.08	6.08	3.14	4.50	6.67	6.02
Methionine	0.61	± 0.00	0.83	1.10	0.99	1.40	0.73
Phenylalanine	4.23	± 0.00	3.88	4.92	5.47	4.68	6.32
Threonine	2.12	± 0.08	3.03	2.53	3.39	4.85	5.00
Valine	3.73	± 0.00	3.79	3.82	5.02	6.44	7.14
Alanine	3.37	± 0.04					
Arginine	3.62	± 0.16	7.96	10.88	10.59	6.78	6.39
Aspartic acid	4.60	± 0.24					
Cystine	0.08	± 0.04					
Glutamic acid	6.68	± 0.24					
Glycine	2.62	± 0.00					
Histidine	1.42	± 0.04	2.27	2.18	3.45	4.04	2.62
Proline	2.84	± 0.00					
Serine	2.12	± 0.08					
Tryosine	2.26	± 0.04					

Values for chaya fodder are from the present study. Values for other feed stuff are from CPM-Dairy, 2008 feed database.

Table 3. Protein degradability (%) of chaya and other common feed

Protein fractions	Chaya meal	Alfafa hay	Soybean meal	Cottonseed meal	Canola meal
A	5.72	20	12.2	6	10.5
B1	22.23	20	20.8	9	19.5
B2	33.77	44	63.8	69.22	48
B3	24.72	10	1.2	5.84	15.8
C	13.56	6	2	9.95	6.2

33.77% of the protein in chaya and fraction B3, most of which escapes degradation and available at duodenum was 24.72% of the CP. The unavailable fraction (Fraction C) was 13.56% of the protein in chaya.

When the CPM-Dairy model was run for a dairy cow of 450 kg body weight in second lactation solely fed on Chaya hay, it was estimated that 52.3% of the protein was rumen degradedable protein (RDP) and 47.7% was rumen undegradable protein. The amino acid balance (Table 4) indicates that all the amino acid requirements were met feeding of chaya at 2.6% of BW. Amino acids available for absorption and ultimately for production are supplied by microbial protein synthesized in the rumen, dietary protein escaping rumen degradation, and endogenous secretions into the digestive tract (Richardson and Hatfield 1978). Assessing an accurate estimate of amino acids available from dietary protein escaping ruminal degradation is needed to determine total amino acids available to the animal. Amino acids available from dietary protein escaping ruminal degradation can be estimated from the amount of dietary protein escaping degradation, the amino acid composition of insoluble protein, and the true digestibility of dietary amino acids in the intestines. These factors are estimated within the model.

The metabolizable amino acids from bacterial sources ranged from 12.7 g/d (methionine) to 63.9 g/d (lysine) (Table 4). Similarly, the metabolizable amino acids from feed sources (chaya) ranged from 3.8 g/d (methionine) to 36.6 g/d

Table 4. Metabolizable amino acid in lactating cows fed as sole diet of chaya hay

Amino acids	% of required	Metabolizable amino acids (g/day)		
		Bacteria	Feed	Total
Methionine	128	20.9	3.8	24.7
Lysine	150	63.9	36.6	100.5
Arginine	155	54.2	22.5	76.7
Threonine	144	43.5	6.8	50.3
Leucine	117	58.5	32.8	91.3
Isoleucine	147	45.8	19.9	65.7
Valine	132	48.0	23.2	71.2
Histidine	127	21.0	8.8	29.8
Phenylalanine	201	40.2	26.3	66.5
Tryptophan	241	12.7	7.3	20.0

(lysine). The metabolizable protein available to the animal from bacteria was estimated to be 779 g/d and from protein escaping rumen degradation (RUP) to be 621 g/d. The model predicted that feeding sole chaya hay can sustain 13.1 kg of milk per day based upon amino acid protein supply. However, the results indicated that the limiting factor for enhanced milk yield was metabolizable energy (ME). The level of ME in the chaya hay was the first limiting factor in the diet and thus limited the milk yield up to 10 kg/d.

Chaya fodder is rich in most of the amino acids required for milk production and it is particularly rich in lysine, leucine, phenylalanine, valine and isoleucine. It has a high rumen undegradable protein which escapes rumen degradation and thus makes it suitable for high milk producing cattle. Using CPM-Dairy modeling it was predicted that feeding chaya fodder alone can sustain milk yield of up to 10 kg/d in dairy cattle.

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