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Research Article

Nutritional Characterization of Moringa (*Moringa oleifera Lam.*) Leaves for Forage Use

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Abstract

The aim of this work was to evaluate the nutritional content, amino acids, phytochemical compounds (phenols, tannins and flavonoids) in ethanol extract, minerals and fatty acids, in *Moringa oleifera* leaves under the edaphoclimatic conditions typical of the central-northeast region of Jalisco, Mexico. In animal feed, moringa consumed fresh, directly, as part of a diet, or in silage, has favorable properties in the nutrition and health of cattle, chickens, fish, goats, pigs and lambs, and has become in an alternative to the traditional grain-based use. Due to its favorable properties, moringa is one of the most used vegetables for forage use.

Keywords: Chemical Composition; Amino Acids; Fatty Acids; Tannins; Linoleic Acid

Introduction

Livestock and poultry production is an activity of great importance in the food industry. The current trend is the inclusion in the diet of plants and their extracts as supplements that function as substitutes for antibiotics [1], in addition to the fact that there is a limited supply of grass-based forages [2]. Among the most promising plant resources for this purpose is *Moringa oleifera* Lam., A tree native to the southern Himalayas and northwestern India. It belongs to the *Moringacea* family. It grows in tropical areas below 500 masl, although it can adapt to edapho-climatic conditions in places above 1,500 masl. In Mexico it is distributed throughout the Pacific coast [3]. This species is very versatile since all its organs, pods, flowers, bark, roots and leaves can be used, since they contain medicinal properties. This plant has functioned as a nutritional source in diets for the population in different countries of the world, and it has also had forage and fertilizer use [4,5]. It has a high content of protein, carotenoids, vitamins, minerals, chlorogenic acid, gallic acid, kaempferol, glycosides and quercetin in its leaves [6,7]. Consumption of moringa has been shown to increase meat yield in animals, antiparasitic and curative effect [8]. It has been used as a forage resource with yields of up to 21 tons of dry matter/ha [9]; In particular, the moringa leaf has been used as a dietary supplement and activator of the immune system [10], improving productivity and increasing the digestibility of nutrients [11], being supplied in various ways, such as fresh feed, supplement for pasture or silage. Since the nutritional composition of plants is dependent on the climate and place of growth or cultivation [12], the objective of this work is to present the nutritional potential of *M. oleifera* cultivated under the edaphoclimatic conditions of the area of sampling (Central West of Mexico), through the nutritional evaluation, amino acids, mineral and phytochemical composition of the leaves, in the search for alternatives or supplements of grains and cereals commonly used in animal production.

Materials and Methods

The research was carried out in the Nutrition Laboratory of the University Center for Biological and Agricultural Sciences (CUCBA) of the University of Guadalajara, Zapopan, Jalisco, Mexico.

Plant sampling

Moringa leaves were collected in July 2017, from adult trees in a crop located in the municipality of Cuquío (20° 55' 6'' N, 103° 01' 3'' W), Jalisco, Mexico, in the center-northeast of the State at a height of 1,810 masl. The climate is semi-dry, dry and semi-warm winter and spring with mild winter, average annual temperature 17.9 °C, average annual rainfall 839.5 millimeters, rains in June, July and August.

Material Preparation

The leaves were dried at room temperature (25 °C) for 7 days. Subsequently, the material was pulverized in a Restsch-Gnbh brand blade mill, and it was sieved on a 70-mesh screen (opening 0.21 mm), using the material that passed through this mesh.

Proximal chemical analysis

This analysis was made according to Valdez-Solana., *et al.* (2015) [13]. The ground leaves were treated with cold hexane at 4°C to remove fats, since they interfere with the analyzes. Crude protein (CP) (% N x 6.25), crude fiber (CF), fat content, moisture, ash and carbohydrates (or nitrogen-free extract, ELN), calculated by difference, were evaluated on the dislipidized material. Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (FDA) were determined, applying the Van-Soest technique (1991) [14].

Amino acids

The grinded leaf sample was hydrolyzed with HCl (6N, 105 °C, 24 h), neutralized with 6N NaOH and filtered. The amino acids were evaluated using an HPLC (Varian), reversed phase (C18 column) with fluorescence detector, with derivatization in precolumn with Orthophthaldehydothiol (OPT) [15].

Mineral composition

Macrominerals and microminerals were determined by atomic absorption spectrophotometry (Perkin-Elmer spectrophotometer) [16]. all analyzes were performed by 3 repetitions (n = 3).

Fatty acids

The analysis was done according to standard procedures [17], using a gas chromatograph (Hewlett-Packard, HP-5890, series II), equipped with flame ionization detector.

Extraction process

The ground and dry material (100 g. Dry weight) was extracted with 500 mL of ethanol for 5 days at 25 °C and 150 rpm in an incubator (LabTech model LSI-3016R). The material was filtered with Whatman No.4 paper and the filtrate was concentrated in a Buchi rotary evaporator under reduced pressure and 40 °C, keeping it in the freezer until the tests were carried out.

Total phenols

The Folin Ciocalteau assay was applied. A standard curve for Gallic Acid (0-100 µg/mL) was prepared in 20 µg/ mL intervals. Absorbance (λ = 760 nm) was measured [18].

Flavonoids

The $AlCl_3$ Technique was applied [19]. 5 mg of extract were weighed and dissolved in 1 mL of distilled water. It was diluted 1:10 in distilled water. 1250 μL of distilled water were added to each of the standards and test samples, and after adding 75 μL of 5% $NaNO_2$. They were allowed to stand for 6 minutes. 150 µL of 10% $AlCl_3$ were added and the mixture was left to stand for 5 minutes. Later, 500 μL of 1M NaOH were added and finally the volume of each reference and sample was completed at 2.5 mL with distilled water. The sample was allowed to stand 30 minutes and the absorbance at 510 nm was measured. A standard curve was prepared with Catechin.

Condensed tannins

The vanillin method was applied [20], with some changes. 20 g of ground dry leaves were treated with 200 ml of absolute methanol and centrifugation was applied (9,000 rpm, 20 min). Subsequently, 10 g of activated carbon was added, and it was centrifuged again (5000 rpm, 10 min), recovering the supernatant. For the evaluation of the condensed tannins, 5 ml of the crude extract were treated with 25 ml of freshly prepared vanillin reagent (10% vanillin in methanol and 80% hydrochloric acid in methanol, 1: 1). It was left to rest in a water bath for 20 min at 30 °C. Subsequently, the absorbance of the sample was read at 500 nm in a UV spec-

trophotometer (Velab). A catechin standard curve was prepared. It was reported as gEC/100 g dw.

Statistical analyzes

The tests were done in triplicate. Basic statistics were applied to calculate the arithmetic mean and standard error.

Results and Discussion

Chemical composition, secondary metabolites, minerals

Results are shown in table 1. The values shown are those obtained in proximal chemical analysis, phytochemical analysis and mineral profile.

Parameter	*M. oleifera*, %	Soybean meal, % [21]	Maize, % [22]
Crude protein	25.50 ± 0.81	49.22	7.10
Crude Fat (lipids)	7.4 ± 0.62	1.27	4.18
Carbohydrates (NFE)	58.20 ± 2.63	---	75.48
Ash	5.40 ± 0.61	---	1.79
Neutral Detergent Fiber (NDF)	10.35 ± 0.61	14.88	6.69
Acid Detergent Fiber (ADF)	7.36 ± 0.57	6.62	---
Phenols (gGAE/100g)	3.29 ± 0.54	---	0.174
CT (gEC/100g)	0.367 ± 0.41	---	0.048
Flavonoids (gCE/100g)	3.06 ± 0.55	---	0.005
Nitrogen	4.45 ± 0.297	---	---
Phosphorus	0.24 ± 0.070	0.72	0.284
Potassium	6.16 ± 0.087	---	0.276
Sodium	0.25 ± 0.0454	---	0.037
Calcium	2.23 ± 0.075	0.43	0.011
Magnesium	0.38 ± 0.030	---	0.126
Copper	8 ± 1.00 (ppm)	---	0.064
Manganese	51.4 ± 1.36 (ppm)	---	0.001
Iron	99.3 ± 3.05 (ppm)	---	0.003

Table 1: Chemical composition of moringa leaf, compared with commonly used food species.

DM = Dry Matter ; CT= Condensed Tannins; NFE= Nitrogen Free Elements; gGAE= Grams Gallic Acid Equivalent; gCE= Grams Catechin Equivalent.

The data in table 1 show that moringa has a higher protein content than corn but lower than soybean; higher lipid content than corn, but lower than soybean; higher fiber content than corn and similar to soybean. Likewise, the nutritional content is within the standards of the multiple reports that exist on the moringa leaf, in some of them, the chemical composition of 17-22% is established in crude protein, 2-6% crude fat, ash 7-8%, crude fiber 7-21% [23,24]. The chemical composition of moringa has positioned it as a vegetable with nutritional and nutraceutical properties. In addition, essential amino acids and fats are used to obtain foods with higher energy levels [25]. According to the data presented in table 1 coinciding with the numerous bibliographic reports-, the moringa leaf has high protein content (25.50%), as well as an acceptable content of lipids, minerals, low fiber content, presence of secondary metabolites antioxidants, such as phenols, flavonoids and tannins, as well as vitamins, for which it has been consumed and used for various purposes. Usually, the low fiber content indicates a good palatability of the animal feed [26]. The secondary metabolites would provide antioxidant and antibacterial properties to the supplied diet [6]. Moringa improves nutrition, but also improves the immune system of birds and animals, with antimicrobial effect against E. *Coli*, and increase of Lactobacillus in the intestine [27].

In relation to the phytochemical analysis, the values in table 1 are similar in the content of tannins and flavonoids and slightly lower in phenols, when compared with other analyzes. Nweze and Nwafor (2014) [23] found 3.83% flavonoids, 9.19% tannins in addition to other compounds (alkaloids, saponins, carotenoids and others) in ethanol extract; Another study indicates values of 12% phenols and 4% flavonoids [28]; also 4.51% phenols and 2.56% tannins [11]. In a study done on moringa grown in Mexico (Celaya, Guanajuato), they report 3.3% in total phenols and 0.007% in condensed tannins [29]. *M. oleifera*, particularly the leaves, contains a high percentage of secondary metabolites, which can be used as additives in diets or as direct consumption to increase the quality of the meat [30]. Phenols and flavonoids prevent the potential peroxidation of lipids, improve meat quality -chemical composition and color stability, and lighter color due to the production of conjugated linoleic acid- and the composition of fatty acids [31,32]. Antioxidants improve the immune system of animals protecting them against infections and diseases [32]. High polyphenol contents have a beneficial effect on the rumen microbial population [33]. The presence of flavonoids in moringa leaves increases the content of high-density lipoprotein (Good cholesterol) in the egg, decreases low-density lipoprotein and cholesterol [34]. Tannins,

an astringent polyphenolic compound considered antinutritional, common in plants, affect the nutritional value of foods by decreasing palatability and intestinal enzymatic digestion [35], with a decrease in intake. However, ruminants can use lignin or cellulose as an energy source since they degrade it to monosaccharides, not so monogastric animals that cannot degrade it. Tannins have a high antioxidant activity, and the antioxidant potential of moringa leaves is due to the concentration of tannins [36]. Since antinutritional factors, in particular hydrolysable tannins and phenols, should not be eliminated from animal feed in their entirety, since they have antioxidant properties, improve meat quality and reduce methane emission in ruminates [26].

The minerals are at the level of other reports. In one of them [23], they mention 3.03% N, 0.44 P, 2.09% Ca, 0.48% Mg, 1.62% K, in addition to 0.03% Au, and 0.85 S. Also 0.30% P, 3.65% Ca, 0.50% Mg, 1.50% K [37]. Reviewing the minerals in table 1, a higher mineral content is seen in moringa leaves compared to soybean and corn, used as a comparison, in K, Na, Ca, Mg, but lower content in phosphorus. Minerals are also important for evaluating nutritional properties. Su and Chen (2020) [26], citing several authors, mention that the minerals related to animal growth and development are Calcium, a necessary element for bones, teeth, eggshell, proper functioning of the nervous system, reduction of capillary permeability and regulation of metabolism and enzymatic activation. Gold (Au), promotes animal growth since it is a key component of various proteins and participant in various biochemical reactions. Zinc, necessary in enzymes, is important in the oxygenation of tissues and the metabolism of proteins, fats, sugars and nucleic acids. Magnesium, with favorable effects on milk production.

Amino acids

The amino acid profile of the moringa leaf (Figure 1) shows that the highest signal corresponds to threonine (Thr), to a lesser extent Proline (Pro), Glycine (Gly) and Asparagine (Asn).

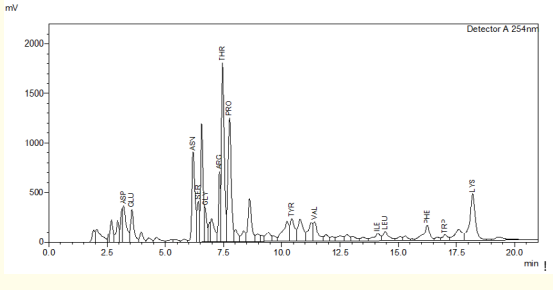

Figure 1: Chromatogram of amino acids present in Moringa oleifera leaf.

The concentrations of the amino acids are presented in table 2, which includes the references soybean and corn.

Amino acid	Quantity		
	M. oleifera	Soybean meal [21]	Maíz [38]
Aspartic acid	2.91	---	---
Glutamic acid	2.55	---	5.70
Asparagine	4.14	---	---
Serine	0.97	2.47	1.80
Glycine	0.63	2.28	2.50
Methionine*	ND	0.66	0.90
Cisteine*	ND	0.69	0.70
Arginine*	1.62	3.64	3.82
Threonine*	7.40	2.25	2.00
Proline	3.01	2.56	1.15
Tyrosine	2.72	1.70	2.07
Valine*	0.86	2.47	3.00
Isoleucine*	0.61	2.33	2.53
Lysine*	2.73	3.22	1.80
Leucine*	0.61	3.86	8.82
Phenylalanine*	1.16	2.49	3.50
Tryptophan*	0.29	2.25	---

Table 2: Amino acids in *Moringa oleifera,* compared to common foods (Soybean and Maize). (% of DM).

*Indispensable Amino acids; ND = Not detected.

The amino acid found in the least amount (limiting amino acid) is tryptophan (0.29%). In contrast, threonine is the one with the highest percentage (7.4%). Of the total amino acids presented in this analysis, it contains threonine, valine, leucine, lysine, isoleucine, arginine, tryptophan and phenylalanine as essential. The content of methionine and cysteine/cystine was low (ND). Other studies done on moringa leaves report 0.297% methionine and 0.01% cystine [37]. Compared to soybeans and corn, the moringa leaf in this analysis has more threonine. However, it contains a lower content of arginine, valine, isoleucine, leucine and phenylalanine. Similar contents of serine, proline, tyrosine, and lysine are appreciated. In particular lysine, a limiting sulfur amino acid in corn, the content of this amino acid in moringa is approximately 1.5 times higher in relation to the content in corn, but slightly higher in relation to soybeans. However, the amino acid content in moringa is moderate, as shown in the table. Like legumes, and in general inputs of plant ori-

gin, moringa is low in sulfur amino acids, methionine and cysteine/cystine. Sulfur amino acids are important in nutrition and growth as they participate in protein synthesis and regulate the percentage of food that is metabolized at the cellular level [39]. Sulfur amino acid deficiency has negative effects on nutrition. Generally, the deficiency in sulfur amino acids is compensated by supplementing the food with fish meal, which is characterized by its high content of methionine and cystine, or gramineous plant (sorghum, corn, wheat) which also improves palatability [40]. However, the content in moringa leaves of the essential amino acids Threonine and Lysine, which are limiting amino acids in cereals such as corn, is noteworthy [41]. Lysine is related to animal growth and threonine to the immune system, the integrity of the intestinal mucosa, and is related to the synthesis of immunoglobulin [42].

Fatty acids

In this analysis, the values of 1.54% polyunsaturated fat, 0.08% monounsaturated fat and 0.72% saturated fat are observed as notable (Table 3).

Fatty acid	Quantity, % (DM)
Palmitic acid	0.59 ± 0.020
Stearic acid	0.12 ± 0.02
Oleic acid	0.08 ± 0.654
Linolenic acid	1.29 ± 0.062
Saturated fat	0.72 ± 0.025
Monounsaturated fat	0.08 ± 0.015
Polyunsaturated fat	1.54 ± 0.020

Table 3: Fatty acid profile of the dried leaves of *M. oleifera* (% of DM).

It is also worth mentioning the higher content of linolenic acid (1.29%) and palmitic acid (0.59%), with respect to stearic acid (0.12%) and oleic acid (0.08%). These values are within the standards reported in similar studies for the moringa leaf, such as the one that determined 4.58% polyunsaturated fatty acids, 0.87% monounsaturated fatty acids and 3.77% saturated fatty acids [43]. The values are lower than those of corn oil, which contains 14% saturated fatty acids, 30% monounsaturated fatty acids and 56% polyunsaturated fatty acids, 54-60% linoleic acid, 25-31% oleic acid, 11-13% palmitic acid, 2-3% linolenic acid [44]. This last reference of linolenic acid, with 2-3% in concentration, is slightly higher than the one evaluated here in moringa leaves, of 1.54%. Other reports express much higher concentrations of linolenic acid than those found here, such as that reported in moringa leaves from South Africa of 44.57%, who mention the presence of 17 fatty acids [37]. These variations could be attributed to different climatic conditions and soil types. This results with almost twice as many polyunsaturated (1.54%) than saturated (0.72%) fatty acids, which is favorable in the diet. Polyunsaturated fatty acids support the functionality of the immune system. On the contrary, saturated fats are associated with cardiovascular diseases [45]. In relation to the profile of long chain fatty acids, moringa leaf has a higher abundance of linolenic acid (C18:3) and palmitic acid (C16:0) than stearic acid (C18:0) and oleic (C18:1). linolenic acid (omega 3) is an essential polyunsaturated fatty acid (organisms cannot produce it), so it must be acquired through the diet. It is a fundamental compound for the functioning of cell membranes, especially neuronal ones. It is associated with a lower risk of developing diseases such as dementia or Alzheimer's, reduces cholesterol levels, has antithrombotic and vasodilatory, anti-inflammatory properties, prevents diabetes and certain types of cancer [46]. On the other hand, palmitic acid is associated with an increase in blood cholesterol, stearic acid does not affect it, and oleic acid is related to a decrease in blood cholesterol [47].

Due to these favorable characteristics, moringa has become one of the most widely used species as a dietary supplement for forage, either directly or ensiled, with favorable effects on milk yield in cows [48], higher intake and digestibility [49], higher content of milk fat [50].

Conclusion

The consumption of moringa (leaves and other anatomical parts) in its various forms has beneficial effects on nutrition and health, in the treatment and prevention of various diseases due to the biochemical compounds that this plant contains. It has a high yield in biomass, and it has been used in animal nutrition, since the quality of the various animal products for human consumption is improved. It can be a source of chemical products destined for health, due to the polyphenolic concentration, vitamins and polyunsaturated fatty acids of the omega type.

Conflict of Interest

There is not any conflict of interest.

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