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Protein Hydrolyzate of *Moringa oleifera* Lam., as a Nutritional Supplement in the Diet of Fattening Chinchilla Rabbits

Lianny Pérez Gómez*, Carmen Hernández Mendoza**, Jorge Martínez Melo**, Jorge Orlay Serrano Torres**, Aurora Pérez Martínez*, Carlos Mazorra Calero**

*****Laboratory of Metabolic Engineering, Bioplants Center, Maximo Gomez Baez University, Ciego de Avila, Carretera a Morón, km 9½. Zip: 69450, Ciego de Ávila, Cuba.
**Faculty of Agricultural Sciences, Maximum Gómez Báez University of Ciego de Ávila, Carretera a Morón, km 9½., Cuba Zip: 69450. Ciego de Ávila, Cuba.
Corresponding author: lianny@bioplantas.cu

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ABSTRACT

Background: Post-weaned young rabbits are subject to significant stress despite their digestive capacity is not yet adapted to adult rations. The utilization of protein hydrolyzate in animal nutrition is important, in order to cope with metabolic shortages. **Aim:** To evaluate the effect of a protein hydrolyzate of *Moringa oleífera* Lam, (PHM), as a food supplement of Chinchilla fattening rabbits.
Methods: The following experimental treatments were applied: 1. Supplementation of protein hydrolyzate of moringa (PHM), 2 Supplementation of aqueous extract of moringa (AEM), and 3. No supplementation (NS). Overall, 27 rabbits were included (three treatments and three repetitions). The productive indicators (final live weight, total weight gain, mean daily gain, and carcass yield), and carcass and blood chemistry indicators (cholesterol and triglycerides), were determined.
Results: According to Duncan ($p<0.05$), the best results in the productive indicators and carcass were observed in rabbits supplemented with PHM (2.572 6 kg final weight, 1.237 kg total gain, and 0.020 7 kg mean daily gain), with significant differences ($p<0.05$), compared to the other treatments. PHM was able to increase the weight of the hind (0.523 kg), and anterior (0.806 kg) portions of the carcass, with significant differences compared to the control (NS). The blood chemistry indicators showed no significant differences ($p> 0.05$).
Conclusions: PHM had a positive effect on food supplementation of fattening Chinchilla rabbits.
Key words: fattening, leaf extract, animal nutrition (Source: *AIMS), Moringa oleifera* Lam

INTRODUCTION

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Rabbits are an important source of highly nutritional meat for humans at low economic costs. The meat from rabbits is healthy: it has high protein, vitamin, and mineral contents, which are easily digested. Besides, it has low calories, with low percentages of fat and cholesterol (Dalle, Cullere, Alberghini, Catellani, and Paci, 2016). Accordingly, it is highly demanded in quality markets.

Moreover, in animal production, the nutritional requirements of animals are higher, the faster the growth speed is (Tomás, 2017). In that sense, there is evidence that the current fattening feeds do not meet the nutritional needs of rabbits, in addition to the inconvenient that after weaning _besides undergoing stress_ the digestive capacity of young animals is not yet adapted to adult rations (Arias, 2019).

In modern nutrition, the generation of greater benefits is permanently explored. As a result, several supplements have been developed from protein hydrolysis, to facilitate their absorption through the blood torrent, without losing their nutritional properties. Protein hydrolyzates (PH) have a broad range of applications, as ingredients in the formulation of special foods for animals (purified diets, food supplements, and others), since they improve protein digestibility, and reduce allergenic properties (Colas, Bernal, Támbara, Pérez, and Sánchez, 2017).

Consequently, the utilization of PH in rabbit production is important in order to meet metabolic shortages. Furthermore, as a result of this application, proper microflora modification should be taken into account.

Moringa oleifera Lam is a tree from family *Moringaceae* and it is currently cultivated in all the tropical, subtropical, and semiarid regions of the world. The leaves of this species have a high nutritional quality, due to its high vitamin, provitamin, and mineral contents (Oyeyinka and Oyeyinka, 2018), particularly due to its high protein content (25-30 % dry weight) (Palada *et al.*, 2017).

The biological value of a protein depends on its amino acidic composition and the proportions among them (Suárez, Kizlansky, and López, 2006). The foliar proteins of *Moringa oleifera* Lam. have 19 of the 20 most common amino acids (except glutamine), which provide a high biological potential (Moyo, Masika, Hugo, and Muchenje, 2011). However, the leaves of the plant contain a considerable amount of crude protein, though mostly insoluble, and with low *in vitro* digestibility due to the proteases in the digestive tract (Teixeira, Carvalho, Neves, Silva, and Arantes-Pereira, 2014). Therefore, an alternative to elevate leaf protein uptake by the animals may be the production of free amino acid and peptide-rich protein hydrolyzates.

The aim of this paper was to evaluate the effect of a protein hydrolyzate from the leaves of *Moringa oleifera* Lam., used as food supplement to enhance the productive performance, carcass traits, and Chinchilla rabbit health during fattening.

MATERIALS AND METHODS

Production and characterization of the protein hydrolyzate, and the aqueous extract of Moringa as food supplement in the diet of rabbits

The main product for evaluation is from plant origin, known as PHM, and produced in the Laboratory of Metabolic Engineering at the Bioplants Center, University of Ciego de Avila, Cuba. This product was obtained from enzymatic hydrolysis of proteins from *Moringa oleifera* Lam., variety Supergenius, using bromelin as protease. To obtain the product, a 10% suspension of dry powder from depigmented leaves of moringa in water (1 g vegetal mass: 10 mL of water), and a quantity of enzymes equal to AE g^{-1} units of dried moringa mass. The mix was shaken constantly for 5 h, pH 7, at 37ºC. The enzymatic reaction was obtained by heating at 100 °C for 15 min. The resulting mix was centrifuged at 2 500 rpm x g, for 10 min. The supernatants were stored for further characterization at 4 ºC. The aqueous extract from moringa (AEM) was obtained according to the same procedure, but without the addition of the enzyme. The raw materials used to obtain these products were only the leaves of moringa, commonly utilized to feed ruminants (Elghandour *et al*., 2017), and monogastric (Owoleke *et al*., 2016), water, and crude extract of bromelin (extract with negative toxicity, according to Báez, Hernández, and Bello, 1998).

Both PHM and AEM were chemically characterized. The content of total proteins was determined using the Bradford method (1976); the total content of carbohydrates by the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Robers, and Smith, 1956); the total phenolic content was determined by reaction, using the Folin-Ciocalteu reagent, according to the method described by Bray and Thorpe (1954); and the total content of free amino acids was quantified by reaction with nihydrine, according to Moore and Stein, 1948 The hydrolysis degree (HG) was determined through the OPA method (Nielsen, Petersen and Dambmann, 2001), using a methionine-calibrated curve. In each case, the analysis was made by triplicate.

Evaluation procedure of the protein hydrolyzate of *Moringa oleifera* Lam. (PHM), as a nutritional supplement in fattening Chinchilla rabbits

Animal experimentation was done on a private rabbit farm, in the municipality of Venezuela, province of Ciego de Avila, between February and May 2019. A completely randomized designed was used, with three treatments and three repetitions each, with 27 animals aged 45 days, and an average starting weight of 1.23 kg. They were lodged at random, in collective cages containing three animals each, which was considered one experimental unit. The treatments used were, 1) PHM: Protein hydrolyzate of moringa, 2) AEM: aqueous extract of moringa, and 3) NS: no food supplementation.

In the three treatments, a single isoenergetic diet was used, based on 80 and 100 g daily per animal in the first and second months, respectively, twice a day. The mix was made by the farmer, using the following foods and proportions: peanut hulls (10%), rice hulls (20%), citrus peel meal (30%), and commercial feeds (40%).

The bromatological value of the foods used to make the mix was taken from the tables of nutritional values (Anon, 2000), which characterize all these components according to the Cuban conditions. Considering the variations that might appear in commercial feed production, in relation to the values expressed in the literature, the values of crude protein in the total mix were quantified, based on the method recommended by Kjeldahl (AOAC, 1995). The result (14.7%) was corrected in the total protein value shown in Table 1.

According to Montejo, López, and Lamela (2010), the CP and CF contents are within the recommended range for rabbit fattening, whose values were reported between 12 and 17%, and 16 and 20%, respectively. The Ca, P, and AEM contents were within the range or slightly higher than the value reported as nutritional requirement for fattening rabbits.

Table 1. Bromatological characterization (Anon 2000) of foods used to manufacture feed for the isoenergetic diet

Raw materials	CP (%)	CF (%)	AEM (Mcal)	Ca (%)	P (%)
Peanut hulls	14.40	35.00	2.53	1.50	0.13
Rice hulls	5.60	39.80	1.87	1.16	0.12
Citrus peel meal	6.70	11.70	2.83	1.81	0.13
Commercial feed	11.00	3.60	4.54	1.94	2.07
Total	14.70[1]	16.37	4.25	1.75	2.05

Quantified through the Kjeldahl method (AOAC, 1995).

The supplements used (PHM and AEM) were daily mixed with the food, before feeding the animals. During the first month of evaluation, 20 mL of each, per rabbit, per day, were used, whereas 30 mL were supplied in the second month. Food was supplied twice a day, in the morning (8: 00 am), and in the afternoon (5:00 pm). Before the experimental stage, the animals underwent a period of adaptation to supplement consumption: it started using 5 mL per animal, daily, and gradually increased to 20 mL of the diet three times a day.

Determination of productive indicators, carcass traits, and health of rabbits

The experimental stage lasted 60 days, during which the individual live weight in every treatment was evaluated every seven days, using a 40 kg ± 5 g accuracy Wcichen scale. Then, the mean weight gain and the mean daily gain were calculated, considering the starting and final live weights, and the experimental days.

At the end of the experimental period, three animals per treatment were weighed and sacrificed, as a representative sample to determine carcass yield and other traits.

Sacrifice was performed between 8:00 and 9:00 am, by cervical dislocation (Cambar, Arias, Aguilar, and Guzmán, 2009). *Post mortem*, the following parts of the animal were collected and weighed separately: hind portion (HP) and anterior portion (AP) of the carcass, intestines (I),

entrails (E), and the head, legs, and fur altogether (HLF). The instrument described above was used for weighing.

Determination of blood chemistry indicators

Prior to sacrifice, blood sera from all the animals (three per treatment) were drawn, in order to analyze the main indicators of blood chemistry. The technique used for all the animals was immobilization and drawing through the thorax, to standardize the effect of the blood tests. A volume of 2 mL of blood was drawn from every rabbit, then the samples were placed in labeled Eppendorf tubes, and stored at 4 °C. The serum samples were transported to the clinical laboratory of the province of Ciego de Avila for determination, through enzymatic methods, of blood cholesterol contents, following the technique described by Lothar (1998); the triglyceride contents were determined according to the method described by Bucolo and David (1973).

Statistical data processing

The statistical treatment was done through IBM SPSS 20. Parametric tests, simple analysis of variance (ANOVA) were performed, when F was significant, the comparison of means was done through the Duncan test. To test data normality, the Kolmogorov-Smirnoc test was performed, whereas the analysis of variance homogeneity was made with the Levene test ($P<0.05$) in all the cases). Three replicas were processed in all the experiments, at least.

RESULTS AND DISCUSSION

The chemical compositions of PHM and AEM are described in Table 2. PHM showed a lower content of soluble proteins than AEM, possibly because free amino acids and short-chain peptides that are undetected by the protein quantification method used, are generated through enzymatic hydrolysis.

Table 2. Chemical characterization of the protein hydrolyzate of *Moringa oleifera* Lam (PHM) and the aqueous extract of moringa (AEM)

Indicators evaluated	PHM	AEM
Total protein (mg/mL^{-1})	0.237	0.503
Total phenols (mg of chlorogenic acid mL^{-1})	0.897	0.182
Free amino acids (mg of leucine mL^{-1})	1.180	0.443
Total carbohydrates (mg of glucose mL^{-1})	9.710	3.320
Hydrolysis degree (%)	19.66	4.28

Greater carbohydrate and total phenol contents were also observed in PHM, in relation to AEM, since, apart from the carbohydrate and phenol contents in the leaves of moringa, PHM is also present in the crude extract of bromelin used during enzymatic hydrolysis. The content of PHM-free amino acids was greater than in AEM, resulting from the hydrolysis of moringa foliar

proteins, along with the free amino acid contents in the enzymatic extract used. PHM is an extensive protein hydrolyzate (hydrolysis degree greater than 10%), which indicates that it can be used as protein supplement in animal or human diets (Vioque, Clemente, Pedroche, Yuste, and Millán, 2001).

Table 3 shows the live weight and live weight gain values achieved at the end of the experiment, and the mean daily gain of rabbits supplemented with PHM and AEM.

Table 3. Productive indicators in rabbits supplemented with PHM, AEM, and NS

Treatments	Final weight (kg)	Weight gain (kg)	Mean daily gain (kg)
NS	2.254 [b]	1.004 [b]	0.017 [b]
AEM	2.235 [b]	0.971 [b]	0.016 [b]
PHM	2.573 [a]	1.237 [a]	0.021 [a]
SE±	0.091	0.047	0.001
P	0.027	0.043	0.046

Unequal letters on the same row indicate a significant differences, according to Duncan ($p \leq 0.05$).

The final live weight of rabbits underwent a 0.338 kg increase, when their diet was supplemented with PHM, in relation to AEM supplementation, and 0.319 kg in the absence of supplementation (NS). Moreover, the increase observed in the weights of rabbits supplemented with PHM was 0.266 kg, higher than the values found in rabbits supplemented with AEM, and 0.233 kg in relation to the NS. Consequently, the mean daily gain observed in rabbits on a diet supplemented with PHM was 0.004 kg, higher than the value using AEM, and 0.005 kg in relation to NS.

The positive effect of protein hydrolyzates on animal yields has been reported in various research. According to Gilbert, Wong, and Webb (2008), these results have been attributed to the fact that, foremost, the hydrolyzates contain short-chain peptides, and certain amino acids (glycine, glutamic acid, and alanine), which stimulate feeding, promote palatability, and increase adaptation to man-made diets. Secondly, the short-chain peptides and amino acids are easily absorbed in the intestine without previous gastrointestinal digestion, and they enhance growth and animal development. Third, the uptake of labile and insoluble amino acids like cysteine or tyrosine in the form of short-chain peptides, increase the availability of these amino acids in the body of animals. Furthermore, hormone-like specific peptides obtained by protein hydrolysis might modulate gastrointestinal motility, endocrine metabolism, and ingesta, and they could also affect animal performance (Martínez-Alvarez, 2013).

Additionally, after weaning, despite being subject to significant stress, the digestive capacity of the animals is not yet adapted to adult rations. The application of these amino acid-rich protein hydrolyzates might meet certain metabolic shortages, and modify the intestinal microflora properly, thus allowing greater weight increase in the animals (Martínez-Alvarez, Chamorro, and Brenes, 2015).

Table 4 shows the results achieved after weight determination of carcass, hind and anterior portions, the intestines, entrails, and the head, legs, and fur as a whole, in rabbits supplemented with PHM and AEM, and with no supplementation.

Table 4 Carcass indicators in rabbits supplemented with PHM, AEM, and NS

Treatments	C (kg)	HP (kg)	AP (kg)	E (kg)	HLF (kg)	I (kg)
NS	0.943 [b]	0.383 [c]	0.573 [b]	0.110	0.467	0.483
AEM	1.120 [ab]	0.456 [b]	0.667 [ab]	0.147	0.503	0.557
PHM	1.313 [a]	0.523 [a]	0.806 [a]	0.137	0.553	0.567
SE±	0.051	0.018	0.031	0.012	0.025	0.027
P	0.012	0.004	0.021	0.115	0.057	0.153

Unequal letters on the same column indicate a significant difference, according to Duncan ($p \leq 0.05$).

The carcass weight (C) with PHM supplementation was 0.193 kg higher in relation to supplementation with AEM, and 0.37 kg, in relation to no supplementation (NS). Furthermore, the weight of the hind portion of the carcass (HP) of rabbits supplemented with PHM was 0.14 kg, higher than the values of animals without supplementation, and 0.067 kg in relation to supplementation with AEM. This is an important result for rabbit breeders, since in this part of the animal, a larger accumulation of meat is produced. As to the anterior portion of the carcass, the rabbits supplemented with PHM had a 0.233 kg increase compared to the non-supplemented rabbits (NS); however, it was 0.139 kg higher compared to the AEM supplemented rabbits, but no significant differences were observed ($P > 0.05$). Moreover, the weight of entrails (E), intestines (I), and head, legs, and fur (HLF) underwent no statistically significant differences between the treatments evaluated ($P > 0.05$), which indicated that the supplements used did not have any effects on the growth of entrails and intestines, mainly.

The previously described results demonstrate the positive effect of protein hydrolyzates in the diet of rabbits, since they favor the absorption of proteins, peptides, and amino acids in the intestines, and at a systemic level, through the blood torrent, with no loss of the nutritional values. Additionally, these protein hydrolyzates can contain functional proteins and bioactive peptides with several benefits, such as supporters of the intestinal system and enhancers of the immune system (Moreno, Montoya, Buelvas, and Ortiz, 2015).

The results from the evaluation of some of the main indicators of blood chemistry with clinical interest in the serum of rabbits supplemented with PHM and AEM, and with no supplementation, are shown in table 5.

Table 5. Blood chemistry indicators in rabbits supplemented with PHM, AEM, and NS

Treatments	Cholesterol (mM)	Triglycerides (mM)
NS	2.100	0.700
AEM	2.113	0.500
PHM	2.133	0.467

SE±	0.081	0.105
P	0.867	0.211

Unequal letters on the same column indicate a significant difference, according to Duncan (p≤ 0.05).

The contents of blood cholesterol and triglycerides did not show any significant differences in the three treatments, according to the Duncan test of multiple comparisons (P > 0.05); however, it would be necessary to evaluate a larger sample to corroborate the veracity of these results.

Although the contents of cholesterol in the blood showed no significant differences in the evaluated treatments, which may be caused by the size of the sample used, there are reports in the literature on the use of moringa leaf extract to reduce such indicator. In that sense, Ghasi, Nwobodo, and Ofili (2000), said that the crude extract of moringa leaves significantly reduces the serum cholesterol levels of rats fed high contents of fat, which might be attributed to the presence of a bioactive plant constituent, namely β-sitosterol. Regarding the contents of triglycerides, the lowest values in all the treatments were observed in the rabbits supplemented with PHM. Several bioactive peptides present in protein hydrolyzates, and animal and plant proteins have stood out for their inhibitory activity of enzyme triacylglycerol lipase in the intestine, which might result in inhibition and/or delay in the assimilation of fat, and consequently, a reduction in the levels of postprandial triglycerides in the blood (Möller, Scholz-Ahrens, Roos, and Schrezenmeir, 2008). On the other hand, several studies have confirmed that the extracts of moringa leaves can reduce the triglyceride levels in the blood (Anwar, Latif, Ashraf, and Gilani, 2007; Ali, Hassan, and Abdrabou, 2015).

CONCLUSIONS

Supplementation with protein hydrolyzate of moringa of fattening Chinchilla rabbits, showed the best results in productive and carcass indicators without harming animal health, thus indicating that an adequate alternative is possible as food supplement. Nevertheless, since these results are preliminary, further studies of meat physiology and quality should be conducted in other categories, breeds, and doses of the hydrolyzate, in order to test its effectiveness.

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AUTHOR CONTRIBUTION

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.