











Nutritive value of adding dried *Cratylia argentea* leaves to the feed of growing rabbits

Valor nutritivo das folhas secas de *Cratylia argentea* na alimentação de coelhos em crescimento

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Abstract: Nutrition in rabbit farming accounts for about 70% of all costs; in this sense, *C. argentea* is an excellent alternative as a feed, due to its bromatological composition. Our goal is to evaluate the nutritional value of diets formulated with the dried leaves of *C. argentea* to feed growing rabbits. Twenty-eight white rabbits of the New Zealand breed, 55 days old, of both sexes, were used. Two treatments (n = 14, 7 males, and 7 females) were evaluated in a randomized block experimental design in a 2 x 2 factorial scheme. The control diet (CTRL) was formulated to meet the nutritional requirements of growing rabbits, and the test diet (DRY) was obtained by replacing 20% of the CTRL diet with dried leaves of *C. argentea*. The experimental period was comprised of 11 days, with seven days of adaptation and four days of total feces collection. The average daily gain, dry matter intake, feed conversion, and final body weight were not affected by replacing the CTRL diet with the dried leaves of *C. argentea*. The coefficient of dry matter digestibility showed better results in CTRL rabbits (female: 72.19%, male: 72.48%). Rabbits fed with the CTRL diet exhibited a higher consumption of crude protein compared to those fed with the DRY diet, however, these rabbits showed higher elimination and lower retention of crude protein. It is concluded that *C. argentea* presents values of 1,400.67 kcal per kg of dry matter, digestible energy, and 3.34% digestible protein per kg of dry matter.

Keywords: alternative feedstuff; digestibility; legume; rabbit production

Resumo: A nutrição na criação de coelhos representa cerca de 70% de todos os custos; nesse sentido, a *Cratylia argentea* é uma excelente alternativa como alimento devido à sua composição bromatológica. Nosso objetivo foi avaliar o valor nutricional de dietas formuladas com folhas secas de *C. argentea* na alimentação de coelhos em crescimento. Foram utilizados 28 coelhos brancos da raça Nova Zelândia,

Received: August 14, 2023. Accepted: January 05, 2024. Published: March 11, 2024.

com 55 dias de idade, de ambos os sexos. Dois tratamentos (n = 14, 7 machos e 7 fêmeas) foram avaliados em delineamento experimental de blocos casualizados em esquema fatorial 2 x 2. A dieta controle (CTRL) foi formulada para atender às exigências nutricionais de coelhos em crescimento, e a dieta teste (DRY) foi obtida substituindo 20% da dieta CTRL por folhas secas de *C. argentea*. O período experimental compreendeu 11 dias, sendo sete dias de adaptação e quatro dias de coleta total de fezes. O ganho médio diário, o consumo de matéria seca, a conversão alimentar e o peso corporal final não foram afetados pela substituição da dieta CTRL por folhas secas de *C. argentea*. O coeficiente de digestibilidade da matéria seca apresentou melhores resultados nos coelhos CTRL (Fêmea: 72,19%, Macho: 72,48%). Coelhos alimentados com a dieta CTRL apresentaram maior consumo de proteína bruta do que os alimentados com a dieta DRY, porém esses coelhos tiveram maior excreção e menor retenção de proteína bruta. Conclui-se que *Cratylia argentea* apresenta valores de 1.400,67 kcal de energia digestível por kg de matéria seca e 3,34% de proteína digestível por kg de matéria seca.

Palavras-chave: alimento alternativo; digestibilidade; leguminosa; produção de coelhos

1. Introduction

Rabbits are monogastric animals that require a great amount of fiber in their diet⁽¹⁾. When rabbits are provided low amounts of fiber, increased body weight (BW) is observed, but a higher incidence of enteritis and mortality also occur; on the other hand, high amounts of fiber in the diet provide a reduction in mortality, but a simultaneous decrease in dry matter intake (DMI) and BW gain is obtained⁽²⁾. According to De Blas and Mateos⁽³⁾, the fiber in the diet fulfills important functions, such as, maintaining digestion consistency and volume, ensuring normal digestive transit, distending the mucosa by stimulating intestinal motility, and serving as a substrate for the cecum microbiota. In addition, diet digestibility could be affected by the level of fiber.

In addition to nutritional factors, it should be considered that the demand for food by animals is relatively constant throughout the year, leading to fluctuations in prices, which can make diet-associated costs more burdensome, prompting a constant search for alternative sources⁽⁴⁾. In this sense, the use of *C. argentea* becomes an interesting alternative, as it is easily obtainable and has a high leaf yield that can be used in animal feeding⁽⁵⁾.

Alfalfa hay is considered a balanced source of fiber for rabbits, capable of meeting the nutritional needs, when included at approximately 30% in the diet, but owing to its high price, it accounts for up to 40% of the cost of the diet⁽⁶⁾. Therefore, other sources of fiber are being researched, as alternatives to alfalfa hay, aiming to potentially reduce the feeding costs, improve comfort, and enhance feed conversion compared to fiber sources in traditional diets⁽⁷⁾. An example is a legume popularly known in Brazil as “Camaratuba” (*Cratylia argentea*). This legume is a native shrub found in the Brazilian Cerrado, in the central, northern, and northeastern regions of the country, as well as in Bolivia and Peru. It is considered a promising source for use as a supplement during the dry season, when facing low soil fertility and prolonged drought⁽⁸⁾. Its nutritional value is superior to most shrub legumes adapted to dry soils, containing only small amounts of tannins. It also offers advantages such as high leaf production (especially young leaves) and good regrowth capacity during the dry season.

Thus, it could be used as an alternative to alfalfa hay, but little is known about its use in rabbit feeding. Therefore, this experiment was conducted with the aim of evaluating the nutritional value of diets formulated with dried leaves of *C. argentea*, and fed to the growing white rabbits of the New Zealand breed.

2. Materials and Methods

The rabbits used in this study were handled by acceptable practices, in accordance with the guidelines outlined in the Code of Practice for Intensive Rabbit Husbandry, adapted to local conditions. The research was carried out in the rabbit breeding sector in Alfenas, in the state of Minas Gerais, at coordinates Latitude: 21° 25' 46" South, Longitude: 45° 56' 50" West. The experimental protocol used for the animals was carried out in accordance with the Institutional Animal Use Committee guidelines (protocol number 12A/2016).

2.1 Animal handling, experimental design, sampling, and analyses

Twenty-eight white New Zealand rabbits, weaned at 35 days of age, of both sexes, were distributed individually into galvanized cages (60 [length] x 80 [width] x 45 [height] cm), which was considered to be the experimental unit. Each cage had a semi-automatic feeder and a nipple-type drinker and all cages were placed in a barn with 50-cm side block walls and a net up to the roof; a curtain was utilized to control the internal temperature. The temperature and relative humidity were recorded in the morning and afternoon, on a daily basis.

Two treatments (n = 14 animals, 7 males, and 7 females) were evaluated in a randomized complete block experimental design in a 2 x 2 factorial scheme (control diet and experimental diet) x (male and female), randomized, considering light, medium, and heavy weights for block allocation. The control diet (CTRL) was formulated according to the nutritional criteria and chemical composition described by De Blas and Mateos⁽³⁾, (Table 1), and the test diet (SECA) was obtained by replacing 20% of the CTRL diet with dried leaves of *C. argentea*, based on values obtained from the extended studies by Restore *et al.*⁽⁷⁾. The diets were manufactured as pellets, to pass through a 4-mm sieve (5-cm pellet), in a local commercial industry (Races Nutrimax Ltda., Alfenas, MG, Brazil). The rabbits had free-choice access to the diet and water.

Table 1 Ingredient and chemical composition of the diet provided to growing rabbits.

Item	
Ingredients, % (as-fed basis)	
Alfafa hay	41.68
Wheat middling	22.71
Sorghum	17.22
Corn meal	10.80
Soybean meal	3.73
Powder molasses	1.40
Soybean oil	1.00

NaCl	0.58
Calcitic limestone	0.09
Dicalcium phosphate	0.08
Choline chloride	0.06
L-Lysine 78%	0.17
DL-Methionine 99%	0.08
Mineral and vitamin premix ¹	0.40
Chemical composition (%)	
Dry matter	88.14
Crude protein	15.02
Digestible protein	10.00
Crude fiber	13.93
Acid detergent fiber	18.65
Starch	14.00
Calcium	0.60
Total phosphorus	0.42
Lysine	0.73
Methionine + Cysteine	0.52
Digestible energy, kcal/kg	2,440.00

¹ Mineral and vitamin premix composed of (per kg of diet): Zn (70.00 mg), Fe (45.00 mg), Cu (8.00 mg), Mn (15.00mg), I (0.42 mg), Co (0.10 mg), vit. B2 (3.60 mg), vit. A (6,000 IU), vit. D3 (1,100 IU), vit. B1 (1.60 mg), niacin (35.10 mg), folic acid (1.00 mg), pantothenic acid (10.00 mg), vit. E (40 IU), antioxidants (0.80 mg).

The leaves of *C. argentea* utilized in the present study were harvested at the partner company. Compound leaves were collected, each one containing three leaflets, with their respective stems (petioles). After harvesting, the leaves were dried in an oven with forced ventilation and a humidity of 65%, until they were dry, without the presence of moisture. A chemical analysis of the leaves was performed in a commercial laboratory and the results are described in Table 2.

Table 2 Chemical composition of dried leaves of *Cratylia argentea*.

Item (%)	
Dry matter	94.55
Crude protein	17.19
Crude fiber	23.08
Mineral matter	9.46
Ether extract	3.14
Acid detergent fiber	23.97
Gross energy, kcal/kg DM	4,124.00

Rabbits between 55 to 59 days of age, were fed a commercial diet to adapt to the facilities. At 59 days of age (day 0), they were individually weighted, sorted by body weight (BW) ranges and sex, and then redistributed into the cages, so both treatments would have rabbits with a similar BW. Digestibility assays were performed between 59 and 70 days of age, and during this period the performance of the rabbits was also assessed.

Total collection of feces was accomplished during four consecutive days, as described by Ribeiro *et al.*⁽⁹⁾. A nylon screen was attached on the sides and bottom of each cage to permit the urine to pass through and for feces to be retained. In the morning, the feces of each experimental unit (cage) was collected, placed in identified plastic bags, and stored in a freezer at -18°C, until the last collection day.

On that day, all the material was weighted, dried in a forced-air oven at 55°C for 72 hours, ground in a Wiley-type mill, to pass through a 1-mm, stainless-steel, curved, round-holed sieve, a curved-rounded sieve, and finally stored in plastic flasks, until analysis. The analyzes carried out were: Crude protein⁽¹⁰⁾, gross energy (GE; determined using a bomb calorimeter [model C500, IKA®-Works do Brasil Ltda., Rio de Janeiro, Brazil]), and dry matter⁽¹⁰⁾. Both diets were also sampled and subjected to the same laboratorial procedures.

2.2 Measuring the performance of rabbits

Diet offered and orts were weighed at 59, 66, and 79 days of age, for further calculations of dry matter intake (DMI) during both the whole experimental and feces collection periods. The rabbits were weighted on days 59 and 70 and the average daily gain (ADG) was calculated. Feed conversion ratio (FCR) was calculated by dividing the DMI by the ADG. Coefficient of DM digestibility (CDMD), was important to be determined, because it helped to comprehend the digestible, assimilable, and metabolized fraction of the feedstuff (as the nutrients were in the DM content). It was determined according to the equation ⁽¹⁰⁾ given below:

$$CDMD = \frac{DMI - feces\ dry\ matter}{DMI} \times 100$$

The apparent digestibility coefficients, that is, the digestible protein (DP) and digestible energy (DE) values of CTRL and DRY diets were calculated based on DM using the equations described below:

$$DP\ (\% DM) = \frac{(DMI \times \% diet\ CP) - (total\ feces\ dry\ matter \times \% fecal\ CP)}{DMI} \times 100$$

$$DE\ \left(\frac{kcal}{kg} DM\right) = \frac{(DM \times diet\ GE) - (total\ feces\ dry\ matter \times fecal\ GE)}{DMI} \times 100$$

Subsequently, DP and ED values of the dried *C. argentea* leaves were calculated using the equation proposed by Matterson *et al.* (1965):

$$DP\ (\% DM - basis) = CTRL\ diet\ DP + \frac{DRY\ diet\ DP - CTRL\ diet\ DP}{inclusion\ of\ C.\ argentea\ \left(\frac{g}{kg}/1000\right)}$$

$$DE\ \left(\frac{kcal}{kg DM}\right) = CTRL\ diet\ DE + \frac{DRY\ diet\ DE - CTRL\ diet\ DE}{inclusion\ of\ C.\ argentea\ \left(\frac{g}{kg}/1000\right)}$$

2.3 Statistical analysis

Data were checked for normality of residuals using the Shapiro-Wilk test and for homogeneity of variances using the Levene test. Subsequently, they were submitted to analysis of variance using the SAS GLM procedure (SAS 9.3). When a significant effect was observed, comparisons of means were performed using the F test. For all the analyses performed, the significance level used was 5%.

3. Results

The performance variables were not affected ($p > 0.58$) by the treatment (Table 3). The dried leaves of *C. argentea* had acceptable palatability for the rabbits, according to their acceptability in the diet and ingestion by the animals.

Table 3 Performance of white New Zealand rabbits of both sexes from 59 to 70 days of age fed a control diet (CTRL) or a diet containing dried leaves of *Cratylia argentea* (DRY).

Item	CTRL			DRY			CV (%)	P-value	
	Female	Male	M	Female	Male	M		Trea	Sex
Initial body weight, kg	1.27	1.37	1.32	1.23	1.38	1.30	16.2	0.763	0.015
Final body weight, kg	1.67	1.83	1.75	1.63	1.80	1.71	14.4	0.581	0.014
Average daily gain, kg/rabbit	0.03	0.04	0.03	0.03	0.03	0.03	19.1	0.845	0.496
Dry matter intake (DMI), kg/rabbit	0.11	0.12	0.12	0.11	0.12	0.12	10.1	0.831	0.108
Feed conversion ratio, DMI/kg	3.33	3.14	3.2	3.17	3.28	3.23	17.2	0.964	0.857

CV = coefficient of variation. M = Mean

However, the DMI did not differ ($p = 0.11$) between male and female rabbits. For GMD, CMS, and CA, no effects ($p > 0.10$) of treatment or sex were observed during the growth period between 66 and 70 days of age.

The energy value obtained through bromatological analysis of *C. argentea* leaves was 1,400.67 kcal/kg. This proved that it was a good energy source for inclusion in rabbit diets.

No difference ($p = 0.50$) was observed between treatments in the ADG (overall mean of 0.038 kg). Machado *et al.*, (2010), also reported that there was no effect on the ADG of rabbits fed with a commercial diet or Tifton 85 hay, between 30 to 72 days of age. The feed conversion ratio did not differ ($p = 0.86$) between treatments.

Digestibility data are presented in Table 4. No interactions ($p > 0.05$) between treatment by sex were observed for digestibility parameters from 59 to 70 days of age, when evaluated. For CDMD, a difference ($p < 0.01$) between treatments was obtained with CTRL rabbits presenting greater values than the DRY ones.

Table 4 Protein digestibility of the control (CTRL) and *Cratylia argentea* dried leaves (DRY) diet provided to white New Zealand rabbits of both sexes from 59 to 70 days of age.

Item	CTRL			DRY			CV (%)	P-value	
	Female	Male	M	Female	Male	M		Treat	Sex
Coefficient of dry matter digestibility, %	72.19	72.48	72.34	65.81	63.87	64.84	7.49	0.001	0.544
Crude protein intake (dry-matter basis), g/day/rabbit	21.25	22.77	22.01	19.27	20.16	19.72	14.81	0.021	0.205
Fecal excretion of crude protein (dry-matter basis), g/day/rabbit	4.34	4.72	4.53	5.63	5.85	5.74	22.04	0.003	0.400
Retained crude protein, %	79.39	79.14	79.27	70.74	70.68	70.71	8.02	0.001	0.953

CV = coefficient of variation. M = Mean.

A difference ($p = 0.02$) between treatments was observed with CP intake, with CTRL rabbits consuming more than DRY. A difference ($p < 0.01$) between treatments was observed for fecal extraction of CP, with DRY rabbits presenting greater values than CTRL.

4. Discussion

During the experimental period, the minimum temperature reached was 16.2°C, and the maximum temperature was 32.4°C; humidity ranged from 27% to 88%. Considering the maximum temperature, the rabbits were subjected to thermal stress (heat), as the comfort zone for growing animals varied from 15°C to 25°C. However, it did not affect their feed intake⁽¹¹⁾.

These results are similar to what was reported by Silva *et al.*⁽¹²⁾, who evaluated the levels of inclusion of Manioc plant dried leaves provided to New Zealand rabbits at 71 days of age; and by Retore *et al.*⁽⁷⁾, when they substituted citrus pulp and soybean hulls by alfalfa hay, provided to rabbits at 68 days of age. Male rabbits usually have greater nutritional requirements when compared to females, which can increase DMI.

Overall, the DMI reported in the present experiment was similar to the mean values reported by Michelan *et al.*⁽¹³⁾ and Ribeiro *et al.*⁽⁹⁾. Rabbits are animals that are fed balanced diets with the addition of alternative fibrous ingredients, without decreasing their performance, which demonstrates the great capacity of the species to take advantage of alternative forages for the high requirement of good quality fiber⁽¹⁴⁾.

According to Klinger *et al.*⁽¹⁾, FCR also did not differ when the control diet containing alfalfa hay was substituted by diets containing 25% or 50% grape pomace, with the mean FCR reported in the present study of 3.24, less than that reported by these authors (3.41).

The reduction in DM digestibility may be affected by fiber content and is directly related to the increased transit speed in the digestive tract as a whole^(11,2). Fiber digestibility is generally not affected by the concentration of dietary fiber, and the amount entering the cecum is not a limiting factor for the fermentation process, as the retention time is relatively

short, permitting the occurrence of degradation of the most easily digestible fractions, such as pectin and hemicellulose⁽¹⁴⁾.

The digestibility of rabbits can be strongly affected by their age, as the utilization of the diet is closely associated with when the animal starts ingesting the diet, leading to greater maturity of the digestive system, with increased enzyme production⁽¹⁵⁾. According to Klingler *et al.*⁽¹⁶⁾, one should also consider the quantity of microorganisms present in the intestinal flora and the composition of the diet used in animal feeding, as this can significantly contribute to the increase or decrease in the digestibility coefficients.

Dias *et al.*⁽¹⁷⁾, when evaluating the effect of the reduction of protein content in the diet, with and without enzymatic supplementation, on the performance of growing rabbits, reported that the mean values were lesser than the ones obtained herein. Intake regulation due to fiber quality is related to the increased lignification of the cellular wall, stimulating the increase of the cecum-colic mobility of rabbits, reducing retention time, and stimulating the consumption of the feed more frequently⁽³⁾.

The use of *C. argentea* in animal nutrition is interesting from our standpoint, as it exhibits good levels of dry matter and energy, correlating its digestibility time with the optimal utilization of nutrients in the diet⁽¹⁸⁾.

Increased digestibility values were reported by Ribeiro *et al.*⁽⁹⁾, mainly due to the higher fiber content when providing corn straw to growing rabbits. Nonetheless, those authors do not seem to have considered that in several species the true digestibility of CP is constant, but there is an apparent decrease as the content of this nutrient decreases⁽¹⁹⁾. Fekete and Bokori⁽²⁰⁾, showed that, as the difference between the fiber content and the CP content of the diet increase, rabbits consume a higher proportion of cecotropes, which would compensate, to some extent, the deleterious effect of fiber on CP digestion.

This fact would help explain some discrepancies found in the literature on CP digestion in rabbits. Gidenne⁽²¹⁾, highlighted that insoluble or indigestible fibers, despite having an important function in intestinal motility and physiological adequacy to cecotrophy, are negatively correlated with dietary energy concentration. The dry leaves of *C. argentea* have gross energy (GE) of 4,124.00 kcal/kg DM, digestible energy (DE) of 1,400.67 kcal/kg DM, and 3.34% digestible CP per kg of DM. Despite the values being below those obtained for alfalfa hay, which has approximately 3,948.00 kcal/kg DM, 1,897.57 kcal/kg DM, and 11.67% CP, respectively, we demonstrate that *C. argentea* can be used as its substitute, with adjusted inclusion levels⁽²²⁾.

5. Conclusion

It is concluded that the use of *Cratylia argentea* presents 1,400.67 kcal DE/kg DM and 3.34% DP/kg DM for growing New Zealand white rabbits, not affecting their performance or influencing their digestibility coefficients. It can be utilized from 59 to 70 days of age.

Declaration of conflict of interest

The authors declare no conflict of interest.

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