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Research Article
The Effects of Storing Ceara Rubber (*Manihot glaziovii*) Leaves in Different Forms on Crude Protein and Carotene Contents

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Abstract

Background and Objective: This experiment was conducted to determine the effect of pelleting on quality and storability of ceara rubber (*Manihot glaziovii*) leaves. **Materials and Methods:** Following a 2×4 factorial experimental design, ground or pelleted ceara rubber leaves were stored for 4, 6, 8 and 12 weeks. In the pelleting process, 15% DM cassava starch was added as a binder. Both ground and pelleted ceara rubber leaves were stored for 4, 6, 8, or 12 weeks in plastic bags. In the end of each period, nutritive quality of ceara rubber leaves was evaluated. **Result:** Pelleting the ceara rubber leaves decreased ($p<0.01$) the crude protein (from 21.7-19.7%) and total carotene (from 84.6-61.5 mg/100 g DM) contents. The crude protein content was not affected by the storage periods but a decrease ($p<0.01$) in the total carotene content after 12 weeks of storage was noticed. **Conclusion:** The pelleting with 15% DM cassava starch decreased the crude protein and total carotene contents of ceara rubber leaves but the storing period did not greatly affect those properties.

Key words: Ceara rubber leaves, crude protein, *Manihot glaziovii*, pellet, total carotene

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ceara rubber (*Manihot glaziovii*) is a glabrous shrub or tree with a height of up to 6 m high, although it is occasionally taller (10-20 m) and often has several weak branches from near the base. It is tapped to produce latex used to make ceara rubber and is named after the Brazilian state of Ceara, where it is grown. Due to the plant's relatively small size, compared to *Hevea brasiliensis* (commonly known as the rubber tree) and its horny outer bark, which makes it difficult to tap, *Manihot glaziovii* became the less preferable source of rubber latex compared to *Hevea brasiliensis*. While its use for rubber has diminished, it is frequently used in West Africa as a shady tree for cocoa plants[1].

Due to its great crude protein (CP) content in the leaves (>25%), ceara rubber has a potency to be used as alternative forage for ruminants. However, its high cyanide acid (HCN) content might be harmful when it is used as a protein source. The cyanide acid content in ceara rubber leaves is greater than that in bitter cassava (*Manihot utilissima*). The cyanide acid content in ceara rubber is approximately 0.22%[2], while bitter cassava contains 0.02-0.03% HCN[3]. The normal range of HCN content corresponds to 800-3200 mg kg^{-1} (0.08-0.32%). These levels are substantially higher than the normal range of HCN reported for fresh cassava roots[4].

In Indonesia, cassava forage is commonly used as forage for ruminants and through the process of preservation, such as hay-making, ensiling and pelleting, the HCN content can be significantly reduced or eliminated. One of the forage preservation methods that is commonly practiced by Indonesian farmers is drying the forages (hay making). Dry forages are used for ruminant feed as an energy source when fresh forages are not available. In addition, hay derived from legume or non-grass forages also may provide protein and minerals, as well as an energy source[5]. Another preservation method is pelleting the feed. Pelleting reduces the segregation among ingredients within the finished feed, ensuring that a balanced fraction is consumed; feed in a pelleted form also reduces the natural losses, such as wind loss and spillage loss[6].

Hay quality is decreased during storage due to improper storing methods, as well as the duration of storage. Packaging or bagging, as one of the storage methods of feed, protects feed material or the final products, making them more durable during storage. The increasing durability during storage is because the feed ingredients or final products are more protected from environment influences (temperature, humidity and oxygen), as well as insect invasion. This study was conducted to determine the effect of grinding (powder form) or pelleting on the nutritive value of ceara rubber leaves stored over twelve weeks.

MATERIALS AND METHODS

Materials: This study was conducted at the Laboratory of Feed Technology, Department of Animal Nutrition and Feed Science, Faculty of Animal Sciences, Universitas Gadjah Mada, Yogyakarta, Indonesia. The leaves of ceara rubber were collected from the Sleman area, Yogyakarta. After the fresh leaves samples were collected, they were dried under direct sunlight and then were ground to pass a 1.0 mm screen (Wiley mill, Standard Model 4; Arthur H. Thomas Co., Swedesboro, NJ). Half of the ground leaves was mixed with 15% binder (cassava starch) and processed to form pellets. Both the ground form and pellets were packed in polyethylene plastic bags with 5 replications and were then stored for 4, 6, 8 and 12 weeks, after which they were analyzed for the proximate composition and carotene. Thus, the design of this study followed a 2×4 factorial experimental design (2 forms of feed and 4 storage periods).

The data collected during this research included the crude protein (CP) and total carotene contents of the fresh and post-storage ceara rubber leaves, in accordance with the predetermined times. The determination of the chemical composition of the ceara rubber leaves was done using the Weende method[7] and the total carotene content was determined using the method described by Harris[8].

The data were analyzed using the General Linier Model Multivariate procedure of SPSS ver. 22 (IBM, USA). Comparisons of the means for the feed forms and storage periods were done by a contrast test with Duncan's new multiple range test[4] when the effects of the feed forms and storage periods ($p \leq 0.05$) were detected.

RESULTS AND DISCUSSION

Chemical composition: The fresh ceara rubber leaves contained 44.28% dry matter (DM), 6.54% ash, 26.14% CP, 8.67% ether extract (EE), 13.14% crude fiber (CF), 45.51% non-nitrogenous extract (NNE) and 108.27 mg/100 g total carotene. Our data showed that the CP content of the ceara rubber leaves was higher than 18.59%[9] but was similar to the 23.28% as reported by Noviadi *et al.*[10]. This wide variability was related to the differences in the cultivars, the stage of

maturity, the sampling procedure, the soil fertility and the climate[11]. With its high CP and low CF contents (>20 and <18%, respectively), the ceara rubber leaves are classified as protein source feedstuffs[11], especially for ruminants.

The DM, CP and total carotene contents of the ceara rubber leaves after sun-drying were 96.74%, 20.95% and 83.07 mg/100 g, respectively. These results showed that the CP and total carotene of the ceara rubber leaves decreased after the drying process by 19.85 and 23.27%, respectively. The decrease in the CP and total carotene of ceara rubber leaves was probably due to loss of volatile components during sun-drying.

Post-storage crude protein content: Table 1 shows the effect of the duration of storage of the ground and pelleted ceara rubber leaves on the CP contents. In general, the ground ceara rubber leaves tended to show a decrease in CP content over the 12 weeks of storage ($p<0.01$, Table 1). Additionally, the pelleted ceara rubber leaves tended to be lower in crude protein than ground ones, which was obviously due to the addition of starch as a binder during the pelleting process.

Nevertheless, both the ground and pelleted ceara rubber leaves are considered a suitable protein source, which are not affected by a storage duration of between 4 and 8 weeks. However, it appeared that the CP content of the ground ceara rubber leaves did not significantly differ when the leaves were stored for 4, 6 and 8 weeks but was significantly lower after 12 weeks of storage. Ravindran *et al.*[11] reported that cassava leaves meal (CLM) maintained its nutritive quality during storage; no mold or insect infestations were found after 8 months of storage. Interestingly, the cyanide content declined during storage but a gradual decline in the CP content was also noticed.

The pelleted form was not significantly different in the protein content over the 12 weeks of storage, which suggested that pelleting was a better mode of storing ceara rubber leaves, at least over 12 weeks, without affecting the nutritive value. The lower CP content of the pelleted ceara rubber leaves was due to the binder addition. In this study, cassava starch was added at 15% of the total weight as a binder. Since cassava starch is low in CP content, its addition into the pelleting process decreased the CP content of the pelleted ceara rubber leaves. In addition, the steaming process that is performed during the pelleting process might decrease the CP content of the pelleted ceara rubber leaves. This low CP content was probably related to the denaturation of protein due to its high temperature during the steaming process. Wahyono *et al.*[12] stated that the CP content of the pelleted cassava leave was 15.13%. The CP content of the pellet of cassava leaves depends on the type and percentage of the binder usage.

Post-storage total carotene content: Table 2 shows the carotene content of the ceara rubber leaves stored for 12 weeks in the powder and pelleted form. In general, the carotene content of the pelleted form was lower ($p<0.01$) than that of the ground form. On the other hand, there was no significant difference in the carotene content of the powder form over the 12 weeks of storage, while in the pelleted form, the carotene content was significantly lower at 12 weeks of

Table 1: Crude protein content (%DM) of the ceara rubber leaves powder and pellet post-storage (weeks)

	Feed forms		
Storage duration (weeks)	Powder	Pellet	Means
4	23.4±0.68	19.0±1.04	21.2±2.45
6	22.4±1.00	19.0±0.34	20.7±1.89
8	22.7±0.76	19.1±0.52	20.9±1.99
12	18.5±0.39	21.8±0.24	20.2±1.78
Means	21.7±2.05[a]	19.7±1.37[b]	

[a,b]The means in the same row with different superscripts differ at $p<0.01$

Table 2: Total carotene content (mg/100 g DM) of the ceara rubber leaves powder and pellet post-storage (weeks)

	Feed forms		
Storage duration (weeks)	Powder	Pellet	Means
4	84.0±2.38	66.3±1.44	75.1±9.55[x]
6	83.1±3.13	66.2±3.35	74.6±9.39[x]
8	86.6±2.24	61.1±2.87	73.8±13.65[x]
12	82.6±4.67	52.3±8.90	67.5±17.34[y]
Means	84.1±3.37[a]	61.5±7.44[b]	

[a,b]The means in the same row with different superscripts differ at $p<0.01$. [x,y]The means in the same column with different superscripts differ at $p<0.01$

storage compared to 4, 6 and 8 weeks of storage. The total carotene content of the ceara rubber leaves in the pellet form was lower ($p<0.01$) than those in the powder form (61.5 vs. 84.1 mg/100 g DM, Table 2).

As with the crude protein content, the low total carotene content of the pelleted ceara rubber leaves was caused by the addition of the binder during the pelleting process. Since cassava starch is low in carotene, adding it with the ceara rubber leaves diluted the total carotene content of the final product. The decreased total carotene content in the pellet also might be due to the partial destruction of the carotene contained in the leaves when steaming (heating) were performed during the pelleting process.

In terms of the storage ability, we found that a significant decrease in the total carotene content occurred when the products were stored for 12 weeks ($p<0.01$, Table 2). This significant decrease in the total carotene content of the ceara rubber leaves occurred due to the nature of the beta carotene when exposed to air. Erawati[13] reported that the double bond in the chemical structure of the beta carotene makes it very sensitive to the oxidation reaction when exposed to air (O_2), light, metal, peroxide and heat during processing. Therefore, prolonged contact between the ceara rubber leaves might increase the loss of total carotene content.

In the United States, most of the dried forages feed come from alfalfa and is called dehy. In Indonesia, this conservation method is applied on cassava leaves that have a similar CP content to alfalfa. Dried forage is ground and used in poultry feed, weaning pigs and horses. In addition to their CP content, cassava leaves powder is a useful source of minerals and vitamins and plays a role as unidentified nutritive factors, as well as source of pigments (carotene and xanthophyll) in yolks[5]. The use of dehy for chicken is very low (between 1-3%) and is usually up to 5% of the total ration. At low levels of inclusion, the feeding value of CLM for poultry is similar to that of dehydrated alfalfa meal. The gains were not significantly influenced by leaves meal inclusion but the feed intake and feed/gain increased as the leaves meals were incorporated above the 5% level. The performance of the bird fed CLM and the dehydrated alfalfa meal is similar[11].

Cassava forage is used as a good protein supplement for low-quality roughages in ruminant feeding. A good quality dried leaves powder should contain at least 17% CP[5]. Supplementing 50 g of CLM, containing 23.42% CP, in the diet of sheep fed *ad libitum* napier grass (10.18% CP) results in 48.57 g of average daily gain[14].

It can be stated that today's knowledge of cassava leaves is equivalent to what was known about the alfalfa approximately 60 years ago. Many, if not most, of the advances made with regard to alfalfa utilization may have similar applications to cassava leaves[11].

CONCLUSION

Ceara rubber leaves can be stored both in the powder and pelleted forms without much reduction in the crude protein and carotene contents. However, storing in the pelleted form resulted in a lower crude protein and carotene content, which was possibly due to the diluting effect of the cassava starch as a binder, as well as to the effect of the high temperature and steam during the pelleting process.

SIGNIFICANCE STATEMENT

This study discovers that pelleting is a best mode of storing ceara rubber leaves, at least over 12 weeks, without affecting the nutritive value. This study will help the researcher to understand the best ways to prevent the nutrient loss of ceara rubber leaves during storage.

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