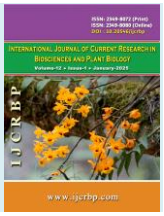
International Journal of Current Research in
Biosciences and Plant Biology

Volume **12** • Number **1** (January-2025) • ISSN: 2349-8080 (Online)

Journal homepage: www.ijcrbp.com



Original Research Article **doi:** https://doi.org/10.20546/ijcrbp.2025.1201.001

Contribution to the production of livestock food from the rachis and shell of cocoa (*Theobroma cacao L.*)

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Article Info

Keywords:
Animals
Cocoa
Food
Rachis
Shell

Abstract

Despite the efforts made by the Ivorian government, meeting the population's animal protein requirements remains largely dependent on external markets. To remedy this import problem, it is essential to find food resources that can cover needs and ensure the development and continuity of the sector. The aim of this study is to use cocoa by-products (shells and rachis) to produce animal feed. To achieve this objective, the physicochemical and nutritive characteristics, anti-nutritional factors and minerals of the shell and rachis were determined. Results showed that lipid, carbohydrate and protein contents were 0.67 ± 0.04 g/100g, 42.06 ± 0.18 g/100g and 5.86 ± 0.03 g/100g for the shell. The rachis, on the other hand, contained 0.97 ± 0.02g/100g lipid, 54.09 ± 0.06 mg/100g carbohydrate and 10.85 ± 0.01 g/100g protein. Tannin content is higher in the shell sample (91.92 ± 4.38 mg/100g) than in the rachis (27.81 ± 2.48 mg/100g). In addition, the anti-nutritional content (oxalates) is higher in the shell (926.75 ± 11 mg/100g) than in the rachis (880 ± 16.80 mg/100g). In other hand, the shell (98.74 ± 0.14%) has a higher antioxidant activity than the rachis (89.65 ± 0.12%). However, with the exception of calcium, the results showed that the rachis was richer in essential mineral elements than the shell. These cocoa by-products could be used as supplements in livestock feed. Concerning microbiological analysis showed that there was no significant difference between the two samples at the 5% threshold ($P > 0.05$). The shell sample showed $(1.3 \pm 0.1)\times10^2$ CFU/g of aerobic mesophilic germs (AMG) versus $(1.7 \pm 0.8)\times10^2$ CFU/g for the spine. These AMGs include *Bacillii cereus*, *Staphylococcus aureus* and moulds, with loads below microbiological criteria. *E. coli*, *Clostridium perfringens* and coliforms were not detected in any of the samples. *Salmonella* was also totally absent from the samples. These results show that the microbiological quality of the samples analyzed is satisfactory.

• *Received: 13 October 2024* • *Revised: 20 December 2024* • *Accepted: 28 December 2024* • *Published Online: 6 January 2025*

Introduction

Côte d'Ivoire has a deficit in animal products (Koffi-Koumi et al., 2001). Despite the efforts made by the Ivorian government, meeting the population's animal protein needs remains largely dependent on external markets (Koffi-Koumi et al., 2001). Livestock productivity remains low, and the country imports almost half of its meat requirements. External supply of livestock makes Côte d'Ivoire one of the biggest importers of ruminants (sheep and cattle) in West Africa (Anonymous, 2001). Animals, particularly sheep, imported from neighboring countries (Burkina and Mali) and Niger arrive by rail and/or road. They generally transit through Abidjan's main livestock market (Adjame) before being redistributed to secondary marketing sites in other districts (Attécoubé, Abobo, Yopougon, etc.).

Poor import conditions (length of journey, overcrowding in vehicles and/or wagons, poor feeding, parking at transit points, etc.) often lead to fatigue, weight loss and even disease in some animals. To make their trade profitable, breeders and other intermediaries first subject the animals to preliminary fattening. A form of short-term breeding, linked to the more or less prolonged presence of the animals on the markets, is practised, with the same sanitary and dietary requirements as those of ordinary livestock farming (grassland breeding, in enclosures and on transhumance). Good livestock nutrition is therefore essential to ensure better sales. Indeed, feed is an important factor in determining the production, weight and sale price of animals (Rivière, 1978). For the same author, the effects of a lack of food can be seen in both the quantity and quality of livestock products. The feed used by these livestock on livestock sales markets is of plant origin, but also comes from leftover feed, etc.). In the past, feeding sheep was often based on the natural vegetation of rangelands and fallow land. However, the reduction in grazing land and its low productivity due to climatic variations, in particular repeated droughts, have led to a situation where traditional food resources are insufficient to cover the needs of the flock. This situation “forces” recourse to the massive use of concentrated feeds and certain imported forages (alfalfa cap), the cost of which remains high given the rising prices of raw materials (corn, soybean meal, etc.) on the national and international markets (Mohamed-Brahmi et al., 2010). Faced with this situation, the search for alternative feed resources, enabling the long-term sustainability of the livestock sector or providing solutions for better coverage of feed requirements and ensuring the development and continuity of the sector is imperative. Some alternative feeds can partially or totally replace these costly feeds while being economically efficient without reducing animal performance (Obeidat et al., 2016). In other hand, for some time now, attempts have been made to valorize wastes for use as feed supplements or substitutes for animal feeds or even for humans (Kanouté, 2022). Given their availability, cost and importance in the Côte d'Ivoire economy, cocoa, particularly the shell and rachis, could be used as a component in livestock feed. The aim of this work is to contribute to the valorization of cocoa shell and rachis, two cocoa by-products, for the production of animal food.

Materials and methods

The plant material consisted of cocoa rachis and shells obtained from cocoa pods collected from fields in the Azguié locality (Fig. 1).

Sampling

Sampling took place in Azaguié (Abidjan Côte d'Ivoire) and sample collection in June 2024. The choice of these sites was justified by the availability of cocoa plantations. In these plantations, 10 kg of cocoa were harvested and deboned, then the shells and rachis were sent to the laboratory of the University of Nangui ABROGOUA for analysis. After harvesting, the shells and rachis were sorted, washed three times with tap water and then rinsed with distilled water. Next, 500 g of these samples were dried and ground to a powder for analysis.

Determination of physico-chemical and nutritive properties

Once the samples had been obtained, they were oven-dried at 45°C for 72 h and then ground using a blender. The resulting grindings were sieved using a 250 µm mesh sieve. The powdered samples were packaged in hermetically sealed, pre-dried, labelled vials. These were stored in a desiccator at 25°C until further use. Thus, the dry matter (DM) and Moisture content were determined gravimetrically in an oven at 105°C until a stable weight was obtained by Association of Official Agricultural Chemists (AOAC, 1990). In the same way, the method used for ash determination is that described

by AOAC (1990), which involves incinerating a sample at 550 ± 15°C for 12 to 24 h until white ash is obtained. The extraction of the lipid fraction was carried out using a Soxhlet Tecator in accordance to Association of Official Agricultural Chemists (AOAC, 1990) method. The total nitrogen determination was carried out using the Kjeldahl method (AOAC, 1990) and total protein was calculated by multiplying the total nitrogen by 6.25, the conversion factor calculated from the amino acid of total sample. The total carbohydrate content was obtained by the difference of protein, moisture, lipid and expressing the sum in grams of total carbohydrates/100 g of fresh sample. The total energy value (TEV) was calculated using the traditional conversion factors for proteins (4 kcal/g), lipids (9 kcal/g), and carbohydrates (4 kcal/g) according to FAO (2018). Fiber content was determined according to the method of AOAC (1990). Two (2) grams of each dried and ground sample were homogenized in 50 ml of 0.25 N sulfuric acid. The mixture was boiled for 30 min and 50 ml of 0.31 N sodium hydroxide added before heating to boiling for 30 min. The extract obtained was filtered on Whatman n°4 filter paper. The residue obtained was oven-dried at 105°C for 8 h and then oven-incinerated at 550°C for 3 h. The determination of tannins was carried out according to the method described by Bainbridge et al. (1996). A volume of 1 ml of methanolic extract was taken and to this volume was added 5 ml of vanillin reagent (50 g of vanillin + 4 ml of hydrochloric acid in 100 ml distilled water). Then the tube was left to stand for 20 min in the dark and the reading absorbance spectrophotometer (PG Instruments, England) was made at 500 nm against the blank. Finally, a calibration curve was made using a range of acid tannic acid with a concentration ranging from 0 to 2 mg/ml. The results were expressed in mg tannic acid equivalent (TAE)/100 g of dry matter (DM). Oxalates were determined according to the method described by Day and Underwood (2022). Two (2) grams of dried and ground sample were homogenized in 75 ml H2SO4 (3M). The resulting mixture was placed under magnetic stirring for 1 h at room temperature (28°C). The mixture was filtered through Whatman No. 4 filter paper. Twenty-five (25) ml of filtrate were hot-titrated with 0.05 M potassium permanganate ($KMnO_4$) solution until the color turned persistent pink. The minerals were determined by atomic absorption spectrophotometry according to the AOAC (1990) digestion method using strong acids. The content of each mineral element was determined using a VARIAN AA.20 brand flame atomic spectrophotometer at a specific wavelength by comparison with standard solutions.

Fig. 1: (A) Cocoa rachis, (B) cocoa shell and (C) cocoa pods.

Microbiological parameters

Ten (10) grams of each sample was weighed in sterile conditions near the flame of a beak bunsen, and put in 90 mL of diluent (peptone water buffered) content in a 100 mL bottle. This mixture was homogenized and a suspension at 10^{-1} East obtained. One (1) mL of the 10^{-1} suspension is subsequently homogenized in 9 mL of diluent in a test tube to obtain the 10^{-2} dilution. By the same technique, subsequent dilutions were made up to 10^{-x} dilution. Thus, the medium used for the counting of aerobic mesophilic germs (AMG) is PCA agar (Plate Count Agar; Oxoïd LTD, Basingstore, Hamsphire, England). The inoculation was done by incorporation into the agar mass and consisted of introducing 1 mL of the decimal dilutions in Petri dishes.. The seeded medium was next incubated at 30°C for 72 h. After this period incubation, the different colonies present in petri dishes containing between 15 and 300 colonies have summer counted (Standard NF V08-051., 1999).

The enumeration of *Escherichia coli* has summer the agar RAPID'*E.coli* (Standard NF ISO 16140, 2003). Incubation has made at 37°C for 24 h. Presumptive colonies *of Escherichia coli* were violet to pink. The search and enumeration of coliforms has was made on the agar lactose biliated with crystal violet and neutral red (VRBL agar) was used for the enumeration of coliforms. Incubation has made for 72 h at 30°C for coliforms totals and 44°C for coliforms fecal (AFNOR Standard, NF ISO 4832 July 1991). The colonies appeared purplish red, round with a diameter of 0.5 mm.

For the counting of yeasts and moulds was accomplished according to the standard French NF ISO 7954-1988. The medium used for the enumeration of yeasts and moulds is the agar Sabouraud with Chloramphenicol. The boxes seeded are incubated at 30°C for 24 to 72 h. After incubation, all colonies characteristic of yeasts (white, creamy, ovoid, smooth and shiny) and mold in appearance (filamentous, producing various colored pigments) have summer counted. Before any counting *Clostridium perfringens* the mother solution or decimal dilutions have summer bets In a bain-marie at 80°C for 10 minutes Then cooled immediately In water cold. This treatment made it possible to destroy the shapes vegetative. The enumeration of Anaerobes Sulfite Reducers (spores) is a numeration presumptive of *Clostridium perfringens*. The medium used is the agar tryptone sulfite to neomycin (TSN) (BioMérieux, France). For inoculation 1 mL of the stock suspension and / or decimal dilutions, have summer taken Then seeded in duplicate in the mass of the previously melted TSN agar then preserved in super-cooling in a tube at 45 ºC. The tubes have summer left to stand until the media have completely solidified. The tubes have summer next incubated in the oven at 46°C. A first reading was carried out after 24 h to prevent total blackening of the tube and a second after 48 h where the big black colonies visible in the tube have summer counted (Harmon et al., 1971).

The medium used for the detection and enumeration of *Staphylococcus aureus* was Baird Parker agar. The incubation has made at 35°C for 24 h. Presumptive colonies of *Staphylococcus aureus* were either shiny black, whole, convex, surrounded by light areas extending in the opaque medium either black shiny, whole, convex, without light zone GOOD defined Or either gray dark (Rosa et al., 2001). *Salmonella* research has summer realized according to the standard NF ISO 6579-2007. The isolation and identification of *salmonella* have summer made by streak seeding on the surface of the agar Hektoen help from a Pasteur pipette from the enrichment medium. Petri dishes have summer next incubated at 37°C. After 24 h incubation, boxes have summer examined in order to look for colonies that may be *salmonella*. Before any counting, the mother solution or decimal dilutions were bets in a bain-marie at 80°C for 10 minutes then cooled immediately. This treatment allowed to destroy the forms vegetative. The medium used for research and the *Bacillus* count was the agar Mossel. The incubation has made at 30 °C for 24 h. Presumptive *Bacillus* colonies were pink with the presence of a clear, opaque halo around the culture or yellow with the absence of an opaque halo (Mossel et al., 1967).

Statistical analyses

All tests relating to the different analyses were carried out in triplicate and the data obtained were expressed by the arithmetic mean affected by the corresponding statistical standard deviation. One-way analysis of variance (ANOVA) was carried out on all the results obtained in order to determine the existence of significant statistical differences between the values of the calculated means. Significant statistical differences were highlighted by the Duncan test at 95% confidence level. This statistical analysis was carried out using XLSTAT Version 2016 software.

Results and discussion

This study was carried out with a view to valorizing cocoa by-products (shells and rachis) for the production of new animal feed. The study showed that the moisture content was 7.3 ± 0.2% and 9.39 ± 0.2% respectively for shells and rachis, while dry matter was 92.71 ± 0.5% and 90.61 ± 0.17% respectively (Table 1). However, moisture is essential to sustain life and its analysis is one of the most widely used measurements in food processing. Moisture has been found to have a stable effect on livestock. Therefore, rachis with a high or considerable moisture content will be of great value for animal food. These values are close to those obtained by Okpako et al. (2008) in fermented cassava peelings (10.30%). In other hand, the low moisture contents observed could be justified by the drying process under study, as well as the drying time. Lipids, proteins and carbohydrates were higher in the rachis sample (0.97 ± 0.02 g/100 g, 10.85 ± 0.01 g/100 g and 54.09 ± 0.06 mg/100 g) (Table 1). The presence of these compounds in shell and rachis would be an advantage for animal food. Lipids are important macronutrients in nutrition. They help increase the energy density of feed (Tenagashaw et al., 2017) and improve the nutritional composition of fat-soluble vitamins (A D E K) by facilitating their availability to the body (Amegovu et al., 2013; Levitsky and Potapova, 2015).

Table 1. Physicochemical composition of cocoa shell and rachis samples.

Composition	Shell	Rachis
Dry matter (g/100 g)	92.71 ± 0.5 [a]	90.61 ± 0.17 [b]
Rate moisture (g/100 g)	7.3 ± 0.2 [b]	9.39 ± 0.2 [a]
Protein (g/100 g)	5.86 ± 0.03 [b]	10.85 ± 0.01 [a]
Lipids (g/100 g)	0.67 ± 0.04 [b]	0.97 ± 0.02 [a]
Sugars reducers (g/100 g)	1.49 ± 0.02 [b]	1.42 ±0.02 [b]
Carbohydrates (g/100 g)	42.06 ± 0.18 [b]	54.09 ± 0.06 [a]
Fiber (g/100 g)	42.12 ± 0.13 [a]	27.96 ± 0.03 [b]
Ash (g/100 g)	9.4 ± 0.12 [a]	6.36 ± 0.27 [b]
Value energy (Cal)	197.6 ± 0.94 [b]	268.49 ± 0.08 [a]

The values obtained are means ± standard deviations determinedin three tests. On an even line, values that carry the same letter do not present any difference significant at the 5% threshold.

Proteins play an essential role in the construction and repair of the animal organism (Hayat et al., 2014). In humid tropical conditions, it has been reported on sheep fattening that nitrogen values of 90 g MAD/UFL are required to obtain GMQs of 50 g/d in these animals (Babatounde et al., 2009). Carbohydrates are also a serious source of vitality in animal feed. In fact, carbohydrates are mainly represented by starch, sugars and cellulose (not easily digestible in food). This carbohydrate content could have beneficial effects in animal feed. However, according to Cheeke and Dierenfeld (2010), in ruminants, too high a proportion of carbohydrates can activate fermentation in the rumen, lowering ruminal pH to levels that are detrimental to the animal's long-term health and productivity. In other hand, the energy value of the rachis (268.49 ± 0.08 kcal) is higher than that of the shell (197.6 ± 0.94 kcal) (Table 1).

Energy is one of the most decisive elements in animal growth. The high total carbohydrate content is the main factor explaining this good energy value. Fiber content is higher in the shell (42.12 ± 0.13%) than in the rachis (27.96 ± 0.03 mg/100 g). This fiber content in cocoa rachis and cocoa shells could increase feed bulkiness and promote poor digestibility and absorption of nutrients such as proteins and minerals, as reported by (Olorunfemi et al., 2006). According to (Sanni et al., 1999), a high ash content is most often attributed to the mineral richness of the feed. Thus, the ash content was higher in the shell (9.4 ± 0.12%) than in the rachis (6.36 ± 0.27%) (Table 1). The ash composition of food samples is very important in determining mineral content. The cocoa shell would be richer in mineral elements than the rachis. The mineral profile of cocoa shell and rachis showed the presence of macro-elements such as potassium (K), calcium (Ca), phosphorus (P), sodium (Na) and magnesium (Mg), and trace elements such as iron (Fe) and zinc (Zn) in significant quantities (Table 2). Minerals play an essential role in animal health. They are involved in several bodily functions such as enzymatic reactions, energy production, transmission of nerve impulses, and multiple biological reactions. Iron is an essential constituent element of hemoglobin and is also involved in numerous enzymatic reactions (Abbaspour et al.,

2014). As a result, cocoa rachis and cocoa shell could be a good match for use as animal food additives. With regard to the analyses carried out on polyphenols, flavonoids and tannins, contents were higher in the shell with high antioxidant activity. The polyphenol, flavonoid and tannin contents, as well as the anti-radical power of the cocoa by-product samples, are presented in Table 3.

Table 2. Mineral content of cocoa shells and rachis.

Parameters	Mineral content (mg/100 g)	
	Shell	**Rachis**
Magnesium	15.57 ± 0.07 [b]	21.03 ± 0.03 [a]
Iron	0.74 ± 0.00 [b]	6.12 ± 0.01 [a]
Phosphorus	8.79 ± 0.43 [b]	17.10 ± 0.03 [a]
Calcium	0.54 ± 0.02 [a]	0.51 ± 0.02 [a]
Sodium	7.25 ± 0.01 [b]	10.26 ± 0.03 [a]
Zinc	0.12 ± 0.00 [b]	0.67 ± 0.01 [a]
Potassium	15.57 ± 0.62 [b]	21.03 ± 0.56 [a]

The values obtained are means ± standard deviations determinedin three tests. On an even line, values that carry the same letter do not present any difference significant at the 5% threshold.

Table 3. Content in compounds phenolic samples.

Sample	Polyphenols (mg/100 g)	Flavonoids (mg/100 g)	Tannins (mg/100 g)	Activity antioxidant (%)
Shell	2.65 ± 0.04 [a]	0.18 ± 0.02 [a]	91.48 ± 4.48 [a]	98.72 ± 0.14 [a]
Rachis	0.95 ± 0.02 [b]	0.1 ± 0.00 [b]	27.97 ± 2.97 [b]	89.65 ± 0.12 [b]

The values obtained are means ± standard deviations determinedin three tests. On an even column, values that carry the same letter do not present any difference significant at the 5% threshold.

Polyphenol contents are estimated at 2.65 ± 0.04 mg/100 g for the shell and 0.95 ± 0.02 mg/100 g for the rachis. Flavonoids content is 0.18 ± 0.02 mg/100 g for the shell and 0.1 ± 0.00 mg/100 g for the rachis. Tannin levels are higher in the hull (91.48 ± 4.48 mg/100 g) than in the rachis (27.97 ± 2.97 mg/100 g). In other hand, antioxidant activity was higher in the shell (98.72 ± 0.14 mg/100 g) than in the rachis (89.65 ± 0.12 mg/100 g). The presence of these phytochemicals in the samples could have a beneficial effect on animal food. Indeed, these antioxidant compounds are known as substances that inhibit or slow down the oxidation of a substrate. They therefore play an important role in the body, whatever their concentration in the feed. The positive correlation coefficients (r = 0.999; r = 0.963; r = 0.998) obtained between antioxidant activity and polyphenols, total flavonoids and condensed tannins testify to the responsibility of these compounds in the antioxidant activity of the samples analyzed (Table 4).

Table 4. Pearson correlation matrix.

Phytochemicals	Activity antioxidant
Polyphenols	0.999 *
Flavonoids	0.963 *
Tannins	0.998 *

*** :** Significant correlation.

These results are in line with those reported by Yong et al. (2009) and Coulibaly et al. (2023). These authors emphasized that condensed tannins were responsible for antioxidant activity. In terms of anti-nutritional factors, oxalates (98.74 ± 0.14% for the shell and 89.65 ± 0.12 for the rachis) and phytates (112.12 ± 2.42% for the shell and 334.75 ± 1.53% for the rachis) (Table 5). These values are below the recommended norms, since the oxalate dose is between 2,000 and 5,000 mg oxalate/100 g food (Mpondo et al., 2012) and the phytate dose between 250 and 500 mg phytate/100 g food. In other hand, it is important to take into account anti-nutritional factors in animal food rations. Indeed, when these factors are not respected, the performance of the animals consuming these rations does not reach the expected levels. The presence of these factors therefore calls for treatments that can limit their presence in animal feed (cooking and drying). In addition, knowledge of a feed's oxalate content is necessary because high levels of these anti-nutritional compounds have deleterious effects on digestibility (Acho et al., 2014).

Oxalates form complexes with essential minerals, making minerals unavailable to the body. In other hand, Table 6 shows the microbiological quality of the samples analyzed. The shell sample showed $(1.3 \pm 0.1)\times10^2$

CFU/g aerobic mesophilic germs (AMGs), compared with $(1.7 \pm 0.8)\times10^2$ CFU/g for the spine. These AMGs included *Staphylococcus aureus*, *Bacillus*, yeasts and moulds at loads below microbiological criteria. *Clostridium perfringens*, Coliforms and *E. coli* were not detected in the two samples analyzed. *Salmonella* was also absent in both types of sample. It could be said that the microbiological quality of the samples analyzed was satisfactory. However, the presence of *Bacillus*, staphylococci and moulds in these samples could present a risk. In addition, these microorganisms produce heat-resistant toxins or mycotoxins (moulds) which, when ingested, can cause gastro-enteric illness and often death in the consumer (Harvey et al., 2007). Toxins and mycotoxins must therefore be measured before any conclusions can be drawn.

Table 5. Compounds antinutritional samples.

Sample	Antinutritional compounds (mg/100 g)	
	Oxalate	Phytates (mg/100 g)
Shell	926.75 ± 11 [a]	112.12 ± 2.42 [b]
Rachis	880 ± 16.80 [a]	334.75 ± 1.53 [a]

The values obtained are means ± standard deviations determinedin three tests. On an even column, values that carry the same letter do not present any difference significant at the 5% threshold.

Table 6. Microbiological load of samples

Parameters	Microbial loads (CFU/g)	
	Shell	Rachis
Aerobic mesophilic germs	$(1.3 \pm 0.1)\times10^{2\,a}$	$(1.7 \pm 0.8)\times10^{2\,a}$
Bacillus cereus	$(1.1 \pm 0.1)\times10^{1\,a}$	$(1 \pm 0.2)\times10^{1\,a}$
Staphylococcus aureus	$(2.3 \pm 0.1)\times10^{1\,a}$	$(2.7 \pm 0.6)\times10^{1\,a}$
E.coli	$<1^{a}$	$<1^{a}$
Moulds and Yeasts	$(1.6 \pm 0.5)\times10^{1\,a}$	$(1.9 \pm 0.1)\times10^{1\,a}$
Coliforms	$<1^{a}$	$<1^{a}$
Salmonella	nd	nd
Clostridium perfringens	$<1^{a}$	$<1^{a}$

The values obtained are means ± standard deviations determinedin three tests. On an even line, values that carry the same letter do not present any difference significant at the 5% threshold. **nd:** not detected.

Conclusions

The Ivorian State, through various projects and programs at the level of the Ministry of Animal Production and Fisheries Resources, aims to make Ivory Coast a self-sufficient country in animal protein. It is in this perspective that this study is part of the aim of promoting the shell and the rachis, cocoa by-products in animal feed in order to boost the search for food resources to ensure the development of this sector. The results showed that the rachis is richer in protein, lipids, carbohydrates and mineral elements with the exception of calcium. These cocoa by-products contain antioxidant compounds that can be useful for animal feed. In addition, compounds such as oxalates and phytates are present in minimal quantities and cannot affect animal health. On the microbiological level, the microbiological quality of the samples remains satisfactory. The results obtained from our study on cocoa by-products could allow us to consider their use as inputs in the composition of livestock feed or food supplements.

Conflict of interest statement

Authors declare that they have no conflict of interest.

Acknowledgement

The authors thank the reviewers for their constructive comments, which helped to improve the manuscript.

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How to cite this article:
Touré, M., Krabi, E. R., Tra Bi, E. C., Kouamé, K. A., Bouatenin, K. M. J-P., Niamké, S., 2025. Contribution to the production of livestock food from the rachis and shell of cocoa (*Theobroma cacao L.*). Int. J. Curr. Res. Biosci. Plant Biol., 12(1): 1-9. **doi:** https://doi.org/10.20546/ijcrbp.2025.1201.001