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Apparent digestibility of nutrients in diets based on dried Okara (Solid residue from soy milk and cheese production) in growing rabbits in Benin

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**ABSTRACT**

In order to valorize okara, a locally available agricultural co-product in Benin, a seven day *in vivo* digestibility trial was conducted at a research station. The objective was to evaluate apparent digestibility coefficients of nutrients in diets based on dried okara (DO), used as a total substitute for soybean meal in growing rabbits. Three iso-protein and iso-energy diets (A0, control diet: 0% DO; A10: 10% DO; A20: 20% DO) were tested on eighteen growing rabbits with an average live weight of 1759 ± 80.18 g, distributed into three homogeneous groups according to a Randomized Complete Block Design. Feed intake slightly decreased with the inclusion of DO (71.4–72.6 g DM/day) compared to the control (78.6 g DM/day). However, overall digestive efficiency remained stable, and the ADC of dry matter (73–79%) and organic matter (74–81%) were not significantly ( $p > 0.05$ ) affected. The digestibility of Crude Protein remained constant (70–73%), confirming the good biological value of okara nitrogen. Crude fiber was better used with diet A20 (56.2%), while a peak in fat digestibility (91%) and total ash digestibility (76.7%) was observed with diet A10. These results show that dried okara can be incorporated up to 20% in the diet of growing rabbits, providing a local and sustainable alternative to soybean meal for the formulation of efficient rabbit feeds in tropical regions.

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## INTRODUCTION

In tropical regions, and particularly in Benin, rabbit farming appears to be an effective solution for meeting the population's animal protein needs. However, dependence on soybean meal (the primary protein feed source, which is very expensive and subject to market fluctuations) remains the main factor limiting the development of this sector. In this context, the valorization of available local agricultural by-products offers an alternative (Alabi *et al.*, 2018). Soybean pulp, or okara, belongs to this group (Alabi *et al.*, 2019). Okara is the solid residue obtain from the production of soy milk and soy cheese (Iyegh-Erakpotobor *et al.*, 2006). It is rich in protein (24–27% DM) and fiber (15–20% DM), making it a valuable feed resource for rabbits (Alabi *et al.*, 2018; Odunlade and Uza, 2021).

However, as Villamide *et al.* (2010) point out, the truth nutritional value of a feed resource lies not only in its chemical composition, but specifically in its digestibility. Indeed, *in vivo* digestibility provides a better understanding of the percentage of nutrients (organic matter, amino acids, fat, and Métabolisable Energy) absorbed in the digestive tract that constitute the fraction available for the animal's metabolism (INRA, 1989).

Although the positive impact of okara on the weight gain performance of rabbits have been demonstrated by Alabi *et al.* (2018) in northern Benin, aspects related to its digestibility remain to be explored. To understand how the synergy between okara fiber and the digestive physiology of fattening rabbits optimizes nitrogen utilization, this study focused on determining the digestibility coefficients of nutrients in complete diets in which soybean meal was totally replaced by okara.

This study was aimed to evaluate the efficiency of the digestive utilization of dried okara as a total substitute for soybean meal in growing rabbits in Benin.

## MATERIALS AND METHODS

### Study area

The study was conducted at the research station of the Laboratory for Animal and Aquatic Health and

Nutrition (LASNAH) of the National Institute for Agricultural Research of Benin (INRAB), located in the Atlantic Department in southern Benin. Atlantic Department is characterized by a Sudanian-Guinean tropical climate (Adam and Boko, 1993), with an annual rainfall ranging from 1.100 to 1.350 mm, an average temperature varying between 26 and 28 °C, and the alternation of four distinct seasons, including two rainy seasons and two dry seasons (Données Mondiales, 2024). The Atlantic Department is a favorable area for rabbit farming, where year-round availability of okara is ensured by the continuous local production of soy cheese by small processing units.

### Preparation and drying of okara

The fresh okara used was obtained from a small soybean processing facility located in the municipality of Abomey-Calavi, Atlantic Department. The collected okara was produced according to the manufacturing process reported by Alabi *et al.* (2019) and transported to a research station for solar drying. Following FAO (1995) guidelines, the fresh okara was spread in thin layers on clean tarps and turned regularly to ensure uniform dehydration.

Drying lasted seventy-two (72) hours, with the okara stored overnight in a ventilated room to prevent re-moistening from dew. Visual inspection and touch were used to ensure the absence of residual moisture in the okara and to confirm that it was completely dry before packaging it in polypropylene bags and storing it in a well-ventilated warehouse until its subsequent use in the production of the test foods.

### Experimental diets

Three (3) isoprotein and isocaloric diets meeting the nutritional requirements of growing rabbits were formulated and produced. In these diets, soybean meal (the reference protein source in the control diet (A0, 0%)) was completely replaced by dried okara (DO) at inclusion levels of 10% (A10) and 20% (A20), respectively. Apart from the two

(02) protein sources mentioned, the other feed ingredients used in the manufacture of the tested diets were grain corn, wheat bran, rice bran, cottonseed meal, palm kernel meals, as well as

mineral and amino acid supplements (oyster shell, lysine, methionine, and salt). The detailed composition of experimental diets is presented in Table 1.

**Table 1.** Ingredient composition of experimental diets

Feed ingredients	Diets (% FM)		
	A0	A10	A20
Dried okara (DO)	0	10	20
Maize grain	27.25	27	26
Wheat bran	18	17	15
Rice bran	8	6.25	5
Soybean meal	10	0	0
Cottonseed meal	5	8	5.5
Palm kernel meal	29	29	26
Oyster shell	2	2	1.75
Lysine	0.1	0.1	0.1
Methionine	0.1	0.1	0.1
Nacl	0.55	0.55	0.55
Total	100.0	100.0	100.0

FM = Fresh matter; DO = Dried Okara; A0 = Diet containing 0% DO (control); A10 = Diet containing 10% DO; A20 = Diet containing 20% DO

### Experimental animals and design

The study involved eighteen (18) male and female rabbits, ten (10) weeks of age, of mixed breeds, and sourced from partner farms supervised by LASNAH. At the beginning of the experiment, the animals had an average live weight of  $1759 \pm 80.18$  g. Based on their live weights, the rabbits were allocated according to a randomized complete block design into three (03) groups of six (06) rabbits, each rabbit representing an experimental unit, thus providing six (06) replicates per treatment. Each group corresponded to one dietary treatment representing a level of DO inclusion in the diet (A0, A10, and A20). Each rabbit was housed individually in a galvanized wire cage measuring 40 cm × 35 cm × 40 cm, equipped with a feeder, a waterer, and a device for collecting total feces.

### In vivo digestibility evaluation

The nutrient digestibility study was carried out in growing rabbits using the protocol described by Atchadé *et al.* (2020). Rabbits were acclimated for seven (07) days on an *ad libitum* basis to their respective diets before being subjected to a twelve (12)-hour fast. After this adaptation period, the actual digestibility measurement protocol extended over seven (07) days. Following initial fast, a five-day (05)

consecutive total collection phase was conducted, during which each rabbit received a fixed daily amount of 100 g DM with free access to drinking water. This period ended with a second twelve hours (12) fast. During the feeding phase, the amounts of feed served and feed leftovers were weighed daily for each individual to determine feed intake (FI). At the same time, total feces were collected at a fixed time each morning, by rabbit, then grouped by dietary treatment before being stored in airtight containers in the refrigerator at +4 °C. At the end of the collection period, the collected droppings were weighed, thoroughly homogenized, and sampled by feed type for laboratory determination of the content of Dry Matter (DM), Crude Protein (CP), Ether Extracts (EE), Crude Fiber (CF), and Total Ash (TA), according to official AOAC methods (2002). The same analyses were performed on the feed offered and feed leftovers.

The analytical results were used to calculate the Apparent Digestibility Coefficients (ADC) for DM, OM, CP, EE, and TA, in experimental, using the following formula:

$$\text{ADC} = \left[ \frac{\text{Quantity ingested} - \text{Quantity excreted}}{\text{Quantity ingested}} \right] * 100.$$

### Statistical analysis

Data were processed in Excel and analyzed using Statistix 10.0.0.9 software. A one-way analysis of variance (ANOVA) was used to examine the effects of the incorporation level of dried okara (n = 3: 0, 10, 20%) on apparent digestibility coefficients (DM, OM, CP, EE, CF, and TA) obtained. Mean values with their standard error (SE) were presented in tables. When significant differences were detected, Tukey's HSD test was as used to separate means at the 5% significance level.

## RESULTS AND DISCUSSION

### Nutritional value of dried okara and experimental diets

The nutrient contents of dried okara (DO) and the experimental diets are presented in Table 2. The analyses conducted showed that okara and the

experimental diets had a high dry matter content ranging between 88 % and 90 %. This suggests good storage stability, as demonstrated by Atchadé *et al.* (2020) for cereals, facilitating storage and use in rabbit farming. These values are close to the 89% obtained by Abdullahi *et al.* (2021) for dried okara, confirming the stability of this byproduct across different contexts.

The organic matter content (95.21% DM) was slightly higher than that of the experimental diets (91–94.5% DM), which may be related to the higher mineral content from the other ingredients used in their formulation. Indeed, Wafar *et al.* (2017) emphasized that an increase in total ash reduces the proportion of organic matter in byproducts. Our results are consistent with those of Odunlade and Uza (2021), who reported organic matter values ranging from 93 to 95% DM for okara.

**Table 2.** Nutritional value of dried okara and experimental diets

Composition	Dried okara	A0	A10	A20
Dry matter (%)	88.83	88.51	88.79	90.01
Organic matter (% DM)	95.21	93.31	90.90	94.45
Crude protein (% DM)	26.37	21.86	21.47	21.73
Ether extracts (% DM)	6.87	4.95	6.25	4.33
Crude fiber (% DM)	11.50	14.21	15.16	15.49
Total ash (% DM)	4.79	6.69	9.10	5.55
Metabolizable energy (kcal/kg DM)*	3 109	2 687	2 575	2 586

A0 = Diet containing 0% DO (control); A10= Diet containing 10% DO; A20= Diet containing 20% DO.

\*Metabolizable Energy (ME) was calculated using formula: Metabolizable energy (ME)= 3951 + (54.4 × ether extracts) – (40.8 × total ash) – (88.7 × crude fiber) (Sibbald *et al.*, 1980)

Dried okara was rich in crude protein (26.37% DM). This profile confirms that okara is a byproduct with high nutritional density capable of replacing conventional protein sources (which are often more expensive) in animal feed (Abdullahi *et al.*, 2021). Similarly, this high protein content confirms the findings of Yuporn *et al.* (2012), who showed that okara is particularly rich in lysine, compensating for deficiencies in cereals. Our results fall within the 25–28% DM range of crude protein in dried okara reported by Čech *et al.* (2022). They corroborate the 26.3% DM observed by Abdullahi *et al.* (2021), but are slightly higher than the 24% reported by Odunlade and Uza (2021). In contrast, they are lower than the protein content of 34.5% DM found by Alabi *et al.* (2018).

Ether extracts content of okara, at approximately 7% DM, contributed to its high metabolizable energy content of 3,109 kcal/kg DM. This high energy value is beneficial for rabbits, since according to De Blas and Wiseman (2010), lipid-derived energy helps to limit the risk of pathogenic caecal fermentations associated with excess starch. Our value is close to the 3033 kcal/kg reported by Abdullahi *et al.* (2021), but higher than the 2,837 kcal/kg found by Odunlade and Uza (2021). It also corresponds to the fat contents of 8–10% reported by Kamble and Rani (2020) and Čech *et al.* (2022), confirming the energy density of dried okara.

Metabolizable energy showed a slight decrease in the diets incorporating okara (A10 and A20) compared to

the control diet AO, mainly due to the increase in total ash observed in diet A10 (9.10% DM). This variation may be explained by the energy dilution effect induced by the mineral content when using by-products, as noted by Villamide *et al.* (2009).

Nevertheless, the Metabolizable energy values (2575 to 2687 kcal/kg DM) remain within the optimal range to support the growth performance of local rabbits without inducing metabolic disorders (Benali *et al.*, 2018; Xiccato and Trocino, 2010).

The crude protein contents of the experimental diets remained similar, at around 21% DM. This isoproteinemia diet is essential for comparing the efficiency of digestive utilization of alternative feed resources, without bias related to the amount of nitrogen ingested (Villamide *et al.*, 1998). Furthermore, although the crude protein level of 21% DM exceeds the minimum protein requirement for rabbit growth, it is not out of line with complete feed formulation practices in tropical regions, which aim to maximize the growth potential of local breeds (Alabi *et al.*, 2018; Alabi *et al.*, 2019).

The crude fiber content was moderate in dried okara (11.5% DM) but increased gradually with its incorporation into the diets, rising from 14.21% DM (AO) to 15.16% DM (A10) and to 15.49% DM (A20).

This linear increase confirms that okara, while being a source of protein, contributes significantly to fiber intake, as highlighted by Abdullahi *et al.* (2021). By reaching 15% DM in the diets containing DO, the digestive safety recommendations reported by De Blas and Wiseman (2010) - which advocate for 15% fiber to prevent caecal pathologies in rabbits - are respected. The crude fiber contents obtained in this study are slightly lower than those reported by Alabi *et al.* (2018) (15% DM) and by Odunlade and Uza (2021) (14.81% DM), respectively.

Based on the results obtained, dried okara proves to be a feed resource with high nutritional value, making it a particularly interesting byproduct for rabbit feeding. However, variations in this nutritional value are mainly explained by differences in production processes, soybean variety used, degree of dehydration, and processing and storage conditions (Kamble, 2020; Čech *et al.*, 2022).

### Nutrient digestibility of rabbits fed dried okara-based diets

Digestibility is a key criterion for assessing the true value of okara in rabbit diets. The apparent digestibility coefficients (ADC) of the nutrients contained in okara-based diets, recorded during the *in vivo* digestibility study in rabbits, are presented in Table 3.

**Table 3.** Nutrient digestibility of rabbits fed dried okara-based diets

Parameters	Diets (Mean ± Standard Error)			p-value
	A0	A10	A20	
Feed intake (g DM/day)	78.57 ± 1.93 <sup>a</sup>	72.57 ± 2.37 <sup>b</sup>	71.40 ± 2.85 <sup>b</sup>	0.0110
Apparent digestibility coefficients (%)				
Dry matter (%)	79.31 ± 2.17	73.41 ± 2.20	78.71 ± 2.88	0.2053
Organic matter (% DM)	81.75 ± 1.93	74.63 ± 2.12	81.46 ± 2.54	0.0646
Crude protein (% DM)	70.46 ± 3.29	69.13 ± 2.62	73.60 ± 3.87	0.6256
Ether extracts (% DM)	85.40 ± 1.80 <sup>b</sup>	91.03 ± 0.91 <sup>a</sup>	85.10 ± 2.24 <sup>b</sup>	0.0409
Crude fiber (% DM)	40.08 ± 6.23	47.52 ± 4.35	56.20 ± 5.95	0.1580

AO = Diet containing 0% DO (control); A10 = Diet containing 10% DO; A20 = Diet containing 20% DO. Values followed by different letters in the same row (a, b) indicate significant differences ( $p < 0.05$ ).  $p$  values = probability value for the significance threshold.

### Feed intake

The results (Table 3) showed that feed intake decreased significantly ( $p = 0.011$ ) with the inclusion of okara (78.57 g DM/day for AO versus 72.57 and

71.40 g DM/day for A10 and A20, respectively). This reduction can be attributed to the more fibrous texture of diets containing okara, which slightly limits voluntary intake, as previously observed by Villamide

*et al.* (2009) with other fiber-rich byproducts. The feed intake recorded for DO-based diets in this study was lower than that reported by Abdullahi *et al.* (2021), who observed an average intake of 74 g DM/day in rabbits fed diets containing dried okara. These values are relatively close to the 70-73 g DM/day reported by Odunlade and Uza (2021) in their study on the nutritional value of soybean meal on digestibility and visceral organs of growing rabbits in Nigeria. However, it remains higher than the 68 g DM/day recorded by Alabi *et al.* (2018).

These variations suggest that palatability and feed intake are strongly influenced by the quality of the okara used, the feed formulation conditions, and also by the age of the rabbits used in these experiments.

#### **Digestibility of dry matter and organic matter**

The digestibility of dry matter and organic matter was not significantly ( $p > 0.05$ ) affected by the inclusion of okara in the diet, with values ranging from 73% to 82% (Table 3). The values for organic matter digestibility (74.6% to 81.7% DM) are fully comparable, and even higher than, the 74-75% DM reported by Benali *et al.* (2018) in local rabbits in Algeria. This confirms that inclusion of okara into diet does not impair the overall digestibility of the ration, even in a context of slightly reduced intake compared to the 76-86 g DM/day observed by those authors.

#### **Crude protein digestibility**

The stability of the apparent digestibility coefficients for Crude Protein (69.13% DM to 73.60% DM;  $p = 0.6256$ ) demonstrates that the nitrogen in okara has a biological value equivalent to that of the conventional protein source, soybean meal. This performance can be explained by excellent enzymatic accessibility: the heat treatment applied during soy milk extraction denatured trypsin inhibitors, thereby facilitating protein hydrolysis by pancreatic proteases and ensuring rapid release of amino acids in the ileum (Villamide *et al.*, 1998).

Protein degradation, facilitated by the combined action of digestive enzymes and caecal microflora,

may explain this efficiency, confirming the good valorization of okara nitrogen in diets containing it. This bioavailability is all the more remarkable as it was not penalized by the increase in fiber content (14.21 % to 15.49 % DM of CB) in diets supplemented with okara. On the contrary, this digestive synergy was evident in diet A20, which exhibited the highest crude protein ADC (73.60 %), illustrating that a 20 % inclusion does not impair digestive utilization in any way. Furthermore, from a physiological standpoint, this demonstrates that okara nitrogen was not irreversibly sequestered in the insoluble lignin fraction (Abdullahi *et al.*, 2021), thereby avoiding the dilution effect usually caused by accelerated transit. Moreover, the balance between lipids and fermentable fiber in diets A10 and A20 stabilized the caecal ecosystem (De Blas and Wiseman, 2010), promoting constant microbial protein synthesis, recycled via cæcotrophy. Indeed, cæcotrophie, a mechanism specific to rabbits, allows recovery of digestive losses and ensures stable nitrogen assimilation even in the presence of higher fiber fractions (De Blas and Wiseman, 2010). Our apparent crude protein digestibility coefficients (69-74 % DM) are significantly lower than the very high values obtained by Odunlade and Uza (2021) (81-90 % DM) with okara inclusion, probably due to differences in diet formulation (in combination with maize, whole soybeans, and brewer's grains) and experimental conditions. However, the conclusion remains consistent in that okara can be included up to 20 % without compromising nitrogen assimilation. The concordance of our results with the recommendations of De Blas and Mateos (2010) (70-75 %) in terms of crude protein digestibility confirms that okara-based diets meet the physiological requirements of growing rabbits.

#### **Fat digestibility**

Unlike the nitrogenous fraction, the apparent digestibility coefficient of Ether Extracts was significantly influenced ( $p = 0.0409$ ) by okara level into diets. This digestibility ranged from 85 % to 91 % DM, with a significant improvement observed at 10 % okara inclusion (A10: 91.0 %), followed by a decrease

at 20 % (A20: 85.1 %). These values reflect an overall high digestibility, confirming that okara lipids are well assimilated by rabbits. The high Ether Extracts ADC obtained with A10 may be closely linked to the higher lipid content of this diet, which likely results from a formulation effect-specifically, the inclusion of a higher level of cottonseed meal (8% versus 5% for the other two diets). This high lipid content promoted increased digestibility by reducing the relative proportion of endogenous lipids in fecal excretion, as noted by Fernandez-Carmona *et al.* (1996). Furthermore, the combination of okara and cottonseed meal, both rich in unsaturated fatty acids, likely promoted effective micellar emulsification, thereby allowing for increased absorption of fatty acids in the small intestine. Furthermore, the argument of Gidenne *et al.* (1992), according to which an increase proportion of cell walls material can physically trap a fraction of the lipids and render them inaccessible to enzymatic action, may explain the decrease in fat digestibility observed with diet A20. Indeed, this diet has a higher crude fiber content (15.49 % DM), suggesting a larger fibrous fraction that may limit lipid hydrolysis.

Unlike to the observations of Odunlade and Uza (2021), who reported a linear increase in fat digestibility up to 94.91 % DM, our results show a relative decrease of 6.5 % in ADC between diet A10 (91.03 % DM) and diet A20 (85.10 % DM). In contrast, they are consistent with those of Benali *et al.* (2018), who reported Ether Extracts ADC values ranging from 86 % to 90 % DM depending on the energy density of the diets.

### **Crude fiber digestibility**

Crude fiber digestibility remained generally low, increasing 40.08 % DM (A0) to 56.20 % DM (A20), reflecting the insoluble nature of this fibrous fraction in rabbit diets. However, the crude fiber in diet A20 was the most digestible in absolute terms. This result suggests that the incorporation of okara at 20 % in the diet improves fiber quality by providing cell walls that are less lignified than those of the control diet A0. Indeed, this contribution undoubtedly facilitated

bacterial access to the fibrous matrix, leading to more intense fermentation in the caecum (Odunlade and Uza, 2021). This process, on the one hand, provided rabbits with more energy through the production of volatile fatty acids and, on the other hand, protected their digestive health by lowering the pH of the environment, thereby preventing the growth of disease-causing bacteria (Gidenne, 2003). The crude fiber ADC values recorded with okara-based diets in the present study are higher than those found by Benali *et al.* (2018), who obtained an average digestibility of 34.64 % DM, confirming that crude fiber remains poorly digestible in rabbits, regardless of the energy density of the diets. The crude fiber digestibility coefficient obtained with diets A20 (56.20 % DM) is fully consistent with the highest values reported by Odunlade and Uza (2021), which reached 55.34 % DM.

In conclusion, the use of okara up to 20 % allows for the utilization of a low-lignin fiber that promotes caecal fermentation without impairing the overall digestive efficiency of the diet.

Thus, okara emerges as an effective alternative feed resource, combining energy utilization and digestive safety in growth rabbits. The incorporation of dried okara into rabbit's diets is feasible, although nutrient digestibility may be modest at high inclusion levels. Rich in fiber and protein, its optimal inclusion in diets (often via pelleting) supports growth while valorization a byproduct. The apparent digestibility of okara's dry matter and nutrients is generally considered modest to moderate, as rabbits are able to utilize efficiently utilizing properly processed soybean residues. Dried okara can be incorporated at varying levels (often up to 15-30 % according to local studies) to achieve satisfactory weight gain in rabbits, serving as a source of protein and energy. This substitution helps reduce feeding costs during fattening, with positive effects on feed efficiency when okara is properly dried, thereby avoiding mold growth and antinutritional factors. Performance outcomes vary depending on the inclusion rate and okara drying quality.

**CONCLUSION**

The results obtained demonstrate that incorporating up to 20 % dried okara into the diet of finishing rabbits does not compromise either feed intake or overall nutrient digestibility. Crude protein digestibility remained stable and within physiological recommendations, confirming the efficient utilization of okara nitrogen. The lipid fraction exhibited high digestibility, although it was sensitive to the incorporation level, while the crude fiber, being less lignified, promoted caecal fermentation and contributed to digestive safety. These observations attest that okara is a viable and economically attractive local resource capable of replacing soybean meal without impairing the rabbits' digestive performance. Its use therefore represents a sustainable alternative for rabbit farming in tropical regions, combining byproduct utilization, digestive safety, and energy optimization.

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