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BENEFICIAL EFFECTS OF *Canavalia gladiata* SEED MEAL FERMENTATION ON PROXIMATE COMPOSITION, BIOACTIVE COMPOUNDS, FATTY ACID PROFILE AND AMINO ACID PROFILE

Gningnini Alain KONE[1]✉, N'Da Amani Sylvère Bertrand KOKO[1], Faustin Parfait KOUTOUAN[1], Konan Raphaël N'GUESSAN[1], and Maryline KOUBA[2]

[1]*Joint Research and Innovation Unit for Agricultural Sciences and Transformation Processes, National Polytechnic Institute Felix Houphouet Boigny, Yamoussoukro P.o Box: 1313, Côte d'Ivoire*
[2]*Joint research unit For Physiology, Environment, and Genetics for the Animal and Livestock Systems, Institut Agro, National Research Institute for Agriculture, Food and Environment, Saint Gilles 35590, France*

✉Email: alain.kone@inphb.ci

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ABSTRACT: Soybean meal is the dominant protein source in animal feed, but rising costs have encouraged the search for alternatives. *Canavalia gladiata*, a tropical legume with good yield potential and resistance to pests, contains about 26% protein. However, its use in non-ruminant diets is constrained by anti-nutritional factors. Fermentation with *Saccharomyces cerevisiae* has been shown to enhance protein content and reduce anti-nutrients in various feed ingredients, making it a promising strategy for improving *C. gladiata* seed. The aim of this study was to evaluate the effect of yeast fermentation on *C. gladiata* seed meal proximate composition, bioactive compounds, fatty acid profile and amino acid profile. In this study, raw seed meal (RCSM) was prepared from *C. gladiata* seed grown in Côte d'Ivoire, while fermented seed meal (FCSM) was obtained through a two-step aerobic and anaerobic fermentation with yeast. Proximate composition, bioactive compounds, antioxidant activity, fatty acid and amino acid profiles were determined using standard analytical methods. Fermentation increased dry matter by 4% and crude protein by 23%, while reducing carbohydrates and ash. It also enhanced total polyphenols by 11.11% and antioxidant activity by 32.14%, though flavonoids decreased slightly and tannin levels increased. Fatty acid profiles showed stability of polyunsaturated fatty acid (PUFA) and n-6/n-3 ratios within optimal ranges. Amino acids increased overall, with notable rises in several essential amino acids, though lysine, cystine, and glutamic acid declined. Overall, yeast fermentation significantly improved the nutritional and bioactive properties of *C. gladiata* seed meal, supporting its potential as an affordable alternative protein source for monogastric animal diets.

Keywords: Bioactive compounds, *Canavalia gladiata*, Fermentation, Nutritional value, Yeast.

INTRODUCTION

Soybean meal (SBM) remains the primary protein ingredient in animal diets due to its rich protein concentration and favorable amino acid composition (Cherdthong et al., 2014; Gunun et al., 2022). However, the recent increase in its market price has limited its use in feed formulation, prompting nutritionists to explore more affordable alternative protein supplements (Gunun et al., 2022).

Canavalia gladiata is a leguminous plant that originated in the Asian continent and spread throughout the tropics (Sasipriya and Siddhuraju, 2012). Eaten as a green vegetable in Asia, this plant has particular agronomic traits, including a high cultivation temperature (Nishizawa and Arii, 2018). They are cultivated on a limited scale throughout Asia, the West Indies, Africa and South America (Ekanayake et al., 2006). Moreover, its average yield is comparable to that of the soybean, and it is relatively resistant to pests and diseases (Nishizawa and Arii, 2018). Concerning nutritional properties, the *C. gladiata* seed contains approximately 26% protein, 3% fat, and 62% carbohydrate (Sridhar and Seena, 2006; Nishizawa and Arii, 2018). But, presence of anti-nutrients limits its use as a feed ingredient for livestock particularly non-ruminant animals (Sasipriya and Siddhuraju, 2013).

Yeast (*Saccharomyces cerevisiae*) has been applied to enhance the nutritional quality of feed ingredients (Polyorach et al., 2016; Promkot et al., 2017; Gunun et al., 2022) and to mitigate anti-nutritional compounds present in some animal diets (Boonnop et al., 2009). Indeed, Cherdthong and Supapong (2019) reported that cassava waste from bioethanol production fermented with yeast had a crude protein content of 25.1% of dry matter, twice that of unfermented cassava waste. Also, Gunun et al. (2022) reported that rubber seed kernel contained crude protein at 21.2%

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of dry matter, while the yeast-fermented rubber seed kernel product contained crude protein at 33.6% of dry matter, an increase in crude protein of 12.4 % of dry matter. Moreover, as a food-safe strain (Son et al., 2023; Wang et al., 2024), *S. cerevisiae* plays a pivotal role as a chassis organism for the efficient production of fatty acids and their derivatives (Wang et al., 2024). The aim of this study was to evaluate the effect of yeast fermentation on *C. gladiata* seed meal proximate composition, bioactive compounds, fatty acid profile and amino acid profile.

MATERIALS AND METHODS

Production of *C. gladiata* seeds

The raw seeds of *C. gladiata* were produced at the graduate school of agriculture experimental station, at the National Polytechnic Institute Felix Houphouet Boigny (INP-HB) in Yamoussoukro, located in central Côte d'Ivoire, from April to July 2024. Moreover, the *C. gladiata* seeds were sun-dried for 5 days after harvest, sorted, and stored in a dry environment until use.

Processing methods of RCSM and FCSM

The raw *C. gladiata* seed meal (RCSM) was prepared from *C. gladiata* seed ground using a universal grinder. The fermented *C. gladiata* seed meal (FCSM) was prepared by fermenting the RCSM with commercial dry yeast (*Saccharomyces cerevisiae*). The fermentation process was carried out according to the method reported by Shi et al. (2017), with some minor modifications. Each kilogram of seed meal as fermentation substrate was mixed and inoculated with one liter of distilled water containing 2% (w/w) dry yeast (*Saccharomyces cerevisiae*). Aerobic fermentation was carried out at 37 °C for 24 hours. After the first step, the aerobically fermented mixture was transferred to a vacuum oven and then fermented under anaerobic conditions at 37 °C for 24 hours. After fermentation, the mixture was oven-dried at 60 °C for 72 hours to obtain FCSM.

Chemical analyses

Proximate composition of RCSM and FCSM was analyzed using the methods of AOAC (2000). Lipids were extracted from samples using the method of Folch et al. (1957). Bioactive compounds were extracted from samples according to the hydroalcholic method described by Sinan et al. (2023). The total polyphenols were quantified by the method described by Lezoul et al. (2020) and Tiho et al. (2017). The total flavonoids were quantified by aluminium chloride colorimetric method described by Tiho et al. (2017). The total tannins were quantified by the Folin-Ciocalteu method described by Hossain et al. (2020). The condensed tannins were quantified by the vanillin acid method described by Price et al. (1978). The antioxidant activity of samples was measured by the 2,20-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{•+}$) radical cation method described by Tiho et al. (2017). Fatty acid profile of RCSM and FCSM was analyzed according to the methods described by Koné et al. (2020). Amino acids from RCSM and FCSM were analyzed by acid hydrolysis method (AFNOR, 2005).

Statistical analysis

Raw and fermented *C. gladiata* seed meals parameters data were subjected to the independent samples t-test at 5% significance, using R version 4.5.1 software.

RESULTS AND DISCUSSION

Proximate composition of RCSM and FCSM

The results of this study showed that fermentation improved the nutritional profile of *C. gladiata* seed meal, particularly in terms of dry matter and crude protein content by 4% and 23%, respectively (Table 1). The increase in dry matter content and the decrease in carbohydrate and ash contents observed in the fermented seed meal compared to the raw meal is likely due to microbial degradation of soluble carbohydrates and moisture reduction during the processing, which has been similarly reported in legume-based substrates (Ojokoh et al., 2013; Arise et al., 2022). Moreover, Yang et al. (2018) reported that the crude protein content significantly increased by 12% in fermented soybean meal produced by a combination of *B. subtilis*, *L. casei*, and yeast, as compared with those in raw soybean meal.

The increase in crude protein content following fermentation indicates a concentration effect often attributed to the microbial breakdown of non-protein components, such as fiber and anti-nutritional factors, as well as the biosynthesis of microbial proteins (Hidalgo-Fuentes et al., 2024). This aligns with findings from previous studies on other legumes, where solid-state fermentation enhanced the crude protein content of cowpea (Ojokoh et al., 2013) and jack bean (Arise et al., 2022).

Table 1 - Proximate composition of RCSM and FCSM in % of dry matter (DM)

Proximate composition^1	RCSM	FCSM	SEM	p-value
Dry matter (in % of crude matter)	91.23^b	94.89^a	0.82	6.67 x 10^{-6}
Crude Protein	25.62^b	30.38^a	1.14	6.87 x 10^{-3}
Ether Extract	4.95	5.09	0.04	1.15 x 10^{-1}
Crude Fiber	8.28	7.76	0.25	3.51 x 10^{-1}
Ash	4.24^a	3.95^b	0.07	6.84 x 10^{-4}
Total Carbohydrate^2	65.19^a	60.58^b	1.13	4.02 x 10^{-2}
ME (kcal/kg of dry matter)^3	3366.73	3330.01	9.99	4.24 x 10^{-1}

RCSM: Raw *C. gladiata* seed meal; FCSM: Fermented *C. gladiata* seed meal; ME: Metabolizable energy; SEM: Standard error of the mean. ^{1} Values are the means of three analyses per sample. ^{2} Total carbohydrate (Carb_Tot) = 100 - [Protein (% DM) + Fat (% DM) + Ash (% DM)] (Tiho et al., 2024); ^{3} ME (kcal/kg of dry matter) = [2.44 × Protein (% DM) + 8.37 × Fat (% DM) + 3.57 × Carb_Tot (% DM)] ×10 (Tiho et al., 2024). ^{a,b} Parameter means within rows with no common superscript differ ($p < 0.05$).

Bioactive compounds of RCSM and FCSM

The fermentation of *C. gladiata* flour induced an increase in total polyphenol content by 11.11% (Table 2). This increase can probably be explained by the release of phenolic compounds bound or their biotransformation into more active molecules by microbial enzymes such as tannases and glycosidases (Yang et al., 2023). As polyphenols are major antioxidants, this increase could strengthen the antioxidant potential of fermented seed, corroborating several studies on fermented vegetables and legumes (Asensio-Grau et al., 2020; Emkani et al., 2022; Knez et al., 2023). The enhancement of total polyphenols is consistent with previous findings in fermented legumes such as soybeans and pigeon peas, where microbial fermentation has been shown to release bound phenolics from the cell wall matrix or synthesize new bioactive compounds (Duenas et al., 2005; Hur et al., 2014).

Interestingly, a slight decrease in flavonoid content by 7.17% was observed after fermentation. This reduction may be attributed to the partial degradation, transformation, or use of certain flavonoids by fermenting microorganisms (Maria John et al., 2014; Zhao et al., 2021; Emkani et al., 2022).

Contrary to the commonly anticipated effects of fermentation (Adebo et al., 2022; Yang et al., 2023; Hidalgo-Fuentes et al., 2024), an increase in tannin content was observed in the present study. Similarly, a rise in total tannin levels has also been reported during the fermentation of pea protein concentrate (Ç̧abuk et al., 2018). This may be explained by either the liberation of bound tannins from the seed matrix (Ç̧abuk et al., 2018) or the biosynthesis of tannin-like phenolics by certain microbial strains during the fermentation process (Emkani et al., 2022). In contrast, condensed tannin content remained unchanged, suggesting that these compounds are more chemically stable and less affected by fermentation conditions (Makkar, 2003). This finding agrees with previous studies reporting the resilience of condensed tannins during biotransformation processes (Francis et al., 2002).

Finally, the significant enhancement in antioxidant activity by 32.14% following fermentation could be directly linked to the observed increases in total polyphenols and tannins. According to Zhao et al. (2021) and Kim et al. (2024), the antioxidant activity is positively correlated with yeast fermentation. Both classes of compounds are known to contribute to antioxidant capacity through mechanisms such as hydrogen atom donation, metal chelation, and inhibition of lipid peroxidation (Shahidi and Ambigaipalan, 2015). This link is well established: these families of compounds contribute strongly to free radical scavenging capacities and oxidative stabilization (reduction of oxidative stress) in various fermented systems (Zhao et al., 2021; Emkani et al., 2022). This result supports the hypothesis that fermentation could be an effective tool to enhance the biofunctional potential of *C. gladiata*, particularly its antioxidant properties.

Table 2 - Bioactive compounds of RCSM and FCSM

Bioactive compounds ^{1}	RCSM	FCSM	SEM	P-value
Total Polyphenols (mg GAE/g of dry matter)	82.21^b	91.34^a	2.30	1.82 x 10^{-2}
Total Flavonoids (mg QE/g of dry matter)	52.98^a	49.18^b	0.90	5.33 x 10^{-3}
Total tannins (mg TAE/g of dry matter)	0.34^b	0.45^a	0.02	4.91 x 10^{-11}
Condensed Tannins (mg CatE/g of dry matter)	0.08	0.07	0.01	4.60 x 10^{-1}
Antioxidant Activity (µmol TE/g of dry matter)	0.56^b	0.74^a	0.04	1.35 x 10^{-8}

RCSM: Raw *C. gladiata* seed meal; FCSM: Fermented *C. gladiata* seed meal; GAE: Gallic acid equivalent; QE: Quercetin equivalent; TAE: Tannic acid equivalent; CatE: Catechin equivalent; TE: Trolox equivalent; SEM: Standard error of the mean. ^{1} Values are the means of three analyses per sample. ^{a,b} Parameter means within rows with no common superscript differ ($p < 0.05$).

Fatty acid (FA) profile of RCSM and FCSM

The unsaturated fatty acids (74.81% and 71.88%, respectively for raw and fermented seed meal) were more abundant in *C. gladiata* seed oil than saturated fatty acids (25.17% and 28.53%, respectively for raw and fermented seed meal) (Table 3). These results confirm those of Qian et al. (2025), who reported that *C. gladiata* seed oil contained 69.1 to 81.8% unsaturated fatty acids and 18.2 to 30.9% saturated fatty acids. The fermentation of *C. gladiata* resulted in a decrease in unsaturated fatty acids by 3.92%, and an increase in saturated fatty acids (SFA) by (13.35%), notably palmitic acid (8.31%) and stearic acid (53.58%). This development is consistent with several recent studies that report that fermentation of food breaks down nutrients, including proteins associated with allergic reactions, making them easily digestible, and it also creates beneficial nutrients, including fatty acids (Borresen et al., 2012; Song et al., 2013; Kim et al., 2015). *Saccharomyces cerevisiae* plays a role in food production and also exhibits fatty acid composition changes depending on its environment and growth conditions (Son et al., 2023). The yeast can synthesize fatty acids, particularly saturated fatty acids like palmitic acid (16:0), and unsaturated fatty acids like oleic acid (18:1), with the latter's synthesis influenced by oxygen availability (Wang et al., 2024).

In contrast, the balance between polyunsaturated fatty acid (PUFA) contents, notably C18: 2n-6 and C18: 3n-3, were little affected by fermentation, as indicated by the relative stability of the C18: 2n-6/C18: 3n-3 and n-6/n-3 ratios, increasing respectively from 1.89 to 2.01 and from 1.89 to 1.98. The C18:2n-6/C18:3n-3 ratio is considered balanced, particularly in animal feed, when it is less than 4 as recommended by ANSES (2011) and Simopoulos (2016) to optimize the immune response. Simopoulos and Di-Nicolantonio (2016) concluded that n-3 fatty acids participate to a decrease adipose tissue development and lead to weight loss, while n-6 fatty acids increase adipose tissue synthesis and lead to obesity. After examining various effects of n-6/n-3 ratios on experimental animals, Simopoulos and Di-Nicolantonio (2016) indicated that the preferred ratio for an optimal health could be 1:1 or 2:1. This n-6/n-3 ratio interval 1:1 to 2:1 was increased, and set to be optimal between 1:1 and 5:1 according to Gonzalez-Becerra et al. (2023). Anyhow, high n-6/n-3 ratios between 10:1 and 20:1 should be stickily avoided, because they increase the risk of inflammatory diseases and obesity (Simopoulos and Di-Nicolantonio, 2016; Gonzalez-Becerra et al., 2023).

Table 3 - Fatty acid profile of RCSM and FCSM in % of total fatty acid.

Fatty acid [1]	RCSM	FCSM	SEM	P-value
Myristic acid, C14:0	0.51^{a}	0.45^{b}	0.02	2.13×10^{-2}
Pentadecanoic acid, C15:0	0.60	0.62	0.02	6.50 x 10^{-1}
Palmitic acid, C16:0	17.70^{b}	19.17^{a}	0.36	1.08 x 10^{-2}
Stearic acid, C18:0	2.93^{b}	4.95^{a}	0.45	6.52 x 10^{-6}
Arachidic acid, C20:0	0.87	0.83	0.03	5.23 x 10^{-1}
Behenic acid, C22:0	0.43	0.38	0.02	1.11 x 10^{-1}
Tricosanoic acid, C23:0	0.21	0.20	0.00	7.05 x 10^{-2}
Lignoceric acid, C24:0	1.50	1.47	0.02	4.10 x 10^{-1}
Hyenic acid, C25:0	0.42^{b}	0.46^{a}	0.01	2.19 x 10^{-3}
Total of saturated fatty acids (SFA)	25.17^{b}	28.53^{a}	0.79	4.45 x 10^{-3}
9-hexadecenoic acid, C16:1 n-9	0.18^{b}	0.22^{a}	0.01	6.08 x 10^{-4}
Palmitoleic acid, C16:1 n-7	1.62^{b}	3.68^{a}	0.46	3.03 x 10^{-5}
Oleic acid, C18:1 n-9	43.81^{a}	39.97^{b}	0.90	2.56 x 10^{-3}
Cis-vaccenic acid, C18:1 n-7	2.95	2.84	0.03	5.44 x 10^{-2}
Gondoic acid, C20:1 n-9	0.75	0.69	0.02	2.16 x 10^{-1}
Total of monounsaturated fatty acids (MUFA)	49.92	48.01	0.56	7.74 x 10^{-2}
Linoleic acid, C18:2 n-6	16.23^{a}	15.40^{b}	0.22	2.76 x 10^{-2}
Arachidonic acid, C20:4 n-6	0^{b}	0.23^{a}	0.05	6.30 x 10^{-4}
Total of n-6 FA	16.23	15.63	0.17	7.37 x 10^{-2}
Linolenic acid, C18:3 n-3	8.61^{a}	7.77^{b}	0.20	6.77 x 10^{-3}
Total of polyunsaturated fatty acids (PUFA)	24.89^{a}	23.47^{b}	0.37	2.57 x 10^{-2}
Total of unsaturated fatty acids (UFA)	74.81^{a}	71.48^{b}	0.72	4.99 x 10^{-2}
PUFA/MUFA	0.50	0.49	0.01	6.17 x 10^{-2}
UFA/SFA	2.9^{a}	2.5^{b}	0.10	6.75 x 10^{-4}
C18:2 n-6/C18:3 n-3	1.89^{b}	2.01^{a}	0.02	4.87 x 10^{-3}
n-6/n-3	1.89^{b}	1.98^{a}	0.03	5.30 x 10^{-3}

RCSM: Raw *C. gladiata* seed meal; FCSM: Fermented *C. gladiata* seed meal; FA: Fatty acid; SFA: Sum of saturated fatty acids; MUFA: Sum of monounsaturated fatty acids; PUFA: Sum of polyunsaturated fatty acids; UFA: Sum of unsaturated fatty acids; n-6/n-3: Sum of n-6 fatty acids/sum of n-3 fatty acids ratio; SEM: Standard error of the mean. [1] Values are the means of three analyses per sample. [a,b] Parameter means within rows with no common superscript differ ($p < 0.05$).

Amino acid (AA) profile of RCSM and FCSM

The quantity of AAs was much higher in fermented *C. gladiata* seed meal with *S. cerevisiae* than in raw *C. gladiata* seed meal (93.36 vs 87.52 g for 100 g of protein, respectively) (Table 4). Fermentation, one of the earliest techniques for preserving perishable foods, also enhances their nutritional quality by increasing amino acid levels (Elhalis et al., 2023; Yang et al., 2024). Additionally, the fermentation of food breaks down nutrients, including proteins associated with allergic reactions, making them easily digestible, and it also creates beneficial nutrients, including amino acids (Borresen et al., 2012; Song et al., 2013; Kim et al., 2015). The present study confirmed increases in several essential AAs and non-essential AAs in the FCSM, when compared with the RCSM. In fact, all AAs increased by 4.36% to 15.04% in fermented *C. gladiata* seed meal, excepted, the contents of lysine, cystine and glutamic acid that decreased respectively by 8.74%, 19.33% and 2.84%. Moreover, Kim et al. (2015) showed that the amounts of essential amino acids and non-essential amino acids were little increased respectively by 1.98% and 3.98% in fermented *C. gladiata* with 3% *A. oryzae* at 25 ± 2 °C and 55 ± 5% at ambient humidity for 36 h and dried. Also, Yang et al. (2018) indicated that total amino acid contents significantly increased by 5% in fermented soybean meal produced by a combination of *B. subtilis*, *Lactobacillus casei*, and yeast, as compared with those in soybean meal. In addition, the contents of phenylalanine, valine, and methionine were increased by 9-42% after fermentation. The increase in the content of sweet amino acids such as glycine and alanine after fermentation could increase the fermented *C. gladiata* seed meal in sweetness (Yang et al., 2024).

Table 4 - Amino acid profile of RCSM and FCSM in g/100 g protein

Amino acid	RCSM	FCSM	SEM	p-value
Arginine	5.50^b	6.04^a	0.12	2.51×10^{-5}
Histidine	3.06^b	3.44^a	0.09	1.49×10^{-3}
Isoleucine	4.02^b	4.57^a	0.12	1.76×10^{-4}
Leucine	7.65^b	8.41^a	0.17	7.41×10^{-4}
Lysine	6.18^a	5.64^b	0.14	2.13×10^{-2}
Methionine	0.96^b	1.13^a	0.04	2.56×10^{-2}
Phenylalanine	4.71^b	5.08^a	0.09	1.46×10^{-2}
Serine	5.39^b	5.98^a	0.14	3.83×10^{-3}
Threonine	4.53^b	4.97^a	0.10	1.53×10^{-3}
Tryptophan	1.08	1.13	0.03	4.87×10^{-1}
Valine	4.65^b	5.31^a	0.15	8.56×10^{-5}
Total of essential AAs	47.73^b	51.70^a	0.95	5.91×10^{-3}
Alanine	4.65^b	5.08^a	0.10	4.60×10^{-4}
Aspartic acid	11.17^b	11.91^a	0.17	1.12×10^{-4}
Cystine	1.19^a	0.96^b	0.05	2.14×10^{-3}
Glutamic acid	11.62^a	11.29^b	0.07	1.76×10^{-4}
Glycine	3.97^b	4.57^a	0.14	4.66×10^{-4}
Proline	3.68^b	4.18^a	0.12	1.57×10^{-3}
Tyrosine	3.51	3.67	0.06	1.88×10^{-1}
Total of non-essential AAs	39.79^b	41.66^a	0.45	7.09×10^{-3}

RCSM: Raw *C. gladiata* seed meal; FCSM: Fermented *C. gladiata* seed meal; AAs: Amino acids; SEM: Standard error of the mean. [1] Values are the means of three analyses per sample. ^{a,b} Parameter means within rows with no common superscript differ ($p < 0.05$).

CONCLUSION

To our knowledge, the fermentation of *C. gladiata* seed meal with yeast (*Saccharomyces cerevisiae*) has not been previously reported. The results of this study indicate that yeast fermentation could be an effective biotransformation approach for enhancing the protein quality and amino acid bioavailability of *C. gladiata* seed meal, thereby making its nutritional profile more competitive. Yeast-based fermentation appears to be a promising method for improving the bioactive potential of *C. gladiata* seeds. Consequently, the rise in bioactive compounds may positively influence the functional properties of the fermented seed meal, particularly with regard to shelf-life extension and gut health in animal feeding applications. This shows its potential as a value-added alternative protein source for monogastric animal diets.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Gningnini Alain Koné; E-mail: alain.kone@inphb.ci; ORCID: https://orcid.org/0000-0002-5143-3311

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

Gningnini Alain Koné designed the study, contributed to data analysis and manuscript writing, and coordinated the contributions of all other authors; N'Da Amani Sylvère Bertrand Koko and Konan Raphaël N'Guessan contributed to study design, data collection, and manuscript writing; Faustin Parfait Koutouan designed the study and manuscript writing; Maryline Kouba contributed to the study design and critical revision of the manuscript, as well as to the analysis of the samples.

Ethical regulations

Not applicable.

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Competing interests

The authors declare no competing interests in this research and publication.

Abbreviations

SBM: Soy Bean Meal; INP-HB: National Polytechnic Institute Felix Houphouet Boigny; RCSM: Raw *C. gladiata* Seed Meal; FCSM: Fermented *C. gladiata* Seed Meal; AAs: Amino Acids.; CP: Crude Protein; AOAC: Association of Official Analytical Chemists; GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent; TAE: Tannic Acid Equivalent; CatE: Catechin Equivalent; TE: Trolox Equivalent; ABTS: 2,20-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; Abs: Absorbance; DF: Dilution Factor; AFNOR: French Association for Standardization; ISO: International Organization for Standardization; ANSES: French Agency for Food, Environmental and Occupational Health & Safety; ME: Metabolizable Energy; FAO: Food and Agricultural Organization of the United Nations; DM: Dry Matter; FA: Fatty acid; SFA: Sum of Saturated Fatty Acids; MUFA: Sum of Monounsaturated Fatty Acids; PUFA: Sum of Polyunsaturated Fatty Acids; UFA: Sum of Unsaturated Fatty Acids; SEM: Standard error of the mean.

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