UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II

Dipartimento
Medicina Veterinaria
Produzioni Animali

Scuola di Dottorato in Scienze Veterinarie

XXXVII CICLO

PhD Thesis

Sustainability of Animal Nutrition and Environmental Protection Approach by Recycling Fruit By-Products

Coordinator
Prof. Paolo
de Girolamo

Candidate
Dr. Dieu donné
Kiatti

Tutor
Prof. Serena
Calabrò

February 2025

To my mother Agnès Pissibiga Kiansi, who has given her all since childhood by giving me the best advice and always finding ways to encourage me to overcome life's difficulties and dream of a bright future.

To my father Yoa Dassihoun Kiatti, brothers and sisters for all their support.

To my kind son Kiatti Souambo Prince Dylan, to whom my absence from home in pursuit of this thesis has caused pain. I guarantee you to be a good father who will help you achieve your goals.

Table of contents

List of abbreviations and acronyms

Ace/Pro	Acetate to propionate ratio
Ace	Acetate
ADF	Acid detergent fiber
ADF_{om}	Ash free acid detergent fiber
ADL	Acid detergent lignin
AOAC	Association of Official Analytical Chemistry
BCFA	Branched chain fatty acid
BP	Beet pulp
BPs	By-products
But	Butyrate
BW	Body weight
CABP	Cashew apple by-products
CM	Corn meal
CNT	Cashew nut testa
CP	Crude protein
CR_Pom	Cashew (var. red) pomace
CR_WF	Cashew (var. red) whole fruit
CT	Condensed tannins
CTR	Control
CY_Pom	Cashew (var. yellow) pomace
CY_WF	Cashew (var. yellow) whole fruit
DM	Dry matter
DMD	Dry matter degradability
DMDe	Enzymatic dry matter digestibility
DMDm	Microbial dry matter digestibility
DMDt	Total dry matter digestibility
DW	Dry weight
DWG	Daily weight gain
EE	Ether extract
EU	European Union
FAO	Food and Agriculture Organization
FCR	Feed conversion ratio
FE	Feeding efficiency
FS	Free sugars
GDP	Gross domestic product
HT	Hydrolysable tannins
IPBP	Industrial pineapple by-products
Iso-But	Iso-Butyrate
Iso-Val	Iso-Valerate
IVGPT	*In vitro* gas production technique
LW	Live weight
M_Ker	Mango kernel
M_Peel	Mango peel

M_Te	Mango testa
ME	Metabolizable energy
MSE	Mean square error
NDF	Neutral detergent fiber
NDF_{om}	Ash free neutral detergent fiber
NSC	Non-structural carbohydrate
OM	Organic matter
OMCV	Cumulative volume of gas related to incubated organic matter
OMD	Organic matter degradability
P_Peel	Pineapple peel
P_Pom	Pineapple pomace
Pro	Propionate
R_{max}	Maximum fermentation rate
SBM	Soybean meal
SC	Smooth Cayenne
SC_BE	Smooth cayenne bud end
SC_C	Smooth cayenne core
SC_Cw	Smooth cayenne crown
SC_P	Smooth cayenne pomace
SC_Pe	Smooth cayenne peel
SCFA	Short chain fatty acid
SGZ	Sudano-Guinean Zone
SL	Sugarloaf
SL_BE	Sugarloaf bud end
SL_C	Sugarloaf core
SL_Cw	Sugarloaf crown
SL_P	Sugarloaf pomace
SL_Pe	Sugarloaf peel
SZ	Sudanian Zone
TLU	Tropical livestock units
T_{max}	Time at which maximum rate was reached
TMR	Total mixed ration
TS	Total sugars
tVFA	Total volatile fatty acid
Val	Valerate
Var	Variety
VFA	Volatile fatty acid
WA	West Africa
WAD	West African Dwarf
Yield	Cumulative volume of gas related to degraded organic matter
Z	Zone

List of tables

Chapter 2

Chapter 3

Chapter 4

Chapter 5

List of figures

Abstract

The crop production is continuously increasing to meet the world population demand which is also growing. Among the crops, fruit and vegetable production generates a huge amount of waste from the farm to the table during the harvesting time, transportation, and processing which is thrown due to their perishability and lack of knowledge to reuse them in other fields. The decomposition of these wastes contributes to environmental pollution and climate change. However, the breeders, particularly in tropical and subtropical areas, are facing the challenge of feeding their animals year-round due to the long dry season and the key role of natural pasture in the production system. In addition, the cost of conventional feedstuff, such as maize, and its competition between humans and animals, weakens the livestock production system in tropical and subtropical areas. The thesis's general goal was to propose sustainable animal nutrition strategies in West Africa to preserve the environment and the circular economy through cashew, pineapple, and mango by-product recycling in livestock nutrition.

To achieve the aim, the AOAC methods and in vitro gas production technique (IVGPT) were applied to the by-products to determine chemical composition, polyphenol, sugar, and in vitro fermentation patterns. Sheep rumen liquor and pig caecum content, in ruminant and monogastric respectively, were used for the in vitro fermentation trials evaluating the digestibility, fermentation kinetics, gas, and end-product production. To study by-products in monogastric nutrition, gastrointestinal digestion was performed using enzymes (pepsin and pancreatin) before microbial digestion. Some by-products were also tested in vivo on West African Dwarf (WAD) lambs to evaluate intake, apparent digestibility, growth performance, and feeding behavior.

In particular, the thesis opens (Chapter 1) with a general introduction dressing the literature on animal nutrition in West Africa including by-products, successively followed by the studies of each specific objective (Chapter 2 – 5) and finishes with some considerations and future possible investigations (Chapter 6) to reach the principal goal in the nearby future.

The first experimental study (Chapter 2) focused on cashew apple by-products (CABP; whole, up, down, middle part, and pomace) from red and yellow varieties collected in the Sudanian Zone (SZ) and Sudano-Guinean Zone (SGZ) of the West Africa area. The study showed that the by-product characteristics depend more on the sampling area than on variety. The dry matter (DM) in the SZ and SGZ varied from 12.76 to 26.10 % and 7.41 to 22.9 %, respectively. The pomace showed the highest crude protein, lipids, and neutral detergent fiber (NDF) content (SZ: 9.48, 3.94, and 31.7 % DM; SGZ: 14.0, 4.94 and 34.1 % DM, respectively) but the lowest non-structural carbohydrate (NSC) and sugar for both zones. Regarding the in vitro fermentation, the organic matter degradability (OMD) was higher in the middle part (73.7 %) and whole apple (61.6 %) of SZ and SGZ, respectively. In contrast, the pomace from both zones showed the lowest in vitro fermentation parameters. The total polyphenols were more concentrated in the CABP from SZ (whole: 2736 µg/g DW; pomace: 3813 µg/g DW) compared to those from SGZ (whole: 1755 µg/g DW; pomace: 1374 µg/g DW). Results suggest that CABP should be collected in each

cultivation zone regardless of variety, separating pomace from other by-products and might be used as alternative feedstuff for small ruminants during the dry season in the West Africa region.

The individual pineapple by-products (PBP; crown, bud end, peel, core, and pomace) from two West African varieties (Smooth Cayenne - SC and Sugarloaf - SL) were characterized in the second experiment (Chapter 3). The results showed a low DM superimposable between varieties, averaging 17.7 %. On a DM basis, pomace showed the highest protein content (SC 8.10 % and SL 8.81 %, $p < 0.001$), whereas the crown showed the highest ($p < 0.001$) NDF content (47.6 % and 39.0 % for SC and SL, respectively). Due to the high sugar content, the core and pomace showed high *in vitro* OMD (SC: 85.1 % and SL: 84.0 %), estimated metabolizable energy (ME; SC: 7.91 KJ/kg and SL: 7.66 KJ/kg), and volatile fatty acid production (SC: 96.9 mmol/g and SL: 90.6 mmol/g). Based on chemical composition and *in vitro* digestibility results, this study suggests that pineapple by-products have the potential to be used in ruminants' nutrition, considering the crown, bud end, and peel as fiber sources and the core and pomace as substitutes or supplements to concentrate feedstuffs.

In the third experimental study (Chapter 4), three diets containing different levels (CTR: 0 %, Test 1: 14 %, and Test 2: 28 %) of industrial pineapple by-products (IPBP) were tested either in vitro with IVGPT and in vivo on No. 24 WAD lambs in three groups completely randomized design. The in vitro results related to the inclusion of IPBP in lambs' diet revealed the improvement of ME (8.32 and 9.13 vs. 7.68 MJ/kg, for Test 1, Test 2, and CTR, respectively), fermentation rate, and branched chain fatty acid production. The in vivo experiment showed that IPBP in the WAD lamb diet increased nutrient intake and apparent digestibility (except OM). The OM intake and apparent digestibility varied from 504 to 597 g/day and 81.6 - 84.3 %, respectively. The animals' growth performances (daily weight gain, DWG) ranged between 61.3 to 75.5 g/day and were slightly higher in the Test 2 group without significant differences. The feeding time and number of ruminating chews per bolus increased while chewing time per ruminated bolus decreased when IPBP inclusion increased in the WAD lamb diet. These findings suggest that IPBP can be included in the WAD sheep diet up to 28 % without detrimental effects.

The by-products evaluated for pig nutrition (e.g., cashew, pineapple, and mango) were tested in a two-step in vitro trial (Chapter 5). A low DM content specifically in the pomace, peel, and whole apple (13.0 – 27.2 %) was found while higher lipids were observed for cashew nut testa (26.4 % DM) and mango kernel (11.2 % DM). The by-products investigated fall within the interval of referenced feeds such as corn meal, Soybean meal, and beet pulp for structural carbohydrates (NDF: 7.6 – 47.1 % DM) and protein (7.65 – 51.2 % DM) except mango by-products due to its low protein (2.51 – 4.69 % DM) content. The total DM digestibility, short-chain fatty acid, and gas production were low for cashew by-products stopped after 48 h of incubation while the same parameters for other by-products stopped at 72 h were not so far to the referenced feeds. Specifically, the results suggest that pineapple pomace, cashew whole apple, pomace, and testa can be considered as feedstuff in fattening pigs, presenting characteristics partly similar to beet pulp. Mango peel and kernel should be combined with a protein source, such as Soybean meal, to feed pigs.

In conclusion, the studies conducted in the current thesis suggest the possibility of using West African fruit by-products derived from cashew, pineapple, and mango processing in livestock nutrition. However, the studies point out the low dry matter content of these by-products and encourage future investigations on preservation issues. The future research must include also in vivo experiments with animal product quality evaluation to produce more data related to cashew, pineapple, and mango by-products which will encourage their adoption in livestock feeding plans.

Riassunto

La produzione di colture è in continuo aumento per soddisfare la domanda della popolazione mondiale anch'essa in crescita. Tra le colture, la produzione di frutta e verdura genera un'enorme quantità di rifiuti dai campi alla tavola, durante il periodo di raccolta, trasporto e lavorazione che vengono scartati a causa della loro deperibilità e mancanza di informazioni per riutilizzarli in altri campi. Inoltre, la decomposizione di questi rifiuti contribuisce all'inquinamento ambientale e al cambiamento climatico. Gli allevatori, in particolare nelle aree tropicali e subtropicali, si trovano ad affrontare la sfida di alimentare i loro animali tutto l'anno a causa della lunga stagione secca e del ruolo chiave del pascolo naturale nel sistema di produzione. In aggiunta, il costo dei mangimi convenzionali, come il mais, e la competizione tra umano e animali, in queste aree indebolisce il sistema di produzione del bestiame.

L'obiettivo generale della tesi è stato quello di proporre strategie sostenibili per l'alimentazione animale nell'Africa occidentale con un approccio che mira alla conservazione dell'ambiente e all'economia circolare attraverso l'utilizzo dei sottoprodotti di anacardo, ananas e mango nella dieta degli animali da reddito. Per raggiungere l'obiettivo, le metodiche proposte dall'AOAC e la tecnica di produzione di gas *in vitro* (IVGPT) sono state applicate a detti sottoprodotti per determinarne la composizione chimica, il contenuto in polifenoli e zucchero e le cinetiche di fermentazione *in vitro*. Liquido ruminale di pecora e contenuto cecale di suino sono stati utilizzati per le prove di fermentazione *in vitro* che permettono di ottenere dati di digeribilità, caratteristiche di fermentazione, e produzione di gas e acidi grassi volatili.

Per studiare i sottoprodotti nella dieta dei monogastrici, è stata effettuata una digestione gastrointestinale utilizzando enzimi (pepsina e pancreatina) prima della digestione microbica. Alcuni sottoprodotti sono stati anche testati *in vivo* su agnelli nani dell'Africa occidentale per valutare l'ingestione, la digeribilità, le performance di crescita e il comportamento alimentare. In particolare, la tesi si apre (Capitolo 1) con un'introduzione generale che presenta una rassegna sulla nutrizione animale in Africa occidentale, con particolare attenzione ai sottoprodotti; seguono gli studi sperimentali (Capitoli da 2 a 5) effettuati per raggiungere specifici obiettivi; il manoscritto termina con alcune considerazioni finali e suggerimenti per indagini future (Capitolo 6) da realizzarsi per raggiungere l'obiettivo principale.

Il primo studio sperimentale (Capitolo 2) si è concentrato sui sottoprodotti dell'anacardo (CABP: mela intera, parte superiore, patte inferiore, parte centrale e sansa) di campioni appartenenti a due varietà, rossa e gialla, raccolte nella zona sudanese (SZ) e nella zona sudanese-guineana (SGZ) dell'Africa occidentale. Lo studio ha evidenziato che le caratteristiche dei sottoprodotti dipendono più dall'area di campionamento che dalla varietà. La sostanza secca (SS) nella SZ e nella SGZ è variata rispettivamente dal 12,76 al 26,10% e dal 7,41 al 22,9%. La sansa ha mostrato il contenuto più elevato di proteine grezze, lipidi e NDF (SZ: 9,48, 3,94 e 31,7% SS; SGZ: 14,0, 4,94 e 34,1% SS, rispettivamente) ma il più basso contenuto di carboidrati non strutturali (NSC) e zuccheri in entrambe le zone. Per quanto riguarda la fermentazione *in vitro*, la degradabilità della sostanza organica (DSO) è stata più elevata nella parte centrale (73,7%) e nella mela intera (61,6%) di SZ e SGZ, rispettivamente. Al contrario, la sansa di entrambe le zone

ha mostrato i parametri di fermentazione *in vitro* più bassi. I polifenoli totali erano più concentrati nei CABP da SZ (mela intera: 2736 μg/g DW; sansa: 3813 μg/g DW) rispetto a quelli da SGZ (mela intera: 1755 μg/g DW; sansa: 1374 μg/g DW). I risultati suggeriscono che i CABP dovrebbero essere raccolti in ogni zona di coltivazione indipendentemente dalla varietà, la sansa, separata dagli altri sottoprodotti, potrebbe essere utilizzata come mangime alternativo per piccoli ruminanti durante la stagione secca nella regione dell'Africa occidentale.

I sottoprodotti dell'ananas (PBP: corona, estremità del germoglio, buccia, torsolo e sansa) ottenuti da due varietà dell'Africa occidentale (Smooth Cayenne - SC e Sugarloaf - SL) sono stati caratterizzati nel secondo esperimento (Capitolo 3). I risultati hanno mostrato una bassa SS sovrapponibile tra le varietà, con una media del 17,7%. Sulla base della SS, la sansa ha mostrato il più alto contenuto proteico (SC 8,10% e SL 8,81%, $p < 0,001$), mentre la corona ha mostrato il più alto contenuto di NDF ($p < 0,001$) (47,6% e 39,0% rispettivamente per SC e SL). A causa dell'elevato contenuto di zucchero, il nocciolo e la sansa hanno mostrato più elevati valori di degradabilità della sostanza organica (SC: 85,1% e SL: 84,0%), energia metabolizzabile (ME; SC: 7,91 KJ/kg e SL: 7,66 KJ/kg) e produzione di acidi grassi volatili (SC: 96,9 mmol/g e SL: 90,6 mmol/g). Sulla base della composizione chimica e dei risultati *in vitro*, questo studio suggerisce che i sottoprodotti dell'ananas possono essere utilizzati nell'alimentazione dei ruminanti, considerando la corona, l'estremità del germoglio e la buccia come fonti di fibra e il nocciolo e la sansa come sostituti o integratori dei mangimi concentrati.

Nel terzo studio sperimentale (Capitolo 4), tre diete contenenti diversi livelli di sottoprodotti industriali dell'ananas (IPBP) (CTR: 0%, Test 1: 14% e Test 2: 28%) sono state testate *in vitro* con la IVGPT e *in vivo* su 24 agnelli WAD divisi in tre gruppi con un design completamente randomizzato. I risultati *in vitro* relativi all'inclusione dei IPBP nella dieta degli agnelli hanno rivelato un miglioramento del contenuto energetico (ME: 8,32 e 9,13 vs. 7,68 MJ/kg, rispettivamente per Test 1, Test 2 e CTR), della velocità di fermentazione e della produzione di acidi grassi a catena ramificata. L'indagine *in vivo* ha mostrato che i IPBP nella dieta degli agnelli WAD ha aumentato l'ingestione di nutrienti e la loro digeribilità apparente (fatta esclusione per la sostanza organica_SO). L'ingestione di SO e la digeribilità apparente variavano rispettivamente da 504 a 597 g/giorno e da 81,6 a 84,3%. Le performance di crescita degli animali (aumento di peso giornaliero, IMG) variavano tra 61,3 e 75,5 g/giorno e sono risultati leggermente più elevati nel gruppo Test 2 senza però mostrare differenze significative. Il tempo di alimentazione e il numero di masticazioni ruminali per bolo sono aumentati mentre il tempo di masticazione per bolo ruminato è diminuito quando l'inclusione di IPBP aumentava nella dieta degli agnelli WAD. Questi risultati suggeriscono che i IPBP possono essere inclusi fino al 28% nella dieta di pecore WAD senza effetti dannosi.

I sottoprodotti valutati per l'alimentazione dei suini (anacardo, ananas e mango) sono stati in uno studio *in vitro* in due step (Capitolo 5). È stato riscontrato un basso contenuto di SS specificamente nella sansa, nella buccia e nella mela intera (13,0-27,2 %) mentre sono stati osservati lipidi più elevati nel tegumento della noce dell'anacardo (26,4% SS) e nel nocciolo di mango (11,2% SS). Sulla base della composizione chimica, si può affermate che i sottoprodotti studiati rientrano nell'intervallo dei mangimi di riferimento, come la farina di mais, la farina di soia e la polpa di barbabietola se si considerano i carboidrati strutturali (NDF: 7,6 - 47,1% SS) e le proteine (7,65 - 51,2% SS), fatta eccezione per i sottoprodotti del mango a causa del loro basso contenuto in proteine (2,51 - 4,69% SS). La digeribilità totale della SS, la produzione di acidi grassi a catena corta e la produzione di gas risultano bassi per i sottoprodotti di anacardo incubati per 48 ore, mentre gli stessi parametri per altri sottoprodotti incubati per 72 ore non mostrano valori distanti dai mangimi di riferimento. In particolare, i risultati suggeriscono che per l'ananas la sansa di ananas, e per l'anacardo la mela intera la sansa e il tegumento della noce possono essere considerati come mangimi per suini da ingrasso, presentando caratteristiche in parte simili alla polpa di barbabietola. La buccia e il nocciolo di mango dovrebbero essere combinati con una fonte proteica, come la farina di soia, per coprire i fabbisogni dei suini. In conclusione, gli studi condotti nella presente tesi suggeriscono la possibilità di utilizzare sottoprodotti derivati dalla lavorazione di frutti prodotti Africa occidentale - anacardo, ananas e mango - nell'alimentazione del bestiame. Tuttavia, gli studi evidenziano il basso contenuto in sostanza secca di questi sottoprodotti e incoraggiano future indagini per la risoluzione dei problemi di conservazione. Ricerche future devono includere anche gli esperimenti *in vivo* che comprendano la valutazione della qualità dei prodotti animali per ottenere dati aggiuntivi relativi ad anacardo, ananas e mango ed incoraggiare la loro adozione nei piani di alimentazione del bestiame.

Résumé

La production végétale est en constante croissance pour répondre à la demande de la population mondiale qui ne cesse de croître. Parmi les cultures, la production de fruits et légumes génère une énorme quantité de déchets de la ferme à la table pendant la période de récolte, du transport et la transformation qui sont jetés en raison de leur périssabilité et du manque de connaissances pour les réutiliser dans d'autres domaines. La décomposition de ces déchets contribue à la pollution de l'environnement et au changement climatique. Cependant, les éleveurs, notamment dans les zones tropicales et subtropicales, sont confrontés au défi de nourrir leurs animaux toute l'année en raison de la longue saison sèche et du rôle clé des pâturages naturels dans le système de production animale. De plus, le coût des aliments conventionnels, comme le maïs, et sa concurrence entre l'homme et l'animal, fragilisent le système de production animale dans les zones tropicales et subtropicales. L'objectif général de la thèse était de proposer des stratégies de nutrition animale durable en Afrique de l'Ouest pour préserver l'environnement et l'économie circulaire à travers le recyclage des sous-produits de l'anacarde, de l'ananas et de la mangue dans l'alimentation du bétail.

Pour atteindre cet objectif, la méthodes AOAC et la technique de production de gaz en *in vitro* ont été appliquées aux sous-produits afin de déterminer la composition nutritionnelle, la concentration des polyphénols, les sucres et les paramètres de la fermentation en *in vitro*. Le liquide ruminal d'ovin et le contenu caecal de porcs, respectivement pour les ruminants et les monogastriques, ont été utilisés pour les essais de fermentation *in vitro* évaluant la digestibilité, la cinétique de la fermentation, la production de gaz et des produits finaux de digestion. La digestion gastro-intestinale a été réalisée avec des enzymes (pepsine et pancréatine) avant la digestion microbienne pour les sous-produits étudiés dans le cadre de la nutrition des monogastriques. Cependant, certains sous-produits ont également été testés en *in vivo* sur des agneaux nains d'Afrique de l'Ouest (Djallonké) pour évaluer l'ingestion, la digestibilité apparente, les performances de croissance et le comportement alimentaire.

En particulier, la thèse s'ouvre (chapitre 1) par une introduction générale dressant la littérature sur la nutrition animale en Afrique de l'Ouest incluant les sous-produits, suivie successivement par les études de chaque objectif spécifique (chapitre 2 – 5) et se termine par quelques considérations et les futures investigations possibles (chapitre 6) pour atteindre l'objectif principal dans un avenir proche.

La première étude expérimentale (chapitre 2) a portée sur les sous-produits de la pomme de cajou (SPPC ; pomme entière, partie supérieure, inférieur, centrale et résidus d'extraction de jus) des variétés rouges et jaunes collectées dans la Zone Soudanienne (ZS) et la Zone Soudano-Guinéenne (ZSG) de l'Afrique de l'Ouest. L'étude a montré que les caractéristiques des sous-produits dépendent plus de la zone d'échantillonnage que de la variété. La matière sèche (MS) dans la ZS et la ZSG variait respectivement de 12,76 à 26,10 % et de 7,41 à 22,9 %. Les résidus présentaient les teneurs les plus élevées en protéines brutes, en lipides et en fibres insoluble dans le détergent neutre (NDF) (ZS : 9,48, 3,94 et 31,7 % MS ; ZSG : 14,0, 4,94 et 34,1 % MS, respectivement) mais

présentaient les teneurs les plus faibles en glucides non structuraux (NSC) et en sucre pour les deux zones. En ce qui concerne la fermentation *in vitro*, la dégradabilité de la matière organique (DOM) était plus élevée dans la partie centrale (73,7 %) et la pomme entière (61,6 %) des ZS et ZSG, respectivement. En revanche, les résidus des deux zones présentaient les paramètres de fermentation *in vitro* les plus faibles. Les polyphénols totaux étaient plus concentrés dans SPPC de la ZS (pomme entière : 2736 µg/g MS ; résidus : 3813 µg/g MS) par rapport à ceux de la Z SG (pomme entière : 1755 µg/g DW ; résidus : 1374 µg/g DW). Les résultats suggèrent que SPPC devrait être collecté dans chaque zone de culture, quelle que soit la variété, en séparant les résidus des autres sous-produits et pourrait être utilisé comme ressource alternative pour les petits ruminants pendant la saison sèche en l'Afrique de l'Ouest.

Les sous-produits de l'ananas (SPA) à savoir la couronne, la partie basale, l'épluchure, le cœur et les résidus de jus) de deux variétés d'Afrique de l'Ouest (Cayenne Lisse - CL et Pain de Sucre - PS) ont été caractérisés dans la deuxième expérience (chapitre 3). Les résultats ont montré une faible teneur de MS superposable entre les variétés, en moyenne 17,7 %. Sur la base de la MS, les résidus ont montré les teneurs en protéines plus élevées (CL : 8,10 % et PS : 8,81 %, $p < 0,001$), tandis que les couronnes ont montrées NDF les plus élevée (47,6 % et 39,0 % ; $p < 0,001$ pour CL et PS, respectivement). En raison de la teneur élevée en sucre dans les cœurs et les résidus, ces sous-produits ont montré une DOM en *in vitro* plus élevée (CL : 85,1 % et PS : 84,0 %), ainsi que de l'énergie métabolisable estimée (EM ; CL : 7,91 KJ/kg et PS : 7,66 KJ/kg) et de la production des acides gras volatils (AGV ; CL : 96,9 mmol/g et PS : 90,6 mmol/g). Sur la base des résultats de la composition nutritionnelle et de la digestibilité en *in vitro*, cette étude suggère que les sous-produits d'ananas ont le potentiel d'être utilisés dans l'alimentation des ruminants, en considérant la couronne, la partie basale des fruits coupés et les épluchures comme sources de fibres et le cœur et les résidus de jus comme substituts ou complément aux concentrées utilisés en alimentation animale.

Dans la troisième étude expérimentale (chapitre 4), trois rations contenant différents niveaux (CTR : 0 %, Test 1 : 14 %, et Test 2 : 28 %) de sous-produits industriels d'ananas (SPIA) ont été testés en *in vitro* et en *in vivo* sur 24 agneaux Djallonké constitués en trois groupes complètement randomisés. Les résultats *in vitro* ont révélés l'amélioration de l'EM (8,32 et 9,13 vs. 7,68 MJ/kg, pour le Test 1, le Test 2 et le CTR, respectivement), du taux de fermentation et de la production d'acides gras à chaîne ramifiée. L'expérience *in vivo* a montré que SPIA dans la ration des agneaux Djallonké augmentait l'ingestion des nutriments et la digestibilité apparente (à l'exception de la MO). L'ingestion de la MO et la digestibilité apparente variaient respectivement de 504 à 597 g/jour et de 81,6 à 84,3 %. Les performances de croissance des animaux (gain moyen quotidien, GMQ) variaient entre 61,3 et 75,5 g/jour et étaient légèrement supérieures dans le groupe Test 2 sans différences significatives. Le temps d'alimentation et le nombre de coups de mastication du bol ruminé augmentaient tandis que le temps de mastication par bol ruminé diminuait avec l'inclusion des SPIA dans la ration des agneaux. Ces résultats suggèrent que SPIA peut être inclus jusqu'à 28 % dans la ration des agneaux Djallonké sans effets néfastes.

Les sous-produits (d’anacarde, d'ananas et de la mangue) évalués pour la nutrition des porcs ont été testés dans un essai in vitro en deux étapes (enzymatique et microbienne) (chapitre 5). Une faible teneur en MS spécifiquement dans les résidus, les épluchures et des pomme entière (13,0 - 27,2 %) a été observée tandis que des lipides les plus élevés ont été observés pour le tégument de noix de cajou (26,4 % MS) et le noyau de mangue (11,2 % MS). La composition nutritionnelle des sous-produits étudiés se retrouvait dans l'intervalle des aliments de références utilisés tels que la farine de maïs, le tourteau de soja et la pulpe de betterave pour les glucides structurels (NDF : 7,6 - 47,1 % MS) et les protéines (7,65 - 51,2 % MS) à l'exception des sous-produits de la mangue en raison de leur faible teneur en protéines (2,51 - 4,69 % MS). La digestibilité totale de la matière sèche, la production d'acides gras à chaîne courte et la production de gaz étaient faibles pour les sous-produits d’anacarde arrêtés après 48 h de fermentation alors que les mêmes paramètres pour d'autres sous-produits arrêtés à 72 h étaient comparables aux aliments de référence. Plus précisément, les résultats suggèrent que les résidus d'ananas et de pommes entières d’anacarde, et le tégument de noix de cajou peuvent être considérés comme des aliments pour les porcs à l'engraissement, présentant des caractéristiques en partie similaires à la pulpe de betterave. Cependant les sous-produits de la mangue (épluchures et noyau) doivent être combinés avec une source de protéines, comme le tourteau de soja, pour nourrir aux porcs.

En conclusion, les études menées dans le cadre de la présente thèse suggèrent la possibilité d’utiliser les sous-produits de fruits d’Afrique de l’Ouest issus de la transformation de l’anacardes, de l’ananas et de la mangue dans l’alimentation du bétail. Cependant, les études soulignent la faible teneur en matière sèche de ces sous-produits et encouragent de futures recherches sur les questions de conservation. Les recherches futures doivent également inclure des expériences *in vivo* avec l’évaluation de la qualité des produits animaux (lait et/ou viande) pour produire davantage de données relatives aux sous-produits de l’anacarde, de l’ananas et de la mangue qui encourageront leur adoption dans les plans d’alimentation du bétail.

Chapter 1.

General Overview

1.1. Background

In West African countries, the production of animal and agricultural products needs to increase to meet the demand of a growing population, which is around 400 million people and is expected to reach 800 million by 2050 (UN, 2019). Moreover, the demand for red meat was estimated to reach 6–7 million tons per year by 2030, which is greater than 1.7 million tons per year of current supply. In this regard, the livestock production system involves more than 15 % of the West African population contributing between 8 to 15 % of the overall gross domestic product (GDP) and 44 to 50 % of agricultural ones (De Haan et al., 2016; Eeswaran et al., 2022). In 2020, the Food and Agriculture Organization (FAO) estimated that the West Africa livestock population is around 103 million TLU (tropical livestock units – 1 TLU corresponds to a hypothetical animal of 250 kg of live body weight) (Molina-Flores et al., 2020) with up to 70 % of small ruminants which have an important socio-economic impact (Amole et al., 2022).

The main ruminant production systems in West Africa are extensive and semi-intensive. In these kinds of systems, the animal performances largely depend on the quality of natural pasture, which continuously fluctuates from abondance (rainy season) to scarcity (dry season) and changes in function of the climatic conditions of the zone: Saharan (desert), Sahelian (arid), Sudanian (semiarid), and Guinean (subhumid/humid) (Kruska et al., 2003; Ayantunde et al., 2014). Considering the climatic conditions, farmers use alternative techniques to feed animals throughout the year, e.g. crop residues, shrubs and trees, cultivated forages and cereals, and not well-characterized agro-industrial by-products.

Crop residues are the leftovers in the cultivation areas after harvesting the interest products for humans. They are mainly represented by stover of cereals such as millet (*Pennisetum glaucum* L.), sorghum (*Sorghum bicolor* L.), maize (*Zea mays* L.), straw from rice (*Oryza sativa* L.), haulms of leguminous crops such as cowpea (*Vigna unguiculata* L.) and groundnut (*Arachis hypogaea* L.) (Amole et al., 2022). The crop residues were quantified and estimated to provide 360 kg of dry matter (DM) per year for each TLU (Eeswaran et al., 2022). However, the crop residues are insufficient to feed the animals during the dry season and contain low protein and energy to cover the animal requirements.

Trees and shrubs fodders in natural pastures or silvopastoral systems are used to feed the animals during the dry season in West Africa. Trees and shrubs such as *Fadherbia albida*, *Ficus sycomorus gnaphalocarpa*, *Afzelia africana*, *Pterocarpus erinaceus*, *Combretum molle*, *Annona senegalensis*, *Gliricidia sepium*, *Ficus thonningii*, *Antiaris africana*, *Phyllanthus discoideus*, *Morinda lucida*, and *Mallotus oppositifolius* have been identified as the most promising source of nutrients in this geographical area (Avornyo et al., 2018; Koura et al., 2021). These feedstuffs are rich in protein but also contain high levels of structural cell wall and tannin which can negatively affect feed intake and nutrient digestibility (Habib et al., 2016).

The cultivated fodders and cereals are utilized in ruminants' nutrition in West Africa to cross the dry season and increase animal production, even if they are observed at a low scale. The cultivated fodders are divided into two groups: grass and legumes. The main cultivated grasses are annual (maize and sorghum) or perennial plants (*Panicum maximum, Digitaria unfolozis,* and *Bracharia ruziziensis*); the most widespread legumes are *Vigna unguiculata*, *Stylosanthes hamata*, *Mucuna* spp., *Leucaena leucocephela*, and *Cajanus cajan* (Amole et al., 2022; Eeswaran et al., 2022). However, the grasses are cultivated separately or associated with legumes (Bayala et al., 2014). The cereals, principally maize and sorghum grains, are integrated in supplemented diet or total mixed ration (TMR) with other animal feedstuffs (Odusanya et al., 2017; Azando et al., 2022). However, their addition to the animal feeding plan increases the feeding cost, which is reported to account for 60 – 70 % of the total cost of livestock production. Its inadequacy in quality and quantity could lead to scarce nutritional status, low weight gain, reproductive disorders, low production, poor health conditions, and a low feed conversion ratio (Fajemisin et al., 2015). Arable land limit, cereal prices, and competition between animals and humans for these products limit farmers' adoption of this feeding technique.

The agro-industrial by-products derived from food processing are commonly not used in human consumption. According to FAO global food loss and waste is around one-third of total production for human consumption (Gustavsson et al., 2011; Corrado et al., 2019). However, only three principal groups (fruits and vegetables, cereals, and root and tubers) account for around 80 % of the total (Porter et al., 2016). For example, in 2018, annual fruit and vegetable production was 675 million tons, with 10-60 % as waste during processing. So, fruit and vegetable processing generate around 1.3 billion tons of waste yearly (Omre et al., 2018). The several wastes generated from fruit and vegetable processing are pomace, peel, core, seed, stem, skin, outer leaves, cake meal, and molasses (Bedoić et al., 2019). Contrary to the dried cereals processing wastes which are easily recyclable, fruits and vegetable wastes rot quickly and are mostly dumped close to the factories causing health hazards to surrounding populations by environmental and under water pollution. All food waste was hypothesized to be useful in another field such as the animal production sector creating a circular economy (Pinotti et al., 2021). Moreover, due to the livestock growing in developing countries and the feed scarcity during the dry season, the utilization of waste as animal feed will be more valuable and will contribute to decreasing the cereals competition, feeding cost, and environmental pollution as well (Bedoić et al., 2019).

The different animal feeding aspects developed above are in accordance with the #12 and #13 goals of the 2030 Agenda for Sustainable Development proposed by the United Nations (https://sdgs.un.org/goals) which states that "*it is necessary to minimize the negative effects on human health and the environment pollution by promoting sustainable management and the efficient use of natural resources, reducing food losses along supply chains and managing all waste throughout its life cycle at a global level*". To achieve this goal, each country, state, and region of the world should identify the principal waste sources and define their ways of utilizing them. The current research focused on the West Africa area, where tropical and subtropical fruit waste could be utilized in ruminants' nutrition to support the breeders to feed their animals year-round and contribute to limiting the environmental impact. The first step to reach the goal is to characterize the potential by-products for chemical composition, secondary compound content, and *in vitro* and *in vivo* evaluation.

1.2. Characterization of principal tropical and subtropical fruit by-products

Knowing the by-products' chemical composition is the first step to understanding how they must be collected at the processing factories, which usually transform a wide range of fruits/food for humans. The by-product chemical composition could be compared to some similar conventional feedstuff to introduce or substitute them in livestock feeding plans without causing detrimental effects. In addition, the knowledge of micro-nutrients and secondary compounds content in the by-products is useful to boost their advantage and limit their disadvantage in animal physiology. However, for a complete evaluation, the effect of by-products and their potential substitution for conventional feedstuff should be tested using *in vitro* and *in vivo* studies.

1.2.1. Chemical composition

Tropical and subtropical areas are home to various fruits such as *Citrus* spp., banana (*Musa acuminata* L.), mango (*Mangifera indica* L.), cashew (*Anacardium occidentale* L.), pineapple (*Ananas comosus* L.), guava (*Psidium guajava* L.), date (*Phoenix dactylifera* L.), melon (*Cucumis melo* L.), watermelon (*Citrullus lanatus* L.), etc. They are consumed by humans directly or after processing, which, in this last case leads to different types of by-products (e.g.: peel, pulp, pomace, seed). The characterization using the chemical composition (also called proximate analyses) of tropical and subtropical fruit waste for animals' nutrition showed a wide variation between them depending on human consumption habits, which vary a lot, processing technique applied and cultivation areas influence. Citrus pulp, one of the most studied by-products (Paya et al., 2012; García-Rodríguez et al., 2019; Lashkari and Taghizadeh, 2013), revealed low crude protein (5 - 11 % DM) and structural carbohydrate (NDF: 19-30 % DM and ADF: 12 - 22 % DM) content, but high non-structural carbohydrate (NSC: up to 50 %). The latter is the primary energy source necessary for the rumen microorganism's growth and development and contributes to improving the metabolizable energy supply. Also, mango, pineapple, date, guava, and banana by-products are low in crude protein (4 - 9 % DM) and high in structural carbohydrate (NDF: 32 - 73 % DM and ADF: 22 - 58 % DM), with large varying values, except mango seed kernel (NDF: 11 - 25 % DM and ADF: 3 - 6 % DM) (Melesse et al., 2018; Azevêdo et al., 2011; Correia et al., 2006; Lashkari and Taghizadeh, 2013). The group of by-products constituted by papaya (peel and pomace) and cashew (whole apple and pomace) were reported to be rich in protein (12 - 19 % DM) and structural carbohydrate as well (Melesse et al., 2018; Azevêdo et al., 2011; Lashkari and Taghizadeh, 2013; Raseel et al., 2018; Araújo et al., 2020). Notwithstanding, a large variation in crude protein level (3 - 19 % DM) was observed for melon and watermelon peels (Rico et al., 2020), it was reported that papaya, melon, and watermelon seeds contain more crude protein (17 - 31 % DM) than other fruits by-products (Azevêdo et al., 2011; Rico et al., 2020). The same authors report higher ether extract (EE: 14 - 51 % DM) content in the seed generated by fruit processing. The ether extract (crude lipids) and acid detergent lignin are the components that can have negative effects (e.g., reduced digestibility) when their proportion in the total diet is high.

1.2.2. Macro and micro elements

Tropical and subtropical fruits contain high concentrations of various vitamins such as macro and microminerals and other phytonutrients. Related to these ingredients, fruits were associated with properties that prevent cancer, cardiovascular disorders, obesity, and diabetes (Wanapat et al., 2024). The processed waste of these fruits might contain vitamins and macro and micro elements which can be useful in the animal sector. The content in macro and micro elements of tropical and subtropical fruits by-products was reported in comparison to the requirement and maximum tolerance limit in ruminants' nutrition (Wadhwa et al., 2015). The citrus pulp, pineapple waste, and watermelon peel were high in Ca, ranging from 0.34 - 0.49 % DM. The watermelon peel was also reported to be rich in P and Mg. Most of these by-products are rich in K (0.34 - 2.55 % DM) and poor in Na, except for guava seed and watermelon peel (Wadhwa et al., 2015; Melesse et al., 2018). However, these fruit by-products contain sufficient microelements to be fed to the ruminants, but banana peel contains Fe, Cu, and Zn levels higher than the maximum tolerance limit. So, the tropical and subtropical fruits by-products might be an alternative to supplement the macro and microelements in ruminants' diets during the high production, and pregnancy period and to cover the low quality of some conventional feedstuffs, contributing to reducing the feeding cost.

1.2.3. Secondary compounds

Polyphenols are products of the secondary metabolism of plants to protect themselves from infections, animal attacks (insects and grazing animals), and stress caused by solar radiation (Quideau et al., 2011). The presence of aromatic rings with one or more hydroxyl groups is the main characteristic of polyphenols. The polyphenols identified are around 8000 grouped in terms of their chemical structure into flavonoids, non-flavonoids, and tannins (Correddu et al., 2020). The flavonoids are the most represented with more than 5000 identified compounds. They include several subgroups such as flavones (e.g.: apigenin), flavonols (e.g.: quercetin, myricetin), flavanones (e.g.: naringenin, hesperidin), isoflavones, and anthocyanidins (Bravo et al., 1998). Regarding the non-flavonoids, the most represented compounds are the simple phenols (e.g.: cresol, thymol, and resorcinol), phenolic acids (e.g.: gallic, vanillic, and syringic), and stilbenes (Correddu et al., 2020). Tannins are phenolic compounds divided into hydrolyzable tannins (HT) and condensed tannins (CT). The HT is constituted by carbohydrates (glucose mainly) with esterified hydroxyl groups and phenolic acids (gallic acids or hexahydroxy diphenic acid). The CT known as proanthocyanidins are polymers of the flavan-3-ol (dimers, trimers, tetramers, but also very high polymerized structures). The highly hydroxylated molecules of CT can form insoluble complexes with carbohydrates and proteins and confer astringency taste due to the precipitation of salivary proteins (Correddu et al., 2020). Therefore, polyphenols can reduce intake, digestibility, and animal performance if they are at a high level in the diet. However, the polyphenols widely present in the plant kingdoms can be found in fruits, products, and wastes. For example, gallic acid, ferulic acid, *p*-coumaric, catechins, epicatechins, hesperidin, quercetin, diosmin, narirutin, didymin, sinesetin, and kaempferol have been found in citrus processed by-products with 104-223 g/kg DM of total phenols (Ozturk et al., 2018; Jalal et al., 2023). The fruits' origin, maturity status, and the processing technique applied can influence the principals and the total amount of polyphenols amount in the generated by-products. Contrarily to the negative effects of polyphenols on intake and digestibility mentioned above, other authors observed positive effects of by-products rich in polyphenols included in ruminants' diet such as the improvement of milk yield and quality, and animal health (Correddu et al., 2020; Jalal et al., 2023). Moreover, the use of by-products in ruminant feed helps to reduce urea and methane emissions and to control gastrointestinal parasites due to the secondary compounds (Borges et al., 2016; Correddu et al., 2019). The discrepancy findings regarding by-product utilization as animal feed might be due to the inclusion level and different animal feeding behaviors. It was reported that goats developed more strategies against polyphenols than sheep and cattle (Alonso-Díaz et al., 2012). So, the by-products rich in polyphenols have advantages and disadvantages in ruminants' physiology and must be used with more attention.

1.2.4. In vitro study

Many tropical and subtropical fruit by-products have been tested using *in vitro* techniques. The *in vitro* study on citrus revealed that they could be used as replacement feedstuff in ruminants' diets even their large variation in metabolizable energy, short chain fatty acid (SCFA: 0.809-0.924 mmol/200 mg DM), dry matter degradability (DMD: 64-78%) and organic matter degradability (OMD: 55-77%) (Paya et al., 2012; Lashkari et Taghizadeh, 2013). Both authors have utilized the rumen fluid of sheep previously fed at maintenance level but with different incubation times (48 h vs. 72 h). However, García-Rodríguez et al. (2019) reported a high DM degradability of citrus pulp from 94 % to 99 % with increasing the fermentation time up to 144 h. On the other hand, pineapple pulp and papaya peel were reported as possible energy sources due to the high *in vitro* volatile fatty acid (VFA) production and NSC content (Melesse et al., 2018). Moreover, mango peel has been shown to enhance the effectiveness of rumen fermentation in several aspects, including *in vitro* gas production, organic matter degradation, VFA production, and microbial protein synthesis (Okoruwa et al., 2014). The mango peel has been reported to increase *in vitro* VFA production and stimulate microorganism activity in a water-shaking bath at 39°C for 24 h with dairy cow rumen fluid (Garavaglia and Tedesco, 2015). However, different factors can influence fruit by-products *in vitro* characterization: chemical composition (ether extract, lignin, and ash level) and the phenolic compounds (Guil-Guerrero et al., 2016); fruit pre-treatment and donor of the rumen liquor also need to be mentioned. So, the fruit by-products could be reasonably incorporated into animals' diets, but to avoid the detrimental effect and nutrient utilization, the *in vivo* test needs to have occurred as well.

1.2.5. In vivo study

The *in vivo* study is the step where the acceptability, intake, nutrient utilization, and performance (meat and milk yield quality) are evaluated according to a new feedstuff's inclusion in the animal diet. The main nutrient digestibility has been reported to decrease in the ruminant when the fruit processing by-products increased in the diets (Jalal et al., 2023). Including high levels of fruit by-products in ruminant diets can elevate the total polyphenol content, leading to the formation of stable complexes with proteins and carbohydrates that may reduce digestibility (Jayanegara and Palupi, 2010). Tannins, in particular, bind the proteins in the rumen and limit their availability to microorganisms; however, as these protein-tannin complexes, known as by-pass proteins, move to the gut, the change in pH allows the proteins to be released and absorbed, which can sometimes enhance overall protein absorption.

Citrus by-products have been tested *in vivo* experiments with ruminants. The replacement of corn silage by orange peel silage at 250 and 500 g/kg in the total mixed ratio (TMR) in multiparous lactating Holstein cows (Itavo et al., 2020) provided a similar dry matter intake, milk production, and protein content compared to the control. Dried citrus pulp can replace energy sources in the ration of growing cattle at 20 - 30 % DM without negative effects (Alnaimy et al., 2017). Bakr (2020) argues that citrus pulp can be used to feed lactating animals (cows, sheep, and goats), fattening calves, lambs, and goats with different percentages (from 8 to 100 %) as a replacement to energy sources such as corn, barley, and wheat.

Banana by-products were reported to be useful in tropical ruminants' nutrition as complementary feed. Previous studies observed that dairy cows can be fed fresh banana peel at 14-21 kg and contribute to increasing milk production (Dormond et al., 1998). In small ruminants, maize was successfully replaced (100%) by banana peel without affecting growth performance (Aregheore, 1998).

Mango fruit waste such as seed kernel, peel, and juice residue have been incorporated in the ruminants' diets. De Evan et al. (2022) studied commercial concentrate substitution by multi-nutrient blocks in dairy goats. The multi-nutrient blocks were formulated by mixing 65 % of mango juice pulp and 35 % of mango peel and substituting 29 % of commercial concentrate. They observed similar nutrient digestibility, nitrogen and energy utilization, and milk production compared to the control. However, the goats fed with mango pulp and peel mixture tend to reduce the polyunsaturated fatty acid in the milk. Other authors have reported a high intake of mango pulp, peel, and meal despite its low protein content while the intake of mango kernel was lower (Sanon et al., 2013; Silva et al., 2016). The difference was attributed to the high tannin content detected in the kernel. Moreover, Aragão et al. (2012) reported similar digestibility (46.9 %) when corn meal was replaced by whole mango meal in fattening lambs' diet.

Pineapple processing wastes such as crown leaves, peel, core, and pomace have been identified as a potential feed for ruminants. Due to their rapid decomposition, pineapple waste has been preserved by drying or ensiling and fed to the ruminants. The inclusion of pineapple cannery by-products (skin, crown ends, bud ends, and cores) at 1.5 % DM in the diet of finishing steers revealed any adverse effects on growth and carcass quality (Choi et al., 2021). Liu et al. (2021) investigated the intake, digestibility, and ruminal fermentation properties of pineapple juice residue incorporation (low: 1.4/kg DM and high: 3.6/kg DM) in non-lactating Holstein cows. The authors observed the increased intake when pineapple residues were added at high levels, while other parameters were similar. The ensiled pineapple fruit residues have been tested in sheep and cows as a total substitute for maize silage in TMR (Gowda et al., 2015). The authors concluded that ensiling pineapple fruit residues improved the economics of feeding and helped in overcoming the disposal problem due to the absence of negative effects on nutrient utilization, biochemical profile, mineral profile (Ca, P, Mg, Cu, Zn, Mn) and performance (growth and milk) in sheep and cows. Another study conducted in Thailand reported that ensiled pineapple waste can be used as roughage or improve hay digestibility in Thai native cattle (Suksathit et al., 2011). The contribution of pineapple by-products to improving nutrient intake and digestibility in ruminants is due to their soluble carbohydrate content, although this may increase the risk of ruminal acidosis associated with high inclusion levels.

Cashew apples, which remain after nuts are harvested, are less processed into juice and have been tested in animal nutrition in cashew cultivation areas. They are not totally used because of their short shelf life, astringent taste, and lack of knowledge of their nutritional value. The whole apples and pomace or meal could be useful alternative feedstuff for ruminants. A previous study evaluated the replacement of ground nut cake with sun-dried cashew apple meal incorporated in the concentrate mixture at 10 % in lactating Gir cows (Wadhwa et al., 2015). The replacement did not have a negative effect on intake and milk production compared to the control. In Brazil, Souza et al. (2020) tested different substitution levels (0, 0.25, 0.50, 0.75, 1.00 g/g) of corn by dried cashew by-products as a supplement to grazing cattle. They observed a linear decrease in intake, digestibility, microbial nitrogen synthesis, and retention. In the same region, it was reported that the inclusion of dehydrated cashew apple by-products up to 33 % in sheep diet did not affect intake and nitrogen balance (Araújo et al., 2020). In addition, the research conducted in West Africa suggested that sun-dried cashew apples can be added up to 30 % to West African Dwarf goats without influencing intake, digestibility, and growth performance (Okpanachi et al., 2016). However, Ribeiro Filho and Soto-Blanco (2012) reported that cashew apple can promote ruminant poisoning by ethanol production in the rumen during the cashew carbohydrate fermentation. This poisoning was revealed to be reversible and non-lethal. So, cashew apple by-products must be used carefully in ruminants' nutrition to avoid the decrease in intake and poisoning due to the astringent taste and ethanol production in the rumen, respectively.

In summary, fruit by-products are widely available in tropical and subtropical areas but present different characteristics and responses when they are included in ruminants' diets. The differences observed in the *in vivo* studies using fruit by-products might be due to the chemical composition, the taste of the fruits they come from, their geographical origin, the type of processing, and their content in secondary compounds.

1.3. Objectives of the thesis

Overall, this thesis aims to propose sustainable animal nutrition plans for West African farmers with an approach to protecting the environment and the circular economy through recycling agricultural by-products locally processed, particularly cashew and pineapple to include them in animal feeding plans.

To achieve this goal, four objectives were set, such as:

1. explore the potential use of cashew apple by-products in small ruminants' nutrition by *in vitro* technique;
2. study the *in vitro* fermentation characteristics of two varieties of pineapple leftovers;
3. evaluate the effect of pineapple by-products by substituting conventional feedstuff in the diet of West Africa Dwarf lambs through *in vitro* and *in vivo* studies;
4. test the utilization of tropical and subtropical fruit by-products (cashew, pineapple, and mango) in pig nutrition through a two-steps *in vitro* study.

Considering the above-mentioned objectives, the following hypotheses were formulated:

1. cashew apple by-products of West Africa differ by variety (yellow vs. red) and cultivation zone (Sudanian vs. Sudano-Guinean);
2. pineapple by-products differ for variety (Sugarloaf vs. Smooth Cayenne) and parts (crown, bud end, peel, core, and pomace);
3. pineapple by-products can substitute conventional feedstuffs and improve *in vitro* fermentation characteristics and *in vivo* animal performance;
4. cashew, pineapple, and mango can be used in pig nutrition, contributing to the supply of nutrients.

1.4. Introduction to by-products tested and technique used

Pineapple and cashew are among the most cultivated plants in tropical and sub-tropical areas such as West Africa. Worldwide pineapple (*Ananas comosus* L.) fruit production was estimated at 28,179,348 tons (Faostat, 2021), with a high West Africa contribution (Sodjinou et al., 2022). It is highly demanded due to its nutritional composition such as vitamins (i.e., A, C, B1, B6, folic acid, beta-carotene), minerals (i.e., Cu, Mn), antioxidant, digestive enzyme (bromelain), anti-inflammatory, and analgesic properties (Chaudhary et al., 2019; Firatoiu et al., 2021). Pineapple fruits are consumed fresh or processed to juice, jam, candy, ice cream, powder, and wine, leading to around 65 - 80 % waste represented by crown, bud end, peel, core, and pomace (Hemung et al., 2022) which are generally thrown in the environment.

Cashew trees (*Anacardium occidentale* L.) are planted mainly for nuts (true fruit) but generate also apples (peduncle or pseudo fruit) as well. During the harvest, 10-15 tons of apples are obtained for every ton of nut collected (Talasila and Shaik, 2015). The worldwide cashew nuts and apples output of plantations were estimated at 4 million tons and 50 million tons annually respectively with around 20 % of West Africa's contribution; however, 90 % of cashew apples are left in the field during the nuts harvesting due to their perishability and astringent taste (Das et al., 2016).

Mango (*Mangifera indica* L.) is one of the most important tropical fruits in the world due to its succulence, exotic flavor, delicious taste, and the nutrients it contains. Mango production is continuously increasing with African countries accounting for about 40 %, mainly supported by the West African region. However, mangos are consumed freshly (80 %) or processed (20 %) into puree, nectar, leather, pickles, canned slices, and chutney generating 50-65 % of fruits as waste composed of seed and peel (Ravani and Joshi, 2013; Sanon et al., 2013). Figure 1. 1. shows the areas in the Republic of Benin where pineapple, cashew, and mango by-product samples studied in this thesis were collected.

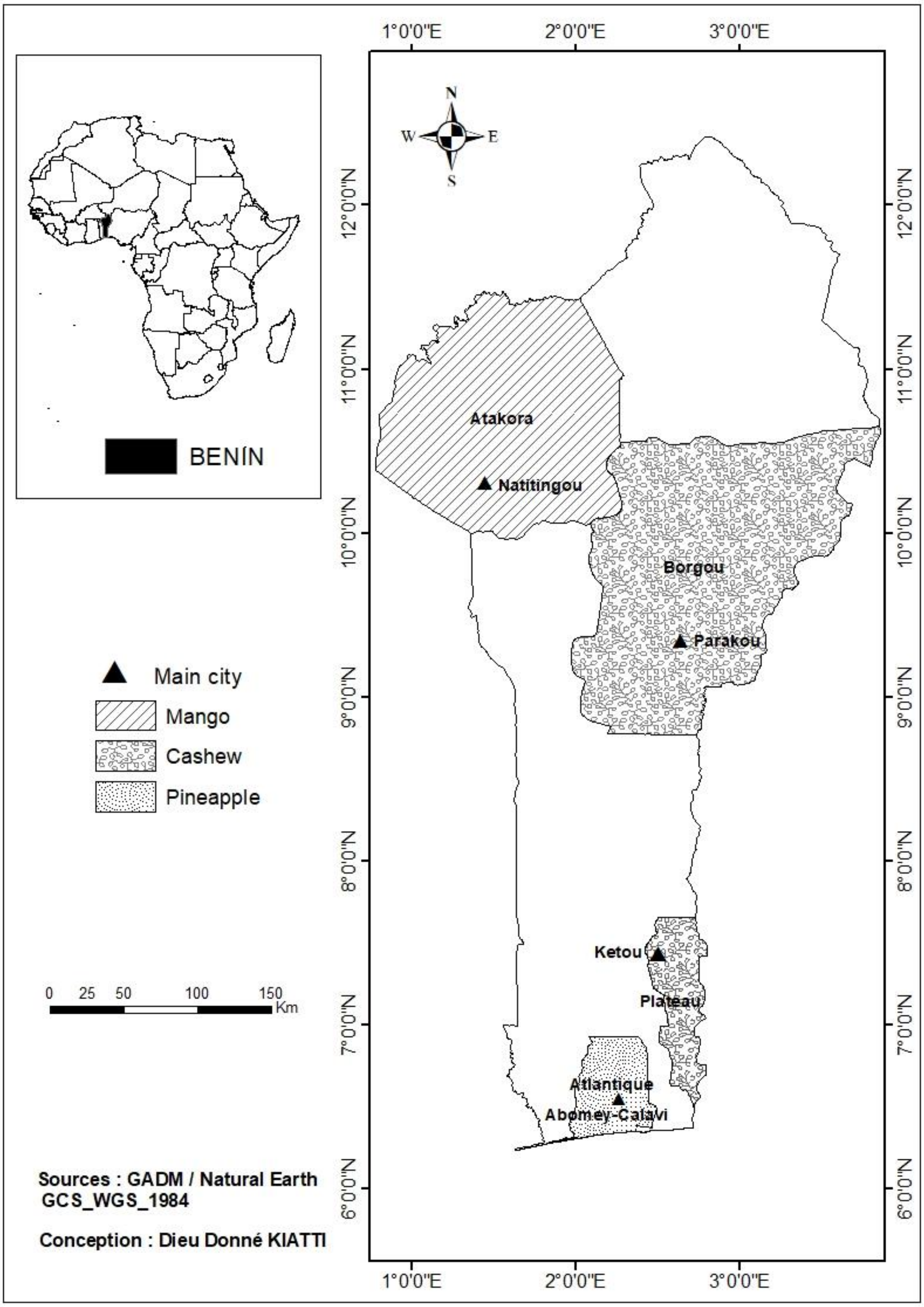

Figure 1. 1. Map of studied by-product areas in the Republique of Benin.

Before proposing innovative and alternative feedstuff for animal nutrition, a previous characterization is necessary, including *in situ*, *in vivo,* and *in vitro* studies. The last one involves the *in vitro* gas production techniques (IVGPT), a laboratory method with many advantages in terms of time, cost, and ethical aspects compared to *in situ* and *in vivo* techniques. The IVGPT consists of testing feedstuffs by creating in the laboratory rumen: the substrates to test are incubated at 39°C under anaerobic conditions with a medium (macro, microminerals, and buffer solutions), reducing agent and rumen liquor (microorganisms' source) (Theodorou et al., 1994). As in the rumen, under favorable conditions, microorganisms use the substrates' nutrients (protein and energy) of the substrate to grow and attach the fibers for digestion and produce gas. Many samples can be tested and compared simultaneously as a single ingredient or mixed in a diet. IVGPT allows to obtain several data, end-point parameters (i.e., pH, gas production, organic matter degradability, fermentation end-products), and fermentation kinetics (Calabrò et al., 2005). Recently, methane detection was also included in the IVGPT trials to evaluate livestock's environmental impact (Guglielmelli et al., 2011). The correlation between chemical composition and IVGPT parameters is mostly significant, indicating the validity of the IVGPT method, which enriches the nutritional characterization of the feedstuffs. The IVGPT has been set up for ruminant species focusing on microorganisms' activities in the rumen, but successively, it was extended to caecum microorganisms for monogastric species (Musco et al., 2015) due to the accuracy of the methods' accuracy. So, the IVGPT is proposed as a valid first step to characterize the novel ingredients and understand their interaction with other feedstuff before their use in animals. However, the *in vivo* experiment must be conducted to evaluate the effect of novel ingredients on palatability, intake, digestibility, animal performance, and feeding behavior before its validation in animal nutrition.

1.5. Structure of the thesis

Figure 1. 2. represents the thesis structure showing the different chapters that it takes throughout. In the general overview (Chapter 1.), as background, we first focused on the livestock production system in West African countries and the feeding strategies adopted by the farmers to cross the dry season, which is critical for grazing ruminants. Successively, a short literature review on some principal tropical and subtropical fruit by-product characterization (chemical composition, macro and micro elements, polyphenols, *in vitro* and *in vivo* study) is reported. Then, we pointed out the main by-products we studied such as cashew apple and pineapple, including mango, and the *in vitro* technique chosen for their characterization. Finally, we listed the objectives set and the hypothesis formulated. Consecutively (Chapter 2. to 5.), four experimental contributions are depicted, and each one is subdivided into abstract, introduction, materials and methods, results, discussion, and references. A general conclusion with future perspectives closes the thesis (Chapter 6.). A general abstract and a list of abbreviations are also included given a summarized finding.

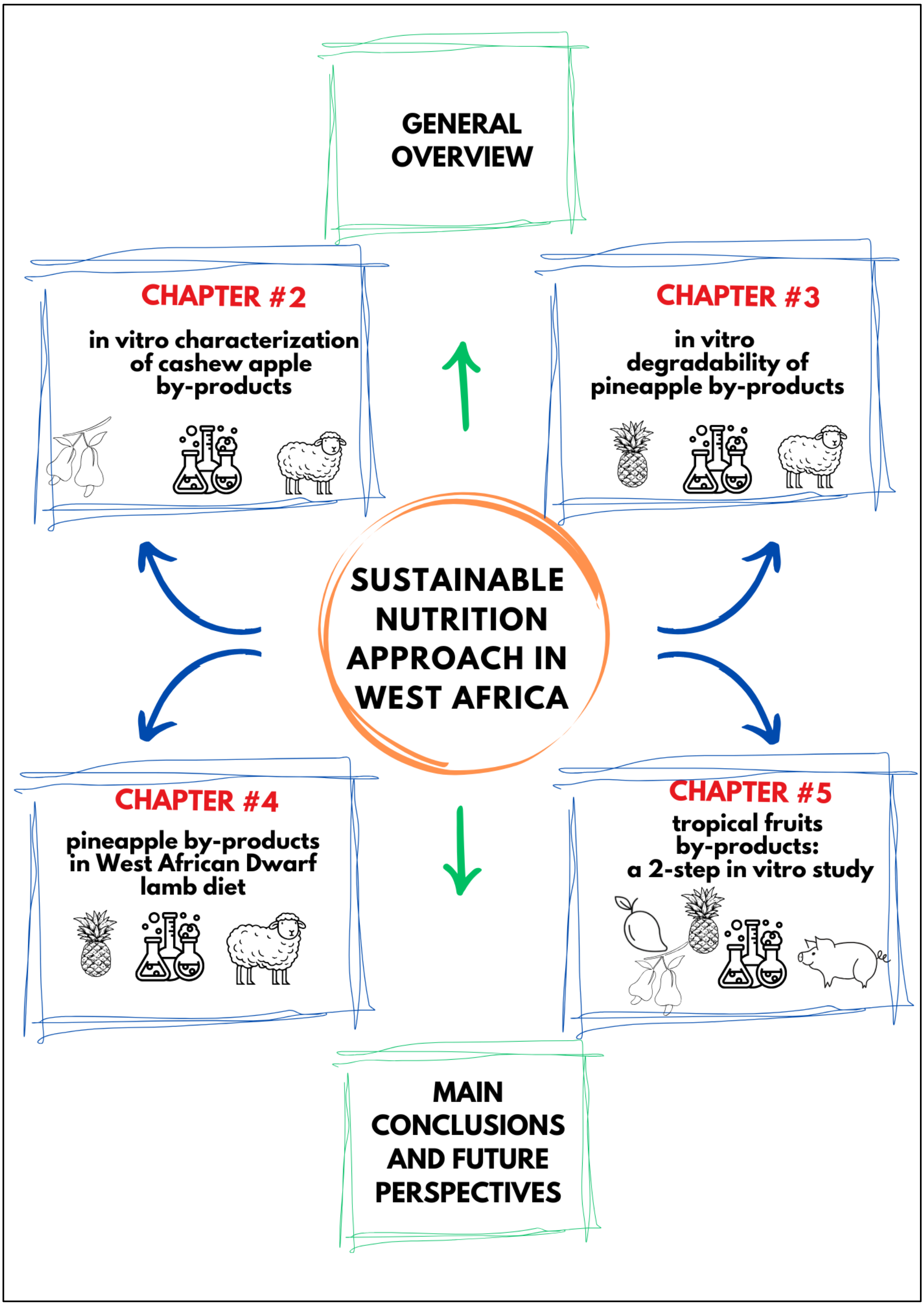

Figure 1. 2. Graphical representation of the thesis structure.

1.6. Reference

1. Alnaimy, A.; Gad, A. E.; Mustafa, M. M.; Atta, M. A. A.; Basuony, H. A. M. Using citrus by-products in farm animals feeding. *Open Access J. Sci.* 2017, *1*(3), 58-67.

2. Alonso-Díaz, M.A.; Torres-Acosta, J.F.J.; Sandoval-Castro, C.A.; Capetillo-Leal, C.M. Amino acid profile of the protein from whole saliva of goats and sheep and its interaction with tannic acid and tannins extracted from the fodder of tropical plants. *Small Rumin. Res.* 2012, 103, 69–74.

3. Amole, T.; Augustine, A.; Balehegn, M.; Adesogoan, A. T. Livestock feed resources in the West African Sahel. *Agron. J.* 2022, *114*(1), 26-45.

4. Aragão, A.S.L.; Pereira, L.G.R.; Chizzotti, M.L.; Voltolini, T.V.; Azevêdo, J.A.G.; Barbosa, L.D.; Santos, R.D.; Araújo, G.G.L.; Brandão, L.G.N. Farelo de manga na dieta de cordeiros em confinamento. *Arq. Bras. Med. Vet. Zootec.* 2012, 64, 967–973.

5. Araújo, A. R.; Costa, J. B.; Rogério, M. C. P.; Carneiro, M. D. S. D. S.; Muniz, L. C.; Fontenele, R. M.; Silva, V. L. Dehydrated cashew apple in different grinding sizes to sheep. *Acta Sci. Anim. Sci.* 2020, *44*, e54398.

6. Aregheore, E.M. A note on the nutritive value of dry ripe plantain peels as a replacement of maize for goats. *J. Anim. Feed Sci.* 1998, 7, 55–62.

7. Avornyo, F. K.; Zougmore, R. B.; Partey, S. T.; Tengan, K. Candidate fodder trees and shrubs for sustainable ruminant production in northern Ghana. *Livest. Res. Rural Dev.* 2018, *30*(9), 1-8.

8. Ayantunde, A.A.; Asse, R.; Said, M.Y.; Fall, A. Transhumant Pastoralism, Sustainable Management of Natural Resources and Endemic Ruminant Livestock in the Sub-Humid Zone of West Africa. *Environ. Dev. Sustain*. 2014, 16, 1097–1117.

9. Azando, E. V. B.; Tchetan, E.; Houehanou, T. D.; Ahoyo, C. C.; Gouissi, M. F.; Adjovi, I. S. M.; Allou, S. Y. D. Traditional breeding of small ruminants in the North-West of Benin: practices and inventory of food resources. *Int. J. Biol. Chem. Sci.* 2022, *16*(3), 1180-1192.

10. Azevêdo, J.A.G.; Filho, S.D.C.V.; Pina, D.D.S.; Detmann, E.; Valadares, R.F.D.; Pereira, L.G.R.; Souza, N.K.D.P.; Silva, L.F.C. Intake, Total Digestibility, Microbial Protein Production and the Nitrogen Balance in Diets with Fruit By-Products for Ruminants. *Rev. Brasil. Zootec.* 2011, 40, 1052–1060.

11. Bakr, M. Citrus pulp as an innovative feed ingredient in ruminant nutrition. A review. *Egyptian J. Anim. Prod.* 2020, 57, 73-80.

12. Bayala, J.; Ky-Dembele, C.; Kalinganire, A.; Olivier, A.; Nantoumé, H. A review of pasture and fodder production and productivity for small ruminants in the Sahel. *ICRAF* 2014, 21.

13. Bedoić, R.; Ćosić, B.; Duić, N. Technical potential and geographic distribution of agricultural residues, co-products and by-products in the European Union. *Sci. Total Environ.* 2019, *686*, 568-579.

14. Borges, D.G.L.; Borges, F.D.A. Plants and their medicinal potential for controlling gastrointestinal nematodes in ruminants. *Nematoda* 2016, 3, 1–7.

15. Bravo, L. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nut. Rev*. 1998, 56, 317–333.

16. Calabrò, S.; López, S.; Piccolo, V.; Dijkstra, J.; Dhanoa, M.S.; France, J. Comparative analysis of gas production profiles obtained with buffalo and sheep ruminal fluid as the source of inoculum. *Anim. Feed Sci. Technol* 2005, *123*, 51-65.

17. Chaudhary, V.; Kumar, V.; Singh, K.; Kumar, R.; Kumar, V. Pineapple (Ananas Cosmosus) Product Processing: A Review. *J. Pharmacogn. Phytochem.* 2019, 8, 4642–4652.

18. Choi, Y.; Park, K.; Lee, S.; Na, Y. Determination of in Situ Degradation Parameters and Feeding Level of Pineapple (Ananas Comosus L.) Cannery By-Product to Hanwoo Steers. *Anim. Biosci*. 2021, 34, 85–92.

19. Corrado, S.; Caldeira, C.; Eriksson, M.; Hanssen, O.J.; Hauser, H.-E.; van Holsteijn, F. Food waste accounting methodologies: challenges, opportunities, and further advancements. Glob. Food. Sec. 2019, 20, 93–100.

20. Correddu, F.; Fancello, F.; Chessa, L.; Atzori, A.S.; Pulina, G.; Nudda, A. E_ects of supplementation with exhausted myrtle berries on rumen function of dairy sheep. *Small Rumin. Res.* 2019, 170, 51–61.

21. Correddu, F.; Lunesu, M.F.; Buffa, G.; Atzori, A.S.; Nudda, A.; Battacone, G.; Pulina, G. Can Agro-Industrial By-Products Rich in Polyphenols be Advantageously Used in the Feeding and Nutrition of Dairy Small Rumin. *Anim.* 2020, *10*, 131.

22. Correia, M.X.C.; Costa, R.G.; Da Silva, J.H.V.; De Carvalho, F.F.R.; De Medeiros, A.N. Use of Dehydrated Pineapple By-Product in Diets for Growing Goats: Digestibility and Performance. *Rev. Brasil. Zootec*. 2006, 35, 1822–1828.

23. Das, I.; Arora, A. Post-harvest processing technology for cashew apples- a review. *J. Food Eng.* 2017, 194, 87–98, https://doi.org/10.1016/j. jfoodeng.2016.09.011.

24. De Evan, T.; Carro, M. D.; Yepes, J. F.; Haro, A.; Arbesú, L.; Romero-Huelva, M.; Molina-Alcaide, E. Feeding mango wastes to dairy goats: Effects on diet digestibility, ruminal fermentation, and milk yield and composition. *Anim. Feed Sci. Technol* 2022, *286*, 115252.

25. De Haan, C.; Robinson, T.; Conchedda, G.; Ericksen, P.; Said, M.; Robinson, L.; Flintan, F.; Shaw, A.; Kifugo, S.; Wane, A. Livestock Production Systems: Seizing the Opportunities for Pastoralists and Agro-Pastoralists. In Confronting Drought in Africa's Drylands: Opportunities for Enhancing Resilience; Cervigni, R., Morris, M.,

Eds: International Bank for Reconstruction and Development/The World Bank, Washington, DC, USA, 2016; pp. 47–70.

26. Dormond, H.; Boschini, C.; Rojas Bourrillon, A. Effect of two levels of ripe banana peel on milk production by dairy cattle. *Agron. Costarricen.* 1998, 22, 43–9.

27. Eeswaran, R.; Nejadhashemi, A. P.; Faye, A.; Min, D.; Prasad, P. V.; Ciampitti, I. A. Current and future challenges and opportunities for livestock farming in West Africa: Perspectives from the case of Senegal. *Agronomy* 2022, 12(8), 1818.

28. Fajemisin, A.N.; Adaramewa, T.; Ogungbesan, M.K. Performance of Yankasa sheep fed Panicum maximum substituted with varying levels of Gmelina arborea forage. In Proceedings of 40th Annual conference of Nigeria society of Animal production held at NAPRI/Ahmadu bello university, Zaria, Nigeria. March 19th, 2015. Pp 589-592.

29. Faostat. New Food Balances; FAO: United Nation, 2021. Available online: http://www.fao.org/faostat/en/#data/FBS.

30. Firatoiu, A.R.; Chiurciu, I.A.; Marcuta, L.; Chereji, A.I.; Soare, E.; Voicu, V.; Marcuta, A. Study on the Production and Marketing of Pineapples Worldwide. In Proceedings of the 37th International Business Information Management Association (IBIMA), Cordoba, Spain, 1–2 April 2021; pp. 4973–4985.

31. Garavaglia, L.; Tedesco, D. Food waste valorization: mango peels enhance *in vitro* rumen microbial fermentation, *Ital. J. Anim. Sci*. 2015, 14, 133–134.

32. García-Rodríguez, J.; Ranilla, M. J.; France, J.; Alaiz-Moretón, H.; Carro, M. D.; López, S. Fermentation kinetics of agro-industrial by-products. *Animals* 2019, *9*, 861.

33. Gowda, N.K.S.; Vallesha, N.C.; Awachat, V.B.; Anandan, S.; Pal, D.T.; Prasad, C.S. Study on evaluation of silage from pineapple (*Ananas Comosus*) Fruit Residue as Livestock Feed. Trop. *Anim. Health Prod*. 2015, 47, 557–561.

34. Guglielmelli, A.; Calabrò, S.; Primi, R.; Carone, F.; Cutrignelli, M.I.; Tudisco, R.; Piccolo, G.; Ronchi, B.; Danieli, P.P. In vitro fermentation patterns and methane production of sainfoin (*Onobrychis viciifolia* Scop.) hay with different condensed tannin contents. *Grass and Forage Sci.*, 2011. 66 (4), 488-500.

35. Guil-Guerrero, J. L.; Ramos, L.; Moreno, C.; Zúñiga-Paredes, J. C.; Carlosama-Yepez, M.; Ruales, P. Plant foods by-products as sources of health-promoting agents for animal production: A review focusing on the tropics. *Agrono. J.* 2016, 108(5), 1759-1774.

36. Gustavsson, J.; Cederberg, C.; Sonesson, U.; van Otterdijk, R.; Meybeck, A. Global Food Losses and Food Waste: Extent, Causes and Prevention. FAO, Rome. 2011.

37. Habib, G.; Khan, N.A.; Sultan, A.; Ali, M. Nutritive value of common tree leaves for livestock in the semi-arid and arid range lands of Northern Pakistan. *Livest. Sci.* 2016, 184, 64-70.

38. Hemung, B.-O.; Sompholkrang, M.; Wongchai, A.; Chanshotikul, N.; Ueasin, N. A Study of the Potential of By-Products from Pineapple Processing in Thailand: Review Article. *Int. J. Health Sci.* 2022, 6, 12605–12615.

39. Ítavo, L. C. V.; Kozerski, N. D.; Ítavo, C. C. B. F.; Dias, A. M.; Petit, H. V.; Benchaar, C.; Voltolini, T.V.; Jobim, C.C.; dos Santos, G.T. (). Orange juice industry by-product silage can increase fat and protein in Holstein cow's milk. *J. Dairy Res.* 2020, 87(4), 400-405.

40. Jalal, H.; Giammarco, M.; Lanzoni, L.; Akram, M.Z.; Mammi, L.M.E.; Vignola, G.; Chincarini, M.; Formigoni, A.; Fusaro, I. Potential of Fruits and Vegetable By-Products as an Alternative Feed Source for Sustainable Ruminant Nutrition and Production: A Review. *Agriculture* 2023, 13, 286.

41. Jayanegara, A.; Palupi, E. Condensed Tannin Effects on Nitrogen Digestion in Ruminants: A Meta-Analysis from *in Vitro* and *in Vivo* Studies. *Media Peternak.* 2010, 33, 176.

42. Koura, B. I.; Yassegoungbe, F. P.; Afatondji, C. U.; Cândido, M. J. D.; Guimaraes, V. P.; Dossa, L. H. Diversity and nutritional values of leaves of trees and shrubs used as supplements for goats in the sub-humid areas of Benin (West Africa). *Trop. Anim. Health Prod* 2021, *53*, 1-14.

43. Kruska, R.L.; Reid, R.S.; Thornton, P.K.; Henninger, N.; Kristjanson, P.M. Mapping Livestock-Oriented Agricultural Production Systems for the Developing World. *Agric. Syst.* 2003, 77, 39–63.

44. Lashkari, S.; Taghizadeh, A. Nutrient digestibility and evaluation of protein and carbohydrate fractionation of citrus by-products. *J. Anim. Physiol. Anim. Nut*. 2013, *97*(4), 701-709.

45. Liu, C.; Asano, S.; Ogata, H.; Ito, S.; Nakase, T.; Takeda, S.; Miyoshi, K.; Numata, Y.; Takahashi, K.; Kajikawa, H. Digestive, Fermentative, and Physical Properties of Pineapple Residue as a Feed for Cattle. *Anim. Sci. J.* 2021, 92, e13535.

46. Melesse, A.; Steingass, H.; Schollenberger, M.; Rodehutscord, M. Component Composition, *in Vitro* Gas and Methane Production Profiles of Fruit By-Products and Leaves of Root Crops. *J. Agric. Sci.* 2018, 156, 949–958.

47. Molina-Flores, B.; Manzano-Baena, P.; Coulibaly, M.D. The Role of Livestock in Food Security, Poverty Reduction and Wealth Creation in West Africa; Food and Agriculture Organization of the United Nations (FAO): Accra, 2020.

48. Musco, N.; Calabrò, S.; Infascelli, F.; Tudisco, R.; Lombardi, P.; Grossi, M.; Addi, L.; Neto, B.P.; Cutrignelli, M.I. *In vitro* fermentation of structural carbohydrate-rich feeds using faecal inoculum from pigs. *Ita. J Anim. Sci.* 2015, 14 (3), art. no. 3875, pp. 557-562.

49. Odusanya, L. Q.; Fasae, O. A.; Adewumi, O. O.; James, I. J. Effect of cassava leaf meal concentrate diets on the performance, haematology and carcass characteristics of West African Dwarf lambs. *Arch. Zootec.* 2017, *66*(256), 603-609.

50. Okoruwa, M.I.; Igene, F.U. Comparison of fermentation kinetics (*in vitro*) of Napier grass and fruit peels for ruminants: the pattern of organic matter degradability, volatile

fatty acid concentration estimated methane and microbial biomass production, *J. Agric. Vet. Sci*. 2014, 7(1), 21–28.

51. Okpanachi U.; OyewoleB. O.; Egbu, C. F.; Ganiyu, O. Y. Effects of Feeding Sun-Dried Yellow Cashew Pulp Based Diets on Performance, Dry Matter and Nutrient Digestibility of West African Dwarf Goats. *Anim. Vet. Sci.* 2016, 4(3-1), 7-12.

52. Omre, P.K.; Singh, S.; Shikha, S. Waste utilization of fruits and vegetables-A review. *South Asian J. Food Technol. Environ.* 2018, 4(1): 605-615

53. Ozturk, B.; Parkinson, C.; Gonzalez-Miquel, M. Extraction of polyphenolic antioxidants from orange peel waste using deep eutectic solvents. *Sep. Purif. Technol.* 2018, 206, 1–13.

54. Paya, H.; Taghizadeh, A.; Lashkari, S.; Shirmohammadi, S. Evaluation of rumen fermentation kinetics of some by-products using in situ and *in vitro* gas production technique. *Slovak J. Anim. Sci.* 2012, *45*(4), 127-133.

55. Pinotti, L.; Luciano, A.; Ottoboni, M.; Manoni, M.; Ferrari, L.; Marchis, D.; Tretola, M. Recycling Food Leftovers in Feed as Opportunity to Increase the Sustainability of Livestock Production. *J. Clean. Prod*. 2021, 294, 126290.

56. Porter, S. D.; Reay, D. S.; Higgins, P.; Bomberg, E. A half-century of production-phase greenhouse gas emissions from food loss & waste in the global food supply chain. *Sci. Total Environ.* 2016, *571*, 721-729.

57. Quideau, S.; De_eux, D.; Douat-Casassus, C.; Pouységu, L. Plant polyphenols: Chemical properties, biological activities, and synthesis. *Angew. Chem. Int. Ed.* 2011, 50, 586–621.

58. Raseel, K.; Chacko, B.; Sunanda, C.; Dildeep, V.; Abraham, J. Nutrient Evaluation of Energy Rich Unconventional Feeds Available in Wayanad. *IJSN.* 2018, 9, 117–118.

59. Ravani, A.; Joshi, D. Mango and it's by product utilization–a review. *Trends Post Harvest Technol.* 2013, 1,1, 55-67.

60. Ribeiro Filho, M. R.; Soto-Blanco, B. Poisoning by cashew apple (Anacardium occidentale L.) in cattle. *Acta Sci. Vet.* 2012, *40*(4), 1-5.

61. Rico, X.; Gullón, B.; Alonso, J. L.; Yáñez, R. Recovery of high value-added compounds from pineapple, melon, watermelon and pumpkin processing by-products: An overview. *Int. Food Res.* 2020, *132*, 109086.

62. Sanon, H.O.; Kanwe, A.B.; Millogo, A.; Ledin, I. Chemical composition, digestibility, and voluntary feed intake of mango residues by sheep. *Trop. Anim. Health Prod*. 2013, 45(2), 665–669.

63. Silva, J.L.; Guim, A.; Carvalho, F.F.R.; Mattos, C.W.; Menezes, D.R.; Coelho, M.C.S.C.; García, D.A.; Pereira, J.D.; Soares, L.F.P. Replacement of corn with mango meal for dairy goats. *Rev. Colomb. Cienc. Pecu* 2016. 29, 178–187.

64. Sodjinou, M.K.B.; Assouma-Imorou, A.; Olounlade, A.O. Technical Efficiency of Pineapple Production and Challenges in Southern Benin. *Afr. J. Agric. Res.* 2022, 18, 522–534.

65. Souza, H. A.; Moraes, E. H. B. K.; Oliveira, A. S.; Batista, E. D.; Santos, K. R.; Sousa, J. N.; Ortelana, A.; Lamaga, K.A.K.; Moraes, K. A. K. Cashew processing product as alternative energy feedstuff for grazing beef cattle under tropical conditions. *Livest. Sci.* 2020, 236, 104022.

66. Suksathit, S.; Wachirapakorn, C.; Opatpatanakit, Y. Effects of Levels of Ensiled Pineapple Waste and Pangola Hay Fed as Roughage Sources on Feed Intake, Nutrient Digestibility and Ruminal Fermentation of Southern Thai Native Cattle. *Songklanakarin J. Sci. Technol*. 2011, 33, 281–289.

67. Talasila, U.; Shaik, K.B. Quality, spoilage and preservation of cashew apple juice: a review. *J. Food Sci. Technol*. 2015, 52, 54–62.

68. Theodorou, M.K.; Williams, B.A.; Dhanoa, M.S.; McAllan, A.B.; France, J.A.; Simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim. Feed Sci. Technol.* 1994, 48 185–197.

69. UN. World Population Prospects, United Nations: New York, NY, USA, 2019.

70. Wadhwa, M.; Bakshi, M. P. S.; Makkar, H. P. Waste to worth: fruit wastes and by-products as animal feed. *CABI Rev.* 2015, 1-26.

71. Wanapat, M.; Suriyapha, C.; Dagaew, G.; Prachumchai, R.; Phupaboon, S.; Sommai, S.; Matra, M. The recycling of tropical fruit peel waste-products applied in feed additive for ruminants: food manufacturing industries, phytonutrient properties, mechanisms, and future applications. *J Agric Food Res* 2024, 101234.

Chapter 2.

Sustainable Ruminant Nutrition In The West Africa By *In Vitro* Characterization Of Cashew Apple By-Products

Article published in Heliyon (2024), 10: e37737

https://doi.org/10.1016/j.heliyon.2024.e37737

2.1. Abstract

Cashew trees (*Anacardium occidentale* L.) are planted primarily for their nuts, but they also generate apples which are mostly thrown away due to their astringent taste. The current study aimed to explore the possible utilization of cashew apple by-products (CABP) in West Africa as an alternative feedstuff for small ruminants' nutrition. To achieve this aim, five parts of cashew apple by-products (whole, up, down, middle part, and pomace) of two cashew varieties (red and yellow) were collected in two different agroecological zones (Sudanian Zone, SZ, and Sudano-Guinean Zone, SZ) to be characterized for the chemical composition, including polyphenols and sugars, and the *in vitro* fermentation pattern. The results showed that CABP characteristics depend more on sampling area than on variety. The dry matter (DM) in SZ and SGZ varied from 12.76 to 26.10 % and 7.41-22.9 %, respectively. The pomace showed the highest crude protein, lipids, and neutral detergent fiber (NDF) content (SZ: 9.48, 3.94, and 31.66 % DM; SGZ: 14.03, 4.94 and 34.12 % DM, respectively) but the lowest non-structural carbohydrate (NSC) and sugar for both zones. Regarding the *in vitro* fermentation, the organic matter degradability (OMD) was higher in the middle part (73.73 %) and whole apple (61.62 %) of SZ and SGZ, respectively. In contrast, the pomace from both zones showed the lowest *in vitro* fermentation parameters. The total polyphenols were more concentrated in the CABP from SZ (whole: 2736 µg/g DW; pomace: 3813 µg/g DW) compared to those from SGZ (whole: 1755 µg/g DW; pomace: 1374 µg/g DW). Results suggest that CABP should be collected in each cultivation zone regardless of variety, separating pomace from other by-products and might be used as alternative feedstuff for small ruminants during the dry season in the West Africa region.

Keywords: waste management, polyphenols, volatile fatty acids, ruminant nutrition, feedstuffs, alternative ingredient sheep.

2.2. Introduction

According to objective #12 of the 2030 Agenda for Sustainable Development proposed by The United Nations (https://sdgs.un.org/goals), it is necessary to minimize the negative effects on human health and environmental pollution by promoting sustainable management and the efficient use of natural resources, reducing food losses along supply chains and managing all waste throughout its life cycle at a global level. The use of agro-industrial residues in animal nutrition is an eco-sustainable feeding strategy of making use of them, especially in countries where the climate conditions do not always guarantee the necessary feed resources to improve livestock product yields and quality.

The cashew (*Anacardium occidentale* L.) belongs to the *Anacardiaceae* family, which includes about 60 genera and 400 species, is a tropical and sub-tropical plant capable of growing on various soil types, even dry ones with poor nutrients (Runjala and Kella, 2017). It is native to tropical America but was successfully introduced in several countries of Asia, Africa, and Central America as an important cash crop (Das and Arora, 2017). Cashew trees are planted principally for nut production (called true fruit) which is outside and linked to the apple (called peduncle or pseudo fruit). The colors of the apples (yellow to red) are the only physical differences observed in cashew plantations that give the name to the varieties (red variety vs. yellow variety) while the nuts do not present any physical differences. The farmers often cultivate different varieties in the same field because only the nuts are of interest. In 2020, the cashew nut production in the world was estimated at 4,180,990 tons (Faostat, 2022) with the West African region (Ivory Coast and Benin) in second position. At the same time, a great number of cashew apples are thrown away annually (ranging from 41 to 62 million tons), considering that 10 - 15 tons of apples are obtained for every ton of nuts harvested (Talasila and Shaik, 2015). Cashew apples are less processed (10 %) to juice, vinegar, jam, chutney, or soft drinks compared to other processed fruits generating the pomace as waste (Cruz Reina et al., 2022), while a large quantity of them (90 %) is thrown away (Das and Arora, 2017; Aidoo et al., 2022). These quantities are expected to increase because the cashew tree inventory has revealed that the plantations are young and will live at least 35 years (Oliveira et al., 2020). However, the perishability of the apples, their rapid fermentation and the presence of phenolic compounds, such as tannins with an astringent taste, limit their total transformation by the food industries even if cashew apple juice is richer in vitamins, sugar, minerals, amino acid, and dietary fiber than the most consumed fruits such as oranges and pineapples (Nair, 2010; Kannan et al., 2021; Asmawati et al., 2022). Clarification, heat treatment, microfiltration, fermentation or ethanol vapors, and CO_2 methods could mitigate the presence of tannin and the astringent taste in cashew apples and their juice (Emelike and Ebere, 2016; Dheeraj et al., 2023). However, these methods are not available in all cashew cultivation areas.

In West African countries, the technique used to reduce the astringent taste in cashew juice consists of cutting the extremities (up and down part of apples), pressing the middle part, and applying a heat treatment (Padonou et al., 2016). Therefore, many different types of cashew waste are produced consisting of whole apples left in the fields during the nut harvesting, the extremities, and pomace generated by the processed apples. The unused cashew waste represents an environmental problem that the decomposition will contribute to global warming and environmental air pollution (Goula and Lazarides, 2015; García-Rodríguez et al., 2019). However, this waste can be utilized for different purposes, such as bioethanol, hydrolytic enzymes, lactic acid, biosurfactants, and as feed for animals (Dos Santos et al., 2012; Kannan et al., 2021). The latter would seem to be the most useful in West Africa due to the cost of animal feed and the scarcity of natural pasture for grazing ruminants during the dry season (Koura et al., 2022). However, some studies in Latin America, focusing on chemical composition, intake, digestibility, and growth parameters, advise replacing cashew apple by-products with other ingredients in ruminants' diets (Ferreira et al., 2004; Rodrigues et al., 2010; Cruz Reina et al., 2022). In addition, recent studies carried out in West Africa revealed that red cashew apple pulp by-products are richer in sodium, potassium, and non-structural carbohydrates than yellow ones which are richer in calcium, magnesium, phosphorus, and lignin (Okpanachi et al., 2018).

Furthermore, tannins, saponin, phytate, oxalate, and flavonoids were reported to be present in cashew apples and their by-products (Filho and Soto-Blanco, 2012; Okpanachi al., 2016) which might have a negative effect on rumen fermentation. Unfortunately, the literature on cashew apple by-products lacks data on the comparative chemical composition, polyphenols, and the rumen fermentation between the by-products of different cashew varieties (red vs. yellow) and their origin (Sudanian vs. Sudano-Guinean) in the West African region. Therefore, there is a need to understand the nutritional composition of the different cashew apple by-products and to consider the influence of variety and environmental conditions in the areas where they are grown. This information would be helpful for farmers when planning animal feed including these by-products in the various agro-ecological zones. The current study aims to explore the potential use of cashew apple by-products (CABP) in ruminants' nutrition in the West Africa region as alternative feedstuff.

2.3. Material and Methods

2.3.1. Study area

The two selected areas, the Sudanian Zone (SZ) and Sudano-Guinean Zone (SZG) are the most suitable areas for cashew cultivation in West Africa and are both present in the Republic of Benin. The municipalities of Parakou and Ketou, located in SZ and SGZ, respectively, were chosen for collecting samples. Parakou is in the North-East of Benin, located between the latitudes 8°55' and 10°53'N, and the longitudes 2°00' and 3°50'E. This area is characterized by two seasons (dry: November to May; rainy: May to October) with annual rainfall varying between 900 and 1000 mm. The average relative humidity and temperature values are 54.9 % and 27.5°C, respectively. Ketou is in the South-East of Benin between the latitudes 7°10'0'' N and the longitudes 2°34'60''E with 1100 - 1400 mm of rainfall within two rainy seasons (April to July and October to November) and two dry seasons (August to September and December to March). The average relative humidity and temperature values are 66.3 % and 26.9°C, respectively (Gnanglè et al., 2011).

2.3.2. Samples collection

Cashew apples were collected separately according to variety (red and yellow) and cultivation area (SZ and SGZ) after nuts harvesting. All the samples were transported within one hour to the Laboratory of Ecology, Health, and Animal Production (Faculty of Agronomy, University of Parakou) to be processed following the local juice processing technique. A steel knife was used to move the extremities parts of apples (up and down), whereas a hydraulic press machine (Tianyu Youdo Machine, model UDZL-W33, dimensions 330 x 330 x 600 mm, Kaifeng, China) was used to press the middle parts, generating juice and pomace. The by-products obtained through this process were: the upper part, lower part, and pomace. In addition, the whole apples and the unpressed middle part were also collected from the apples left over in the cashew plantation field after the harvesting of the nuts. The biggest particles were cut into small pieces for fast drying in a dried oven at 65°C. The cashew apple processing and its by-products generation is presented in Figure 2. 1. All the samples were sent to the Laboratory of Feed Evaluation (Department of Veterinary Medicine and Animal Production, University of

Napoli Federico II, Napoli, Italy), where they were ground at 1.1 mm for chemical composition analysis and *in vitro* fermentation.

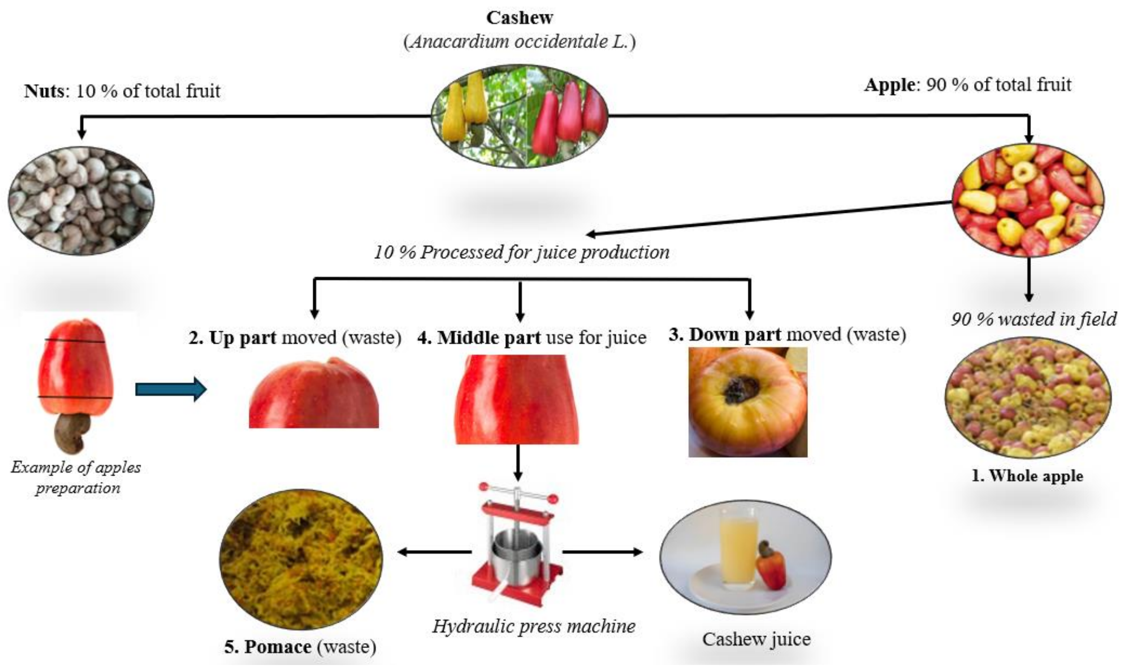

Figure 2. 1. Cashew apple processing into juice in West Africa region with No. 1 to 5 samples collected.

2.3.3. Chemical composition assessment

The AOAC method was used to determine residual dry matter at 103°C (DM, ID: 2001.12), crude protein (CP, ID: 978.04), ether extract (EE, ID: 920.39), and ash (ID: 930.05) (AOAC, 2005). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were also quantified according to Van Soest et al. (1991). The total sugars (TS) and free sugars (FS) were quantified only for whole apples and pomace (AOAC, 2000), the most representative by-products in terms of available amounts. These by-products were infused with distilled water at 1:40 and 1:80 ratio for FS and TS, respectively. The mixture of FS was left at room temperature (20°C) for 30 min while 20 ml of HCl (1:1) was added to each flask of TS and incubated in a thermostatic bath at 70°C for 3 h followed by three drops of 1 % phenolphthalein to cool solution. Before the filtration, 5 ml of Carrez solution I and II were added every 10 min to both FS and TS flasks. The filtrated solutions were titrated using Fehling's solution (A and B) and 1 % of methylene blue. Then the FS and TS were determined as (f x d) / (a - 0.1) where f is the factor due to the power of sugar (being 3.35 for reducing sugars and 5.15 for total sugars), d is the dilution factor, and a is the volume (ml) of the solution used during the titration.

2.3.4. Determination of polyphenols

Polyphenol extraction was carried out as follows (Chiacchio et al., 2022): 0.1 g of each sample was added to 1.0 mL of methanol/water solution (70:30 v/v) and vortexed for 1 min, then centrifuged for 10 min (14800 rpm, 4°C), the supernatants filtered using 0.45 µm PTFE filters and diluted for HPLC analyses. The chromatographic separation was performed (HPLC SHIMADZU equipped with UV/VIS SPD-20° detector; Prominence, USA) (Chiacchio et al., 2022). The identified phenolic compounds were: gallic acid, protocatechuic acid, chlorogenic acid, procyanidin B2, 4-hydroxibenzoic acid, epicatechin, *p*-coumaric acid, sinapic acid, *trans*-ferulic acid, naringin, quercetin, and *trans*-cinnamic acid. Two unidentified phenolic compounds corresponding to 'Unknown 1' and 'Unknown 2' were quantified as the equivalent of gallic acid. The retention times of identified polyphenols varied from 4 min for gallic acid to 28 min for *trans*-cinnamic acid. The analyses were conducted in three replicates and the results were expressed as µg/g of dry weight using a calibration curve with the corresponding pure compounds.

2.3.5. In vitro fermentation

For the *in vitro* trial, 1.0027 ± 0.0010 g of each sample in 3 replications were incubated at 39°C under anaerobic conditions in 120 ml serum bottles containing 74 ml of medium solution, 4 ml of reducing agent and 10 ml of sheep rumen liquor (Theodorou et al., 1994). Three bottles without substrate (blanks) were also incubated to correct the fermentation parameters. In total 63 bottles were incubated. The medium solution and reducing agent used were prepared following the methodology proposed by Lowe et al. (1985) and approved by Theodorou et al. (1994):

- Medium solution: 2.5 L (H_2O) + 0.5 mL (micromineral solution) + 1 L (buffer solution) + 1 L (macro-mineral solution) + 5 mL (resazurin solution, 0.1 g/100 mL H_2O).
- Micromineral solution: 100 mL (H_2O) + 13.2 g $CaCl_2 \cdot 2H_2O$ + 10 g $MnCl_2 \cdot 4H_2O$ + 1 g $CoCl_2 \cdot 6H_2O$ + 8 g $FeCl_3 \cdot 6H_2O$.
- Buffer solution: 1 L (H_2O) + 4 g NH_4HCO_3 + 35 g $NaHCO_3$
- Macro-mineral solution: 1 L (H_2O) + 4.65 g $Na_2HPO_4 \cdot 2H_2O$ + 6.2 g KH_2PO_4 + 0.6 g $MgSO_4.7H_2O$
- Reducing agent: 190 mL (H_2O) + 1.25 g cysteine HCl + 8 mL NaOH (1M) + 1.25 g $Na_2S \cdot 9H_2O$.

Regarding the rumen liquor, it was collected at an EU-authorized slaughterhouse (EC Council, 2004) from three adult males of Merinizzata sheep raised on natural pasture and transported to the laboratory within one hour in pre-warmed thermos (39°C). Before use, the rumen liquor was measured for pH, verifying it was adequate for microorganisms' activity (6.08 ± 0.15). At the laboratory, the rumen contents were mixed, filtered through cheese cloths, and flushed under CO_2 to guarantee anaerobiosis. All the steps of the experimental trial were approved by the Ethical Animal Care and Use Committee of the University of Napoli Federico II (Prot. 2019/0013729 of 08/02/2019). The pressure and the gas produced were recorded with a manual pressure transducer (Cole and Palmer Instrument Co, Vernon Hills, IL, USA). After 48 h the fermentation was stopped by cooling the bottles at 4°C and the pH values per bottle were measured (pH-meter ThermoOrion 720 A+, Fort Collins, CO, USA). The fermentation liquors were sampled and stored at -16°C for volatile fatty acids (VFA) detection. Pre-weighed sintered glass crucibles (Schott Duran, Mainz, Germany, porosity #2) were used to collect the undegraded material by filtration. Then, they were oven-dried (103°C) and burned

(550°C) to calculate the organic matter degraded (OMD, %) and the cumulative volume of gas related to incubated OM (OMCV, mL/g), respectively.

2.3.6. Volatile fatty acid detection

The fermentation liquors were centrifuged twice at 12,000 x g for 10 min (Universal 32R centrifuge, Hettich FurnTech Division DIY, Melle- Neuenkirchen, Germany) for VFA determination (Calabrò et al., 2022). One milliliter of supernatant was mixed with 1 ml of 0.06 mol oxalic acid to inject in gas chromatography (ThermoQuest 8000top Italia SpA, Rodano, Milan, Italy) equipped with a fused silica capillary column (30 m, 0.25 mm ID, 0.25 µm film thickness), with an external standard solution (acetic, propionic, butyric, iso-butyric, valeric and iso-valeric acids). The equation (1) was utilized to quantify in percentages the branched-chain fatty acids (BCFA) produced during the fermentation (Calabrò et al., 2022).

$$BCFA = [(iso - butyric\ acid + iso - valeric\ acid)/total\ VFA * 100] \quad \text{(Eqn. 1)}$$

2.3.7. Data processing

The metabolizable energy (ME, MJ/kg; equation 2) was estimated using the chemical composition data and *in vitro* fermentation parameters (Menke and Steingass, 1988):

$$\mathrm{ME} = 2.43 + 0.1206 * \mathrm{G24} + 0.0069 * \mathrm{CP} + 0.0187 * \mathrm{EE} \quad \text{(Eqn. 2)}$$

where CP, EE, and GP are respectively crude protein, ether extract, and G24 the volume of gas recorded after 24 h of incubation by 200 mg of DM. To estimate the fermentation kinetics, for each incubated bottle, the gas production profiles were fitted to a sigmoidal model (equation 3) (Groot et al., 1996).

$$G = \frac{A}{1+\left(\frac{B}{t}\right)^{C}} \quad \text{(Eqn. 3)}$$

where G is the total gas produced at the end of incubation time (ml/g of incubated OM) at time t (h), A is the asymptotic gas production (ml/g), B is the time at which one-half of A is reached (h), and C is the curve switch. Then maximum fermentation rate (R_{max}, ml/h) and the time at which it occurs (T_{max}, h) were calculated using the equations 4 and 5 respectively (Bauer et al., 2001):

$$\mathrm{R_{max}} = \frac{A*B^{C}*C*T_{max}{}^{(-C-1)}}{(1+B^{C}*T_{max}{}^{-C})^{2}} \quad \text{(Eqn. 4)}$$

$$T_{max} = B * \left(\frac{C-1}{C+1}\right)^{\frac{1}{C}} \quad \text{(Eqn. 5)}$$

2.3.8. Statistical analysis

All the data were applied in JMP® software (Version 14 SW, SAS Institute Inc., Cary, NC, USA, 1989-2019) to statistically evaluate the following fixed effect: cultivation zone, variety, and by-products. The t-test and Tukey's HSD test were used. The equation (6) was utilized as a statistical model fixing the significance level at 5 %.

$$Y_{ijk} = \mu + Z_i + V_j + B_k + (Z * B)_{ik} + \varepsilon_{ijk} \quad \text{(Eqn. 6)}$$

where Y is the experimental data, μ the general mean, Z the effect of cultivation zone (i = Sudanian and Sudano-Guinean), V the variety effect (j = red and yellow), B the by-products (k = whole, up, down, middle, and pomace) and ε is the error term. Only Z∗B interaction was considered because the other interaction including the variety effect was not statistically significant. The correlations between chemical composition, *in vitro* fermentation parameters, and polyphenol content were also performed (JMP® 1989–2019).

2.4. Results

2.4.1. Chemical characterization and nutritive value

The chemical composition of cashew apple by-products (CABP) is presented in Table 2. 1. for the five by-products in the two sampling areas as the average of the two varieties. The results showed that the variety effect was significant only for ADF, ADL, and ME ($p < 0.05$), while the zone and by-product effects were highly significant ($p < 0.0001$) for all the parameters. DM ranged between 26.10 % in SZ pomace and 7.41 % in SGZ down part. A similar trend was observed in the two zones (pomace > up > whole > middle > down), with values always being higher in SZ. Ash content in both areas showed the highest value ($p < 0.05$) in the down part and the lowest in the pomace. The content of CP, EE, NDF and ADF was higher in pomace; all these parameters were always higher in SGZ compared to SZ. Regarding ADL, the content was high in all samples of both areas, rangeding between 8.03 and 17.47 % DM, especially in pomace (11.52 and 17.47 % DM, in SZ and SGZ, respectively). The non-structural carbohydrates (NSC) showed high values in all samples, with the highest values in whole and up part and the lowest level in pomace; different rankings in the two areas resulted. For sugar content (data not showed), significant differences ($p < 0.001$) were observed in the two analyzed by-products, whole and pomace. Total sugar and free sugar (TS and FS, % DM) were higher in the whole apples (SZ: 57.51 and 65.00; SGZ: 54.21 and 53.58, for TS and FS, respectively) compared to pomace (SZ: 43.00 and 44.93; SGZ: 42.73 and 30.15, for TS and FS respectively). Estimated ME ranged between 6.90 and 7.77 MJ/kg DM with any significance ($p > 0.05$) among the principal factors.

Table 2. 1. Chemical composition of cashew apple by-products from Sudanian and Sudano-Guinean zones of West Africa

Item	DM	CP	EE	Ash	NDF	ADF	ADL	NSC	ME
	%	*% DM*							*MJ/kg*
Sudanian Zone									
Whole	14.46^{C}	6.87^{BC}	1.85^{C}	2.95^{B}	16.13^{B}	16.60^{C}	8.68^{AB}	72.19^{AB}	7.23
Up	19.75^{B}	6.17^{C}	1.81^{C}	2.44^{BC}	11.26^{C}	17.04^{C}	9.80^{AB}	78.32^{A}	6.90
Down	12.76^{C}	8.49^{AB}	2.63^{B}	3.89^{A}	18.47^{B}	20.28^{B}	10.40^{AB}	66.56^{B}	7.51
Middle	14.88^{C}	6.04^{C}	1.49^{C}	2.60^{BC}	10.40^{C}	14.94^{C}	8.03^{B}	79.46^{A}	7.00
Pomace	26.10^{A}	9.48^{A}	3.94^{A}	2.11^{C}	31.66^{A}	30.14^{A}	11.52^{A}	52.79^{C}	7.02
Sudano-Guinean Zone									
Whole	11.24^{BC}	8.77^{C}	2.33^{C}	2.38^{B}	18.28^{C}	22.14^{C}	12.38^{B}	68.24^{A}	7.40
Up	16.38^{B}	8.68^{C}	2.35^{C}	2.26^{B}	18.36^{C}	21.39^{C}	11.60^{B}	68.34^{A}	7.77
Down	7.41^{C}	10.15^{B}	3.11^{B}	3.11^{A}	23.82^{B}	28.63^{B}	13.49^{B}	59.81^{B}	7.53
Middle	8.80^{C}	8.33^{C}	3.03^{B}	2.41^{B}	20.97^{BC}	21.02^{C}	10.83^{B}	65.26^{AB}	7.15
Pomace	22.98^{A}	14.03^{A}	4.94^{A}	1.60^{C}	34.12^{A}	33.54^{A}	17.47^{A}	45.29^{C}	7.36
p-value									
Z	<0.0001	<0.0001	0.0007	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0315
Var	0.5685	0.8624	0.5628	0.0517	0.4614	<0.0001	<0.0001	0.6192	0.0120
BP	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	0.3441
Z∗BP	0.6594	0.1100	0.4426	0.0083	0.0002	0.0406	0.3244	0.0255	0.3468
MSE	6.2940	1.2746	0.4498	0.0315	2.9881	2.4680	3.9229	1.3326	0.2997

DM: dry matter, CP: crude protein, EE: ether extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, ME: estimated metabolizable energy, Var: variety, Z: zone, BP: by-product, NSC: non-structural carbohydrate (NSC = 100 – (%NDF + %CP + %EE + %Ash), Z∗BP: Zone ∗ By-products interaction. MSE: mean square error. Along the column, for each zone, different letters indicate the statistical differences ($p < 0.05$

2.4.2. *In vitro fermentation characteristics*

The *in vitro* fermentation characteristics in the five by-products of the two sampling areas (shown as the average of the two varieties) were statistically influenced by the factors considered (Table 2. 2.). The pH recorded at the end of the fermentation trial was different in terms of cultivation zone (6.42 and 6.56, for SZ and SGZ respectively; $p = 0.003$) whereas no differences were observed among by-products. On the other hand, the OM degradability (OMD) and the time at which the maximum fermentation rate occurs (T_{max}) were significantly different ($p<0.0001$) between the cultivation zones. The by-products factor showed a significant effect ($p<0.0001$) in all parameters except pH, A, and T_{max}. In particular, the highest OMD was obtained in the SZ middle part (73.73%) and SGZ whole apples (61.62%), while the pomace showed the lowest degradability in both zones (51.70 and 43.63%, SZ, and SGZ respectively). Moreover, the pomace had the lowest R_{max} in both zones (7.97 mL/h and 7.79 mL/h, for SZ and SGZ, respectively), whereas the down and up parts showed the highest (11.78 and 13.08 mL/h) in SZ and SGZ, respectively. The fermentation process of CABP showed that the gas productions of both zones were more similar from 2 h to 10 h of incubation, after this time the down part in SZ increased the gas production and the middle part in SGZ decreased (Figures 2. 2. And 2. 3., Panel A). For most samples, the fermentation rate reached the maximum around 8 h for SZ and before (around 5 h) for SGZ and then decreased gradually until the stop at 48 h (Figures 2. 2. And 2. 3., Panel B). Pomaces in both zones showed the slowest fermentation rate profile.

Table 2. 2. *In vitro* fermentation characteristics of cashew apple by-products from Sudanian and Sudano-Guinean zones of West Africa

Item	**pH**	**OMD**	**OMCV**	**Yield**	**A**	**B**	**T_{max}**	**R_{max}**
		%	*mL/g*	*mL/g*	*mL/g*	*h*	*h*	*mL/h*
Sudanian Zone								
Whole	6.54	67.68^{BC}	222.8	327.8	243.3	14.40^{B}	7.56	11.40
Up	6.55	70.10^{B}	235.6	338.8	257.6	14.82^{B}	7.96	11.71
Down	6.48	64.96^{C}	237.1	367.2	267.9	14.44^{B}	6.64	11.78
Middle	6.40	73.73^{A}	229.1	311.7	267.2	16.81^{A}	7.84	9.92
Pomace	6.43	51.70^{D}	193.9	347.5	249.7	19.52^{A}	6.63	7.97
Sudano-Guinean Zone								
Whole	6.56	61.62^{AB}	235.7	384.7^{AB}	278.7^{AB}	15.59^{B}	5.03	11.00^{AB}
Up	6.51	59.44^{BC}	227.3	378.5^{AB}	238.0^{B}	12.00^{B}	4.93	13.08^{A}
Down	6.63	51.28^{D}	223.4	439.2^{A}	258.8^{AB}	14.13^{B}	3.82	11.51^{AB}
Middle	6.58	58.57^{BC}	211.5	360.6^{B}	236.0^{B}	13.73^{B}	5.10	10.71^{AB}
Pomace	6.64	43.63^{E}	180.8	429.3^{AB}	286.9^{A}	24.10^{A}	2.29	7.79^{B}
p-value								
Z	0.0031	<0.0001	0.4169	<0.0001	0.3769	0.874	<0.0001	0.6522
Var	0.9240	0.0772	<0.0001	0.0005	0.3770	0.184	0.0158	0.0914
BP	0.5972	<0.0001	<0.0001	<0.0001	0.197	<0.0001	0.0693	0.002
Z∗BP	0.0721	0.0002	0.2628	0.7714	0.0014	0.0014	0.8685	0.7961
MSE	0.0080	2.9021	29.47	139.9	310.1	2.618	1.8159	2.5934

OMD: organic matter degradability, OMCV: cumulative volume of gas related to incubated organic matter, Yield: cumulative volume of gas related to degraded organic matter, A: potential gas production, B: time at which A/2 was reached, T_{max}: time at which maximum rate was reached, R_{max}: maximum fermentation rate, Var: variety, Z: zone, BP: by-product, Z∗BP: Zone ∗ By-products interaction. MSE: mean square error. Along the column, for each zone, different letters indicate the statistical differences ($p<0.05$).

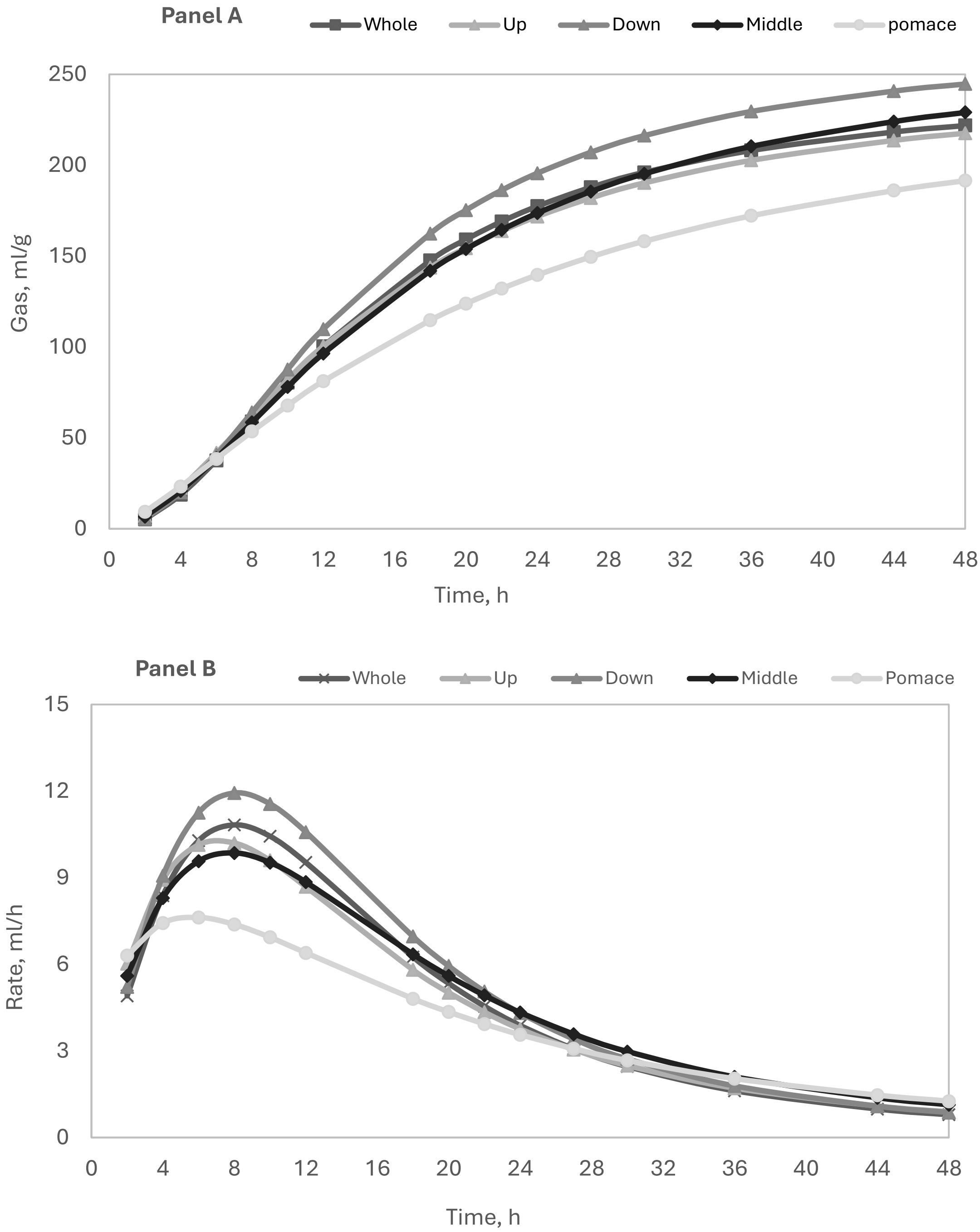

Figure 2. 2. *In vitro* gas production profile (Panel A) and fermentation rate (Panel B) of cashew apple by-products from Sudanian zone of West Africa.

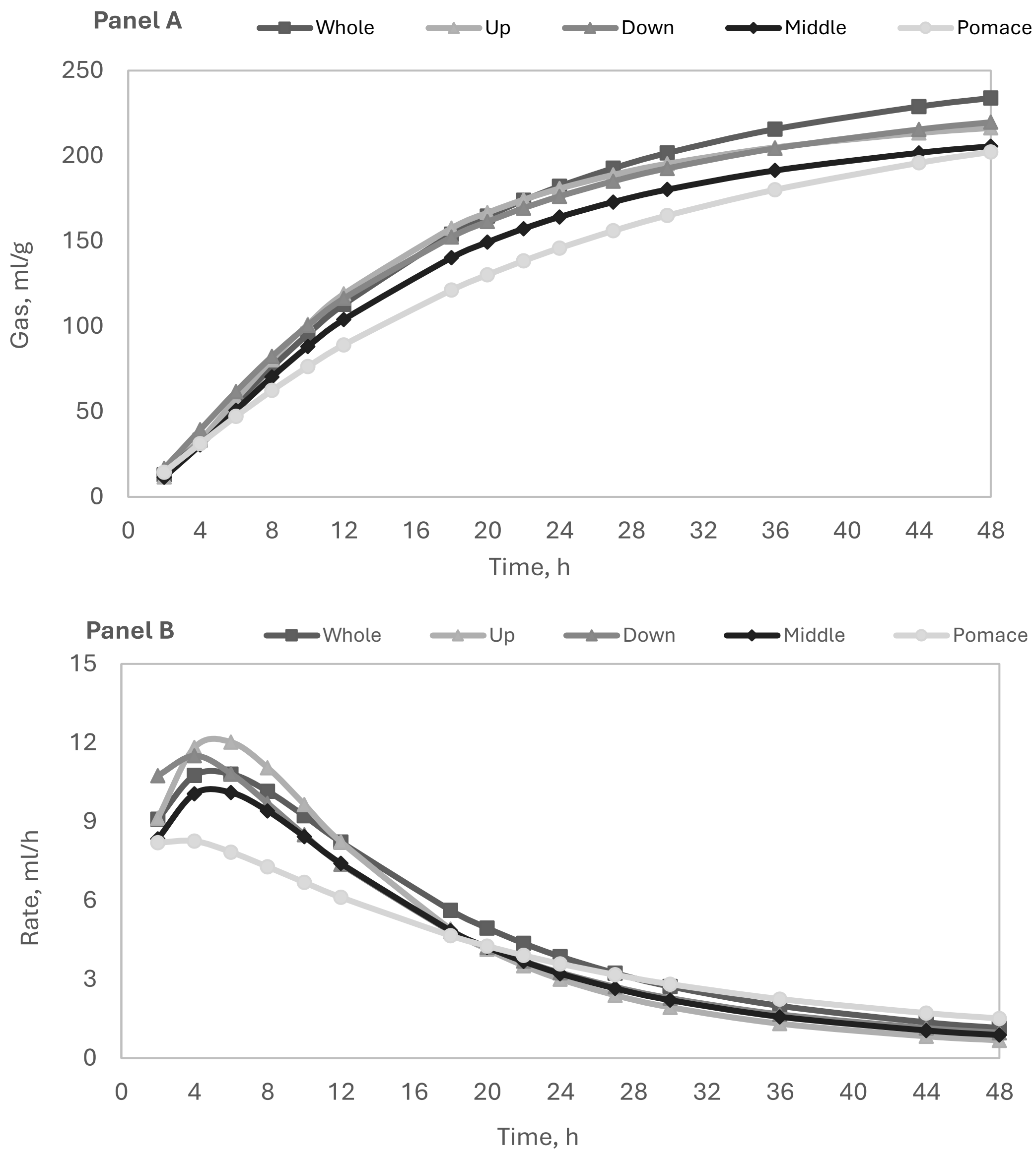

Figure 2. 3. *In vitro* gas production profile (Panel A) and fermentation rate (Panel B) of cashew apple by-products from Sudano-Guinean Zone of West Africa.

The *in vitro* volatile fatty acids determined at 48 h in the five by-products of the two cultivation zones show significant differences for all the factors considered and most of the parameters determined (Table 2.3.). The total volatile fatty acid (tVFA) was higher in SZ for all by-products compared to their corresponding values in SGZ. Total VFA varied from 46.47 mmol (pomace) to 60.97 mmol (middle part) in SZ and from 42.70 mmol (pomace) to 54.50 mmol (up part) in SGZ mainly contributed by acetate and propionate production for both zones. In addition, pomace showed the lowest for most volatile fatty acids except branched chain fatty acid (BCFA) and acetate-to-propionate ratio for both zones.

The correlation between chemical composition and *in vitro* fermentation characteristics showed many significant values (Table 2. 4.). Except for butyrate and R_{max}, the main chemical composition parameters (CP, EE, NDF, ADF, and ADL) were correlated to the *in vitro* fermentation ones, and the significance of the correlation coefficient varied from 0.001 to 0.05. They were positively correlated to pH, acetate, BCFA, Ace/Pro ratio, and the time to reach ½ of potential gas production (B), while they were negatively correlated to OMD, OMCV, propionate, valerate, tVFA production, and T_{max}.

2.4.3. Polyphenols

The polyphenol content determined in pomace and whole cashew apples of SZ and SGZ are reported in Table 2. 5. A very highly significant effect was observed as regards zone and by-products rather than variety. Naringin and protocatechuic acid were the most abundant polyphenols from both zones followed by gallic acid. Two 'unknown' compounds were detected in SZ, and only one in SGZ; both were quite abundant. Conversely, chlorogenic acid was the least abundant phenolic compound in the whole cashew apple by-products and was not found in the whole cashew apple by-products from SZ, whereas 4-hydroxybenzoic and *p*-coumaric acid were the least abundant compounds found in the pomace from SZ and SGZ, respectively. Gallic acid, protocatechuic acid and chlorogenic, acid were significantly ($p<0.05$) more abundant in the pomace compared to the whole by-products from SZ. Consequently, the total polyphenol content in pomace from SZ was significantly higher than in the whole by-product (3,813 vs. 2,736 µg/g DW, $p = 0.0002$). A less clear distribution of polyphenols across the by-products from SGZ was observed, with protocatechuic acid content being 2.5-fold lower in pomace compared to the whole by-product. Therefore, a similar content of total polyphenol was found in whole and pomace by-products from SGZ (1,755 vs. 1,374 µg/g DW, $p>0.05$).

Table 2. 3. *In vitro* fermentation end-products of cashew apples by-products from Sudanian and Sudano-Guinean zones of West Africa

Item	tVFA	Ace	Pro	Iso-But	But	Iso-Val	Val	BCFA	Ace/Pro
				Mmol/g iOM				*%*	
Sudanian Zone									
Whole	53.81^{ABC}	28.35	20.20^{B}	0.20	4.00^{B}	0.22	0.84^{BC}	0.88^{AB}	1.61^{AB}
Up	54.50^{AB}	29.54	23.91^{A}	0.19	4.96^{B}	0.21	1.03^{AB}	0.77^{B}	1.40^{B}
Down	53.20^{BC}	27.92	18.37^{B}	0.22	5.83^{B}	0.24	0.62^{CD}	0.96^{AB}	1.73^{AB}
Middle	60.97^{A}	28.56	21.53^{AB}	0.24	9.21^{A}	0.22	1.22^{A}	1.01^{A}	1.52^{B}
Pomace	46.47^{C}	27.10	14.61^{C}	0.21	3.7^{B}	0.20	0.37^{D}	0.96^{AB}	2.03^{A}
Sudano-Guinean Zone									
Whole	50.08^{BC}	27.79^{AB}	15.34^{BC}	0.19	5.90	0.21	0.64	0.95^{B}	2.15^{B}
Up	54.50^{AB}	29.70^{A}	19.98^{A}	0.23	3.94	0.21	0.44	0.97^{AB}	1.81^{B}
Down	46.60^{CD}	29.27^{A}	12.40^{C}	0.22	3.99	0.20	0.51	1.11^{AB}	3.01^{A}
Middle	48.50^{BCD}	26.21^{AB}	16.29^{B}	0.22	4.98	0.20	0.61	1.01^{AB}	1.92^{B}
Pomace	42.70^{D}	24.63^{B}	13.38^{BC}	0.19	3.80	0.25	0.44	1.17^{A}	2.06^{B}
p-value									
Z	<0.0001	0.1308	<0.0001	0.6381	0.0026	0.5110	<0.0001	<0.0001	<0.0001
Var	0.7435	0.9996	0.1248	0.0117	0.1277	0.0054	0.0011	0.5807	0.0286
BP	<0.0001	0.0004	<0.0001	0.0077	<0.0001	0.6606	<0.0001	0.0001	<0.0001
Z∗BP	0.0525	0.0906	0.0201	0.0453	<0.0001	0.0998	<0.0001	0.5269	<0.0001
MSE	15.474	3.7922	3.1574	0.0005	1.7043	0.0019	0.0186	0.0117	0.0587

tVFA: total volatile fatty acid, Ace: acetate, Pro: propionate, Iso-But: iso-butyrate, But: butyrate, Iso-Val: iso-valerate, Val: valerate, BCFA: branched chain fatty acid, Ace/Pro: acetate to propionate ratio. Var: variety, Z: zone, BP: by-product Z∗BP: Zone ∗ By-products interaction. MSE: mean square error. Along the column, for each zone, different letters indicate the statistical differences ($p < 0.05$).

Table 2. 4. Correlation between chemical composition and in vitro fermentation data (n = 20)

Items	CP	EE	NDF	ADF	ADL
pH	0.7066***	0.6027**	0.5262*	0.5669**	0.6967***
tVFA	-0.8179***	-0.8030***	-0.8279***	-0.7602***	-0.6373**
Acetate	0.7229***	0.6896***	0.7013***	0.7440***	0.6176**
Propionate	-0.5773**	-0.4768*	-0.5869**	-0.6813**	-0.5300*
Iso-Butyrate	0.5933**	0.5730**	0.6123**	0.6709**	0.5726**
Butyrate	-0.3925^{NS}	-0.4616*	-0.3342^{NS}	-0.2789^{NS}	-0.2723^{NS}
Iso-Valerate	0.7276***	0.7340***	0.5620*	0.4593*	0.4354 ^{NS}
Valerate	-0.6655**	-0.7018***	-0.7007***	-0.7144***	-0.6432**
BCFA	0.9148***	0.8753***	0.7968***	0.7734***	0.7133***
Acet/Pro	0.6134**	0.5276*	0.5789**	0.6978***	0.5736**
OMD	-0.8843***	-0.8413***	-0.9416***	-0.9166***	-0.7334***
OMCV	-0.4858*	-0.5870**	-0.6669**	-0.5942**	-0.4546*
T_{max}	-0.7829***	-0.6561**	-0.6964***	-0.7657***	-0.7238***
R_{max}	-0.2030^{NS}	-0.3064^{NS}	-0.4761*	-0.4085^{NS}	-0.2136^{NS}
B	0.4899*	0.5055*	0.6144**	0.5721**	0.4446*

CP: crude protein, EE: ether extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, tVFA: total volatile fatty acid, BCFA: branched chain fatty acid, Ace/Pro: acetate to propionate ratio, OMD: organic matter degradability, OMCV: cumulative gas volume related to incubated organic matter, T_{max}: time at which maximum rate was reached, R_{max}: maximum fermentation rate, B: time at which A/2 was reached. ***, **, *, NS: p<0.001, p<0.01, p<0.05, not significant, respectively.

Table 2. 5. Polyphenol content in pomace and whole cashew apples from Sudanian and Sudano-Guinean zones of West Africa

	Sudanian Zone		**Sudano-Guinean Zone**		**P value**				
µg/g dry weight	**Whole**	**Pomace**	**Whole**	**Pomace**	**Z**	**Var**	**BP**	**Z*BP**	**MSE**
Gallic acid	178.72	103.63	172.05	140.86	NS	**	NS	NS	10.4
Protocatechuic acid	553.1	696.1	934.4^{A}	378.9^{B}	NS	NS	NS	**	495
Chlorogenic acid	ND	30.66	2.30^{B}	8.80^{A}	***	NS	***	.	2.48
Procyanidin B2	28.74^{B}	50.38^{A}	7.91	22.74	***	NS	***	NS	0.37
4-hydroxy benzoic acid	16.97^{B}	25.69^{A}	5.68^{B}	10.52^{A}	***	**	***	NS	0.58
Epicatechin	21.46^{B}	97.00^{A}	22.00^{B}	109.1^{A}	NS	**	***	NS	5.89
p-coumaric acid	7.70^{B}	26.97^{A}	5.98	8.00	***	*	***	***	0.10
Sinapic acid	14.45^{B}	34.34^{A}	9.64^{B}	13.12^{A}	***	***	***	***	0.48
trans-ferulic acid	16.09^{B}	44.28^{A}	12.43	10.34	***	**	***	***	1.40
Unknown 1	246.5^{B}	672.8^{A}	120.27	136.4	***	*	***	***	28.8
Naringin	1206^{B}	1477^{A}	415.2	469.8	***	NS	**	*	188
Unknown 2	364.3^{B}	439.3^{A}	ND	ND	-	*	*	-	4.5
Quercetin	28.50^{B}	48.30^{A}	32.02	43.93	NS	NS	***	NS	2.07
trans-cinnamic acid	53.82^{B}	81.17^{A}	14.6^{B}	37.05^{A}	***	NS	***	NS	0.46
Total	**2,736^{B}**	**3,813^{A}**	**1,755**	**1,374**	***	**NS**	*	***	**967**

Z: zone, BP: By-products, Var: Variety, Z∗BP: Zone ∗ By-products interaction. Along the row, for each zone, different letters indicate the statistical differences ($p < 0.05$). ***, **, *, NS: $p<0.001$, $p<0.01$, $p<0.05$, not significant, respectively. MSE: mean square error.

The correlation analyses to evaluate the effect of polyphenols on chemical composition and *in vitro* fermentation characteristics gave the following significant results:

- epicatechin with chemical composition data: EE (+0.842; p<0.01), structural carbohydrates (+0.874 and +0.906, p<0.01, with NDF and ADF, respectively), and ash (-0.785; p<0.05) and *in vitro* data as well: OMD and OMCV (-0.775, -0.742; p<0.05, respectively);
- quercetin with EE (+0.821; p<0.05) and ADF (+0.760, p<0.05);
- gallic acid with ME (-0.775; p<0.05) and Iso-Val (-0.768; p<0.05);
- naringin with potential gas production (-0.825; p<0.05);
- procyanidin B2 with pH (-0.728; p<0.05);
- total polyphenols with pH (-0.790; p<0.05) and potential gas production (-0.840; p<0.01);
- protocatechuic and Chlorogenic acid with butyrate (+0.866; p<0.01) and iso-butyrate (+0.747; p<0.05), respectively.

In general, the polyphenols present in CABP had little effect on chemical nutrient utilization and fermentation parameters.

2.5. Discussion

2.5.1. Chemical composition

The current study aimed to characterize the cashew apple by-products (CABP) left in the field and during the juice production to promote their use in the nutrition of West African ruminants as an alternative feed principally during the dry season. The natural pasture, varying according to the season, presents the main feed resource for ruminants in West Africa. Unsurprisingly, the decrease in natural pasture during the dry season generally has a negative effect on the performance of the animals. As part of the inventory of alternative feed, the current study collected five cashew by-products from red and yellow varieties in Sudanian and Sudano-Guinean areas of Benin and expected differences in nutrients due to genetics, environmental conditions, and processing methods. In general, by-products collected in the Sudanian Zone (SZ) compared to the Sudano-Guinean Zone (SGZ) showed differences for most chemical parameters, probably due to the different rainfall and soil conditions. The DM content was relatively low in all samples, highlighting the difficulty in considering these by-products for long-term use, as also reported in other studies (Murugan et al., 2015; Tai et al., 2020). The DM content was slightly higher and less variable in SZ as compared to SGZ, and the highest value was found in pomace. The pomace also showed higher values of crude protein, lipids, and structural carbohydrates. The high structural carbohydrate content in the pomace might be due to the low moisture after the juice has been extracted. The CP content observed in other publications (Rodrigues et al., 2010; Raseel et al., 2018; Araújo et al., 2022) on dehydrated cashew apple pomace and bagasse (ranging between 13 and 15 % DM) was similar to the CP content found in SGZ pomace (14 % DM) and higher than the other by-products from SGZ and all of SZ. CP level of up to 19 % DM was reported in the cashew apple waste of Indian varieties (Bhamare et al., 2016; Sreekutty et al., 2017). The ash and structural carbohydrates reported by other authors (Raseel et al., 2018; Araújo et al., 2022) on dehydrated cashew apple pomace and bagasse were higher than our findings. The metabolizable energy, estimated from chemical composition and *in vitro* data, was similar for both zones and between by-products. In other West Africa feedstuffs, a comparable metabolizable energy content was reported in sun-dried yellow and red cashew pulp meal (Okpanachi et al., 2016), partly due to the high level of ether extract (10 % DM), which could affect the *in vitro* rumen fermentation. The differences observed in our results and the literature on CABP would be due to the differences in the parts of by-products considered, processing techniques, and conditions in the areas where they were cultivated. Based on CP and ME content, CABP are comparable to pineapples, citrus pulp, sugar beet pulp, and sugar beet root for CP content and almond hulls, lettuce leaves, apple pomace, banana stem, and jackfruit waste for ME content (Raseel et al., 2018; García-Rodríguez et al., 2019; Kiatti et al., 2023). This similarity could help to collect and pool fruit by-products when they are available in the same area according to the feeding objective (protein source vs energy source).

2.5.2. In vitro fermentation characteristics

In the rumen, the normal pH is maintained at 5.5 - 6.5 with a possible fluctuation to 2.5 - 3.0 depending on the acid produced during the fermentation which is related to the kind of feed. At the end of the fermentation process of cashew by-products, the pH values varied between 6.40 - 6.64 which confirms the effectiveness of the trial (Calabrò et al., 2022). High OM degradability was observed for the middle part and whole apple in both zones, whereas low values were found in the pomace of both regions. The same trend was observed for the fermentation kinetics and volatile fatty acids production, except for the branched-chain and acetate to propionate ratio. The high level of structural carbohydrates, specifically lignin present in the pomaces, could explain the low *in vitro* fermentation pattern. On the other hand, the whole and middle parts of cashew apples had the greatest content of non-structural carbohydrates (NSC) and sugar (FS and TS) which favored the growth of microorganisms, fiber attack, and the production of volatile fatty acids. However, the effects of lignified cell walls on feed digestibility and end fermentation products were widely reported (Pragna et al., 2018; Giamouri et al., 2023). In addition, we should also consider that lignin is one of the arrays of polyphenols present in plant biomass with potential antioxidant activity (Okonkwo et al., 2023). Furthermore, the low OMD and tVFA of pomace in both zones could be explained by the low NSC which was extracted more in the juice limiting the quota of energy more easily available in the rumen for the activity of microorganisms. Microorganisms in the rumen utilize carbohydrates (NSC and fiber) for their own growth producing end-products (VFA) which are then utilized by the animal for maintenance and production (meat or milk). Our results related to OMD in pomaces are consistent with other data reported on cashew apple waste (Raseel et al., 2018). On the other hand, the OMD of cashew whole apples, and other parts (up, down, and middle part) were comparable to previous data reported for different by-products such as peels, core, and pomace from pineapples (Kiatti et al., 2023) and yellow, black, and red maca varieties (Vastolo et al., 2023). The *in vitro* fermentation parameters (OMD, gas, and VFA production) that we found fall within the range of fruit and vegetable by-products also tested using the *in vitro* fermentation technique (García-Rodríguez et al., 2019). The similarities would probably lead to the chemical composition which includes lower NDF and EE content and a larger availability of free sugar in fruit and vegetable waste compared to the valuable, conventional ruminants' feedstuffs.

2.5.3. Polyphenols

Polyphenols are metabolites developed in the plant kingdom to confront environmental constraints such as insects, herbivores, and heat stress. Polyphenols can have a positive or negative effect onanimals' health, physiology, and quality of their products (Formato et al., 2022). It was reported that polyphenols have antioxidant, immunomodulatory, antimutagenic, and anti-inflammatory effects and may reduce antibiotic use in livestock production (Bešlo et al., 2022) thereby guaranteeing the safety of food (meat and milk) for humans. The current study revealed that naringin, protocatechuic, and gallic acid are polyphenols found in abundance in CABP. The total polyphenols of the whole and the pomace from SZ were around two or three times higher than the same by-products from SGZ, respectively. The differences are due to the heat stress characterized by the high temperatures and the low annual rainfall in the Sudanian Zone (SZ) which increases the concentration of polyphenols in the apples. Naringin found in the endocarp of citrus fruits was attributed to an unacceptable bitter taste (Tian et al., 2023) and will be probably the cause as well as the astringent and bitter taste of cashew apples. However, the quantity of naringin in fruit or fruit by-products depends on their maturity. Therefore, the presence of a high level of naringin in the animals' diet will probably increase feed bitterness and reduce its intake. Naringin and quercetin from the flavonoid group of polyphenols have been shown to increase the total of volatile fatty acid and decrease methane production through the reduction of the protozoa and methanogen population in the rumen with no negative effect on rumen fermentation (Formato et al., 2022; Shilpa et al., 2023). Tannins, which are part of a non-flavonoid group, are divided into two groups - condensed tannins and hydrolysable tannins. The condensed tannins are polymers of flavan-3-ol subunits (subunits of procyanidins: catechin and epicatechin; subunits of prodelphinidins: gallocatechin and epigallocatechin) whereas hydrolyzable tannins are water-soluble molecules composed of a glucose core esterified with gallic acid or ellagic acid. Recently, tannins have received more attention as regards the nutrition of ruminants due to their ability to bind dietary protein to the attack of microorganisms in the rumen (protein by-pass). Thus, the use of nitrogen (N) increases and reduces ruminal ammonia formation and N excretion from urine to feces (Yanza et al., 2021; Manoni et al., 2023). Tannins can also bind with carbohydrates to reduce the palatability of ruminants' feed, feed intake, and thereby methane (CH_4) production. However, hydrolysable tannins have been reported to be more effective than condensed tannins in decreasing CH_4 production, and maintaining nutrient digestibility, and VFA production (Manoni et al., 2023). As an example, the effect of gallic acid on CH_4 emissions and N excretion in beef cattle diet containing alfalfa silage was tested and it was shown that feeding gallic acid has the potential to decrease the environmental impact of ruminants (low CH_4 and ammonia emission) without negative effect on degradability, VFA production and animal performance (Aboagye et al., 2019). The same result was found *in vitro* with 75 mg/g DM of gallic acid or ellagic acid in ruminants' diet (Manoni et al., 2023). However, plant species, fruit maturity status, and the cultivation area affect the total amount and individual polyphenols in fruit by-products, while their bioactivity through ruminants' digestion tracts also depends on their molecular weight, conjugation with other derivatives or the hydrolysis of enzymes.

2.6. Conclusion

The investigation of cashew apple by-products (CABP) in the Sudanian and Sudano-Guinean zones of the West Africa region revealed that they can be a potential alternative foodstuff for ruminants during the dry season. Differences between the cultivation zones were more pronounced than differences between varieties. The chemical variables assessed in by-products from the Sudano-Guinean Zone were slightly higher than those from the Sudanian Zone. Pomace showed the lowest OMD and volatile fatty acid production due to the high content of structural carbohydrates, specifically lignin. In addition, CABP contains naringin, protocatechuic, and gallic acid as main polyphenols which are probably the source of the bitter and astringent taste. However, the polyphenols present in CABP had a minor effect on chemical nutrient use and rumen fermentation parameters. So, CABP could be collected in each cultivation zone separating pomace from other parts of by-products without variety consideration. However, future research must focus on preservation techniques such as ensiling and *in vivo* trials regarding how to enhance CABP self-life and evaluate the acceptability and the effect on the performance and health of animals. Extraction of polyphenols in CABP may also be considered to produce feed ingredients that may mitigate methane formation, thus supporting sustainable ruminant production. According to these preliminary results, we could consider cashew apple by-products a promising eco-sustainable feed in ruminant nutrition and an alternative ingredient in West Africa mainly during the dry season.

2.7. Acknowledgements

Thanks to the International Agreement for Co-Operation between the University of Naples, Federico II (Department of Veterinary Medicine and Animal Production), Italy and the National University of Agriculture (School of Animal Production and Health), Republic of Benin. DR 2632 del 27/6/22 (Resp. Prof. Serena Calabrò).

2.8. Reference

1. Aboagye, I.A.; Oba, M.; Koenig, K.M.; Zhao, G.Y.; Beauchemin, K.A. Use of gallic acid and hydrolyzable tannins to reduce methane emission and nitrogen excretion in beef cattle fed a diet containing alfalfa silage. *J. Anim.* Sci. 2019, 97(5), 2230–2244.
2. Aidoo, R.; Kwofie, E.M.; Ngadi, M.O. Circularity of Cashew Apples: Examining the Product-Process Pathways, Techno-Functional, Nutritional/Phytomolecular Qualities for Food Applications. *ACS Food Sci. Technol.* 20222, 7, 1051–1066.
3. AOAC. Official Method 925.35 Sucrose in Fruit and Fruit Product; Association of Official Analytical Chemists: Arlington, VA, USA, 2000.

4. AOAC. Official Methods of Analysis. 1*2 Vol, 18th ed.;* Association of Official Analytical Chemists: Arlington, VA, USA, 2005.

5. Araújo, A.R.; Costa, J.B.; Rogério, M.C.P.; Carneiro, M.S.S.; Muniz, L.C.; Fontenele, R.M.; Silva, V.L. Dehydrated cashew apple in different grinding sizes to sheep. *Acta Scientia. Anim. Sci.* 2022, 44, 1**-7.**

6. Asmawati, A.; Marianah, M.; Atoum, M.F.M.; Sari, D.A.; Iqrar, I.; Hussain, Z.; Setyobudi, R.H.; Nurhayati, N. The Potential of Cashew Apple Waste as a Slimming Agent. *Jordan J. Biol. Sci.* 2022, 15(5) 887–892.

7. Bauer, E.; Williams, B.A.; Voigt, C.; Mosenthin, R.; Verstegen, M.W.A. Microbial activities of faeces from unweaned and adult pigs, in relation to selected fermentable carbohydrates. *J. Anim. Sci.* 2001, 73, 313–332,

8. Bešlo, D.; Došlić, G.; Agić, D.; Rastija, V.; Šperanda, M.; Gantner, V.; Lučić, B. Polyphenols in Ruminant Nutrition and Their Effects on Reproduction. *Antioxidants* 2022, 11(5), 1–22, https://doi.org/10.3390/antiox11050970.

9. Bhamare, K.S.; Dildeep, V.; Senthil Murugan, S.; Chavan, S.J. Nutritive evaluation of Cashew apple waste in broilers. *Int. J. Sci. Nat.* 2016, 7(3), 629–632.

10. Calabrò, S.; Oteri, M.; Vastolo, A.; Cutrignelli, M.I.; Todaro, M.; Chiofalo, B.; Gresta, F. Amaranthus grain as a new ingredient in diets for dairy cows: productive, qualitative, and *in vitro* fermentation traits. *J. Sci. Food Agric.* 2022, 102, 4121–4130, DOI 10.1002/jsfa.11761.

11. Chiacchio, M.F.; Tagliamonte, S.; Visconti, A.; Ferracane, R.; Mustafa, A.; Vitaglione, P. Baobab-fruit shell and fibrous filaments are sources of antioxidant dietary fibers. *Molecules.* 2022, 27, 5563.

12. Cruz Reina, L.J.; Durán-Aranguren, D.D.; Forero-Rojas, L.F.; Tarapuez-Viveros, L.F.; Durán-Sequeda, D.; Carazzone, C.; Sierra, R. Chemical composition and bioactive compounds of cashew (*Anacardium occidentale*) apple juice and bagasse from Colombian varieties. *Heliyon* 2022*,* 8. e09528.

13. Das, I.; Arora, A. Post-harvest processing technology for cashew apples - A review. *J of Food Eng.* 2017, 194, 87–98, https://doi.org/10.1016/j.jfoodeng.2016.09.011.

14. Dheeraj, Srivastava, A.; Mishra, A. Mitigation of cashew apple fruits astringency. *Environ. Sustain.* 2023, 6, 312-329, https://doi.org/10.1007/s42398-023-00276-7.

15. Dos Santos L.F.C.; Luiz Honorato da Silva, F.; Palmeira Gomes, J.; Mariano da Silva Neto, J. Chemical Composition of the Cashew Apple Bagasse and Potential Use for Ethanol Production. *Adv. Chem. Eng. Sci.* 2012, 2, 519–523.

16. EC Council. Regulation 882/2004 on Official controls performed to ensure verification of compliance with feed and food law, animal health and animal; EC Council: UE, 2004.

17. Emelike, N.J.T.; Ebere, C.O. Effect of treatments on the tannin content and quality

assessment of cashew apple juice and the kernel. *Eur J. Food Sci. Technol.* 2016, 4, 25–36.

18. Faostat. New Food Balances; FAO: United Nation, 2021. Available online: http://www.fao.org/faostat/en/#data/FBS.

19. Ferreira, A.C.H.; Neiva, J.N.M.; Rodriguez, N.M.; Lobo, R.N.B.; Vasconcelos, V.R. Nutritive Value of Elephantgrass Silage Added By-Products from the Cashew Juice Industry. *Rev. Bras. Zoot.* 2004, 33(6), 1380-1385.

20. Filho, M.R.R.; Soto-Blanco, B. Poisoning by cashew apple (Anacardium occidentale L.) in cattle. *Acta. Sci. Vet.* 2012, 40, 1–5.

21. Formato, M.; Cimmino, G.; Brahmi-Chendouh, N.; Piccolella, S.; Pacifico, S. Polyphenols for Livestock Feed: Sustainable Perspectives for Animal Husbandry?. *Molecules.* 2022, 27(22), 7752, https://doi.org/10.3390/molecules27227752.

22. García-Rodríguez, J.; Ranilla, M.J.; France, J.; Alaiz-Moretón, H.; Carro, M.D.; López, S. Chemical Composition, *In Vitro* Digestibility and Rumen Fermentation Kinetics of Agro-Industrial By-Products. *Animals* 2019, 9(861), 1–13, doi:10.3390/ani9110861.

23. Giamouri, E.; Zisis, F.; Mitsiopoulou, C.; Christodoulou, C.; Pappas, A.C.; Simitzis, P.E.; Kamilaris, C.; Galliou, F.; Manios, T.; Mavrommatis, A.; Tsiplakou, E. Sustainable Strategies for Greenhouse Gas Emission Reduction in Small Ruminants Farming. *Sustain.* 2023, 15(5), 4118, https://doi.org/10.3390/su15054118.

24. Gnanglè, C.P.; Glèlè Kakaï, R.; Assogbadjo, A.E.; Vodounnon, S.; Yabi, J.A.; Sokpon, N. Tendances Climatiques Passées, Modelisation, Perception et Adaptations Locale au Bénin. *Climatol.* 2011, 8, 27–40, https://doi.org/10.4267/climatologie.259.

25. Goula, A.M.; Lazarides, H.N. Integrated processes can turn industrial food waste into valuable food by-products and/or ingredients: The cases of olive mill and pomegranate wastes. *J. food eng.* 2015, 167, 45–50.

26. Groot, J.C.; Cone, J.W.; Williams, B.A.; Debersaques, F.M.; Lantinga, E.A. Multiphasic analysis of gas production kinetics for *in vitro* fermentation of ruminant feedstuff. *Anim. Feed Sci. Technol.* 1996, 64, 77–89, https://doi.org/10.1016/S0377-8401(96)01012-7.

27. Kannan, V.; Rangarajan, V.; Manjare, S.D.; Pathak, P.V. Microbial production of value-added products from cashew apples - an economical boost to cashew farmers. *J. Pure Appl. Microbiol.* 2021, 15(4), 1816–1832.

28. Kiatti, D.d.; Vastolo, A.; Koura, B.I.; Vitaglione, P.; Cutrignelli, M.I.; Calabrò, S. The Chemical Characteristics and *In Vitro* Degradability of Pineapple By-Products as Potential Feed for Ruminants. *Animals.* 2023, 13(20), 1–15, https://doi.org/10.3390/ani13203238.

29. Koura, B.I.; Vastolo, A.; Kiatti, D.; Cutrignelli, M.I.; Houinato, M.; Calabrò, S. Nutritional Value of Climate-Resilient Forage Species Sustaining Peri-Urban Dairy

Cow Production in the Coastal Grasslands of Benin (West Africa). *Animals* 2022, 12(24), 1–16, https:// doi.org/10.3390/ani12243550.

30. Lowe, S.E.; Theodorou, M.K.; Trincv, A.P.J.; Hespell, R.B. Growth of anaerobic rumen fungi on defined and semi-defined media lacking rumen fluid. *J. Gener. Microbio.* 1985, 131, 2225-2229, https://doi.org/10.1099/00221287-131-9-2225.

31. Manoni, M.; Terranova, M.; Amelchanka, S.; Pinotti, L.; Silacci, P.; Tretola, M. Effect of ellagic and gallic acid on the mitigation of methane production and ammonia formation in an *in vitro* model of short-term rumen fermentation. *Anim. Feed Sci. Technol.* 2023, 305, 1-9.

32. Menke, K.H.; Steingass, H. Estimation of the Energetic Feed Value Obtained from Chemical Analysis and *in Vitro* Gas Production Using Rumen Fluid. *Anim. Res. Dev*. 1988, 28, 7–55.

33. Murugan, S.; Kumar, S.; Rajan, J.K.; Varshney, L.; Kumar, V. Cashew Apple (Anacardium occidentale): Evaluation of Physical and Chemical Composition. *Indian J. Nat. Sci.* 2015, 5, 4255-4259.

34. Nair, K.P.P. Cinchona (cinchona sp). In *The agronomy and economy of important tree crops of the developing world,* Elsevier: Amsterdam, 2010; pp. 111-129.

35. Okonkwo, C.E.; Hussain, S.Z.; Onyeaka, H.; Adeyanju, A.A.; Nwonuma, C.O.; Bashir, A.A.; Farooq, A.; Zhou, C. Lignin polyphenol: From biomass to innovative food applications, and influence on gut microflora. *Ind. Crops Prod.* 2023, 206(3), 117696.

36. Okpanachi, U.; Agu, C.I.; Igoche, L.E.; Oyedapo, F.A. Potentials of two varieties of cashew apple pulp as feedstuff for ruminants. *Niger J. Anim. Prod.* 2018, 45(4), 203–209.

37. Okpanachi, U.; Attah, S.; Oyewole, B.O. Effects of varieties of dried cashew pulp on *in vitro* fermentation parameters and volume of gas produced at different incubation time. *Agric. Food.* 2016, 4, 530–536.

38. Okpanachi, U.; Ayoade, J.A.; Tuleun, C.D. Composition and Anti-Nutritional Factors (Phyto-Nutrients) Present in both Red and Yellow Varieties of Sun-Dried Cashew Pulp. *Am. J. Food Sci. Heal.* 2016, 2, 45–48.

39. Oliveira, N.N.; Mothé, C.G.; Mothé, M.G.; Oliveira, L.G. Cashew nut and cashew apple: a scientific and technological monitoring worldwide review. *J. Food Sci. Technol.* 2020, 57(1), 12–21, https://doi.org/10.1007/s13197-019-04051-7.

40. Padonou, S.W.; Houssou, P.; Agbobatinkpo, P.; Olou, D.; Klotoe, H.A.; Todohoue, C.; Guédou, M.; Adegbola, P.; Aboh, A.; Montcho, K.; Dossou, J.; Mensah, G.A. Fiche technique: La presse hydraulique pour la production du jus de pomme d'anacarde. *MAEP*. 2016, 13 1-27.

41. Pragna, P.; Chauhan, S.S.; Sejian, V.; Leury, B.J.; Dunshea, F.R. Climate change and goat production: Enteric methane emission and its mitigation. *Animals.* 2018, 8(12),

235, https://doi.org/10.3390/ani8120235.

42. Raseel, K.; Chacko, B.; Sunanda, C.; Dildeep, V.; Abraham, J. Nutrient evaluation of energy rich unconventional feeds available in Wayanad. *Int. J. Sci. Nat*. 2018, 9, 117–118.

43. Rodrigues, M.R.C.; Rondina, D.; Araujo, A.A.; Arruda, I.J.; Silva, L.M.; Nunes-Pinheiro, D.C.; Fernandes, A.A.O. Use of Dehydrated Cashew Apple Pomace (Anacardium Occidentale) the Feeding of Lambs Weaning Puberty: Metabolic Responses and Sex Hormone. *Cien. Anim.* 2010, 20, 17–26.

44. Runjala, S.; Kella, L. Cashew apple (Anacardium occidentale L.) therapeutic benefits, processing and product development: An overview. *Pharma Innov J.* 2017, 6(7), 260–264.

45. Shilpa, V.S.; Shams, R.; Dash, K.K.; Pandey, V.K.; Dar, A.H.; Mukarram, S.A.; Harsányi, E.; Kovács, B. Phytochemical Properties, Extraction, and Pharmacological Benefits of Naringin: A Review. *Molecules* 2023, 28, 5623,

46. Sreekutty, P.S.; Senthil Murugan, S.; Dildeep, V.; Chacko, B.; Balusamy, C. Chemical composition of cashew apple waste. *Shanlax Int. J. Vet. Sci.* 2017, 5, 2321-6387.

47. Tai, V.A.; Tuan, B.Q.; Thuy, V.T.T.; Trach, N.X. Use of cashew apple fruit silage in the cattle fattening diet. *Livest. Res. Rural Dev.* 2020, 32, 1-5.

48. Talasila, U.; Shaik, K.B.; Quality, spoilage and preservation of cashew apple juice: A review. *J. Food Sci. Technol.* 2015, 52, 54–62, DOI 10.1007/s13197-013-0931-0.

49. Theodorou, M.K.; Williams, B.A.; Dhanoa, M.S.; McAllan, A.B.; France, J.A. Simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim. Feed Sci. Technol.* 1994, 48, 185–197.

50. Tian, M.; Wang, J.; Hayat, K.; Lu, H.; Cong, L.; Huang, M. Fabrication of pea protein–naringin Pickering emulsion to mask the bitterness of naringin. *Int. J. Food Sci. Technol.* 2023, 58, 3838–3849.

51. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 1991, 74, 3583–3597, DOI: 10.3168/jds.S0022-0302(91)78551-2.

52. Vastolo. A.; Calabrò, S.; Carotenuto, D.; Cutrignelli, M.I.; Kiatti, D.; Tafuri, S.; Ciani, F. Maca (Lepidium meyenii): *In Vitro* Evaluation of Rumen Fermentation and Oxidative Stress. *Fermentation*. 2023, 9(6), 568.

53. Yanza, Y.R.; Fitri, A.; Suwignyo, B.; Hidayatik, N.; Kumalasari, N.R.; Irawan, A.; Jayanegara, A. The utilisation of tannin extract as a dietary additive in ruminant nutrition: a meta-analysis. *Animals* 2021, 11(11), 3317.

Chapter 3.

The Chemical Characteristics And *In Vitro* Degradability Of Pineapple By-Products As Potential Feed For Ruminants

Article published in Animals (2023), *13*(20): 3238

https://doi.org/10.3390/ani13203238

3.1. Abstract

Pineapple (*Ananas comosus* L.) fruit is mainly consumed fresh or after its transformation into juice, jam, candy, beverages, ice cream, powder, or wine. During processing, many leftovers are produced and released into the environment, contributing to pollution. This current study investigated the possible utilization of pineapple leftovers from two varieties (SC-Smooth Cayenne and SL-Sugarloaf) cultivated in West Africa in ruminant nutrition. They were characterized individually (crown, bud end, peel, core, and pomace) regarding chemical composition, *in vitro* fermentation characteristics, and estimated metabolizable energy. Significant differences were observed between the varieties and by-products. The dry matter (DM) content was low and superimposable between varieties, averaging 17.7%. On a DM basis, pomace showed the highest protein content (SC 8.10 % and SL 8.81%, $p < 0.001$), whereas the crown showed the highest ($p < 0.001$) NDF content (47.62 % and 39.01 % for SC and SL, respectively). Due to the high sugar content, the core and pomace showed high *in vitro* organic matter degradability (SC: 85.09 % and SL: 83.98 %), estimated metabolizable energy (SC: 7.91 KJ/kg and SL: 7.66 KJ/kg), and volatile fatty acid production (96.86 mmol/g and 90.62 mmol/g). Based on chemical composition and *in vitro* digestibility results, this study suggests that pineapple by-products have the potential to be used in ruminants' diets, considering the crown, bud end, and peel as fiber sources and the core and pomace as substitutes or supplements to concentrate feedstuffs. Further research should be conducted on the storability of these by-products through *in vivo* trials evaluating animals' performances and the quality of their products.

Keywords: fruit processing by-products; *in vitro* gas production; fiber; energy; volatile fatty acids; waste management; West Africa.

3.2. Introduction

The pineapple plant (*Ananas comosus* L.), belonging to the *Bromeliaceae* family, is originally from tropical and subtropical areas (Asia, South and North America, Africa, and Oceania), where the varieties mainly cultivated are Cayenne, Sugarloaf, Spanish, Queen, Pernambuco, and Perolera (Agbangba, 2016; Vieira et al., 2022). In 2021, worldwide pineapple production is estimated at 28,179,348 tons (Faostat, 2021). Costa Rica, Philippines, and Brazil are the major contributors. In West Africa, Nigeria and Benin are in the 8^{th} and 17^{th} positions, with 1,671,440 and 386,906 tons produced, respectively (Sodjinou et al., 2022). The high production of pineapple is due to its high demand at the global level and to the health-beneficial effect of pineapple fruit and pineapple-based products, such as juice, jam, candy, beverages, ice cream, powder, and wine. These products are known to be rich in many nutrients, such as vitamins (i.e., A, C, B1, B6, folic acid, beta-carotene), minerals (i.e., copper, manganese), and other compounds with antioxidant, digestive (bromelain), anti-inflammatory, and analgesic properties (Chaudhary et al., 2019; Firatoiu et al., 2021). During fruit processing, a large quantity (about 65%) of leftovers is discarded as waste, including the peel (35.5%), core (14.7%), pomace (6.0%), bud end (4.6%), and crown (4.3%) (Upadhyay et al., 2010; Hemung et al., 2022). Concerning the amount of fruit produced worldwide, more than 18 million tons of pineapple leftovers are annually generated. Generally, because of their short shelf life due to high moisture and sugar contents, these refusals are thrown away as waste material or, in a minor part, utilized for bromelain extraction or organic acid and ethanol production (Chaudhary et al., 2019; Gowda et al., 2015; Roda and Lambri, 2019). Their disposal in the environment causes serious problems such as air and underwater pollution. On the other hand, these leftovers may still contain traces of nutrients that can be useful as by-products for animal nutrition (Kumar, 2021; Pinotti et al., 2021). Particularly in West Africa, pineapple by-products could constitute a low-cost feed resource for utilization in the diets of grazing animals, especially as a supplement during the dry season. Therefore, utilizing pineapple by-products in animal nutrition could reduce breeders' production costs and establish a circular economy by recycling the waste from factories that process the fruits.

Some studies investigated the use of pineapple by-products as feed for ruminants in different cultivation areas (i.e., Ethiopia and Southeast Asia) (Melesse et al., 2018; Idayanti et al., 2022), evaluating the chemical composition and estimating the nutritional value. As suggested by some authors (Prado et al., 2003; Sruamisri, 2007), dry or ensiled pineapple by-products could replace 50 % of the roughage or corn silage in ruminants' diets. In addition, little variation was noted between fresh and dry pineapple by-products in terms of chemical composition (Upadhyay et al., 2010). Some studies on pineapple waste (Siti Roha et al., 2013; Raseel et al., 2018) also investigated its sugar content (glucose, fructose, and sucrose), energy, and degradability. Other authors (Choi et al., 2021) focused on the *in-situ* degradability and proposed the inclusion level of pineapple cannery by-products in steer diets. *In vitro,* degradable nitrogen was also investigated in pineapple waste (Siti Roha et al., 2013; Raseel et al., 2018). The *in vivo* experiment with pineapple by-products concluded that they could substitute roughage or concentrates in ruminants' diets, improving dry matter intake, palatability, microbial activities, and animal performance (Hattakum et al., 2019; Mordenti et al., 2021; Palmonari et al., 2021; Bulkaini, 2022).

However, pineapple by-products are still considered as waste because of their short shelf-life for long-term utilization and their unknown nutritive values in other cultivation areas such as West Africa. In this region where the fruits are locally harvested and processed, pineapple by-products could be a useful resource in small ruminants' production systems. This use would be helpful, particularly during the dry season, when the natural pasture becomes scarce (Amole, et al., 2019; Usman et al., 2022). However, before encouraging farmers to use them in animal feeding plans, it is necessary to know their individual nutritional characteristics considering influencing factors such as environmental conditions (i.e., temperature, rainfall, soil fertility), processing techniques, and plant varieties. To fill this gap, this current study aimed to characterize, in terms of chemical composition, estimated metabolizable energy and *in vitro* fermentation characteristics of pineapple leftovers derived from the processing of two varieties (Sugarloaf and Smooth Cayenne) cultivated in the West African area.

3.3. Material and Methods

3.3.1. Sampling area

The pineapple (*Ananas comosus* L.) samples used in this study were cultivated in the Atlantic district of the Republic of Benin (6◦180–6◦580 N and 1◦560–2◦300 E); this area is in the sub-humid zone of West Africa. The Atlantic district is characterized by two rain seasons (March to July and mid-September to December) and two dry seasons (August to mid-September and December to March), with an annual average rainfall of 1200 mm, a temperature varying between 27°C and 31°C, and relative humidity between 69 % and 97 % (INSAE, 2004).

3.3.2. By-product collection

Two varieties of pineapple (Sugarloaf and Smooth Cayenne) were directly collected from the field and transported to the zootechnic laboratory, Faculty of Agricultural Sciences (FSA), University of Abomey Calavi (UAC) for processing according to local use. Figure 3. 1. shows the different steps of local pineapple processing with the various waste generated.

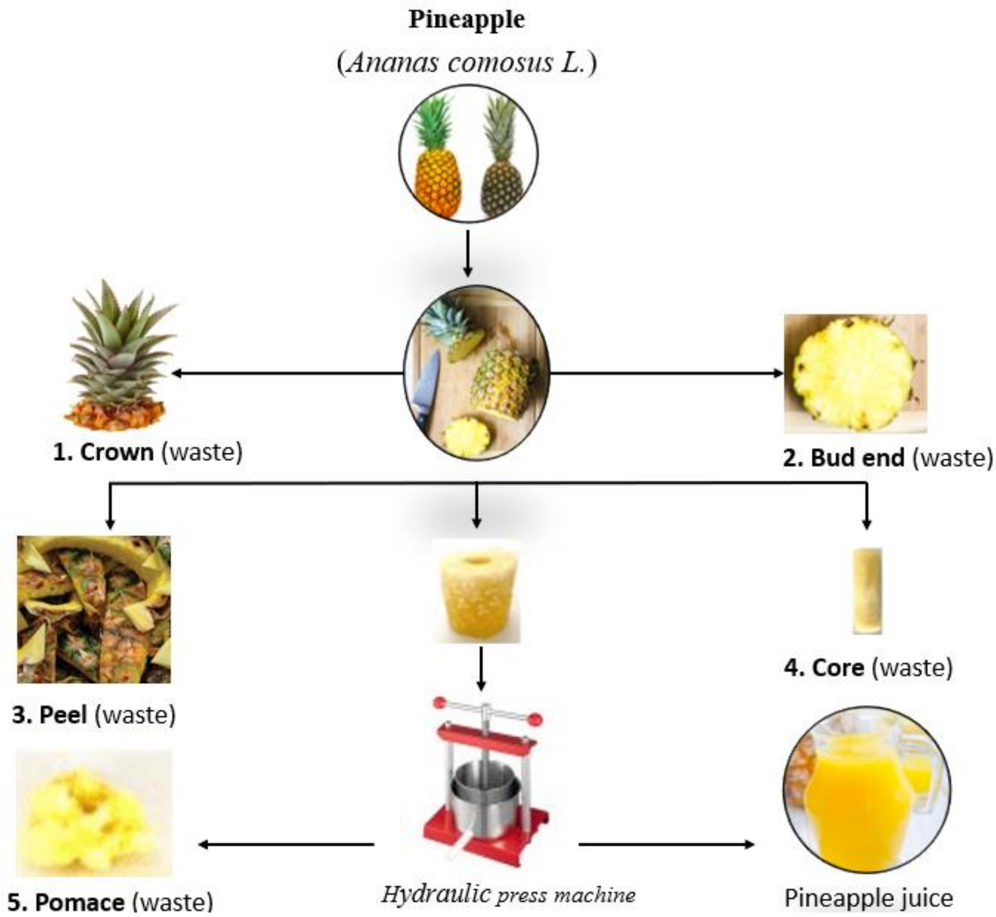

Figure 3. 1. Pineapple home processing into juice in West Africa region with No. 1 to 5 samples collected.

The fruits were collected to represent the cultivation area; a total of 40 fruits from each variety were randomly collected from three farms in the Atlantic districts. First, the fruits were cleaned, and the crowns and bud ends were removed using a steel knife; successively, the peel and core were delicately separated from the pulp. Then, the pulps were pressed to remove the juice and retain the pomace using a hydraulic press machine (Tianyu Youdo Machine, model UDZL-W33, dimensions 330 × 330 × 600 mm, Kaifeng, China) usually used in small pineapple-processing units in West Africa. Finally, five by-products were generated for each variety from the fruits' processing: the crown, bud end, peel, core, and pomace. Each obtained by-product was quantified, and the incidence (%) was estimated considering the weight of the whole fruit. About 300 g of each category of by-products was immediately dried in an oven at 65°C until they reached a constant weight for dry matter evaluation (AOAC, 2005). In total, ten samples (5 by-products × 2 varieties) were transferred to the laboratory of Feed Evaluation of the Department of Veterinary Medicine and Animal Production (University of Napoli Federico II, Napoli, Italy). Before the chemical analysis and *in vitro* trial, all the samples were grounded to pass through a 1.1 mm screen.

3.3.3. Chemical composition

Chemical composition was carried out according to the official methods (AOAC, 2005); in particular, dry matter (DM, 934.01), crude protein (CP, 2001.11), ether extract (EE, 920.39), and ash (942.05) contents were determined. Structural carbohydrates such as neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were also assessed (Van Soest et al., 1991) with an Ankom 220 fiber analyzer (ANKOMTM Technology, Fairport, NY, USA). The filter bags used were burned to correct the NDF and ADF content. The quantitative determination of free sugars (FS) and total sugars (TS) was performed after mixing the by-products in distilled water at 1:40 and 1:80 ratios (w:w) for FS and TS, respectively. For the FS, the mixture was left at room temperature (20° C) for 30 min, whereas for the TS, 20 mL of HCl (1:1) was added and then incubated in a thermostatic bath at 70° C for 3 h; after cooling, three drops of 1 % phenolphthalein were added. At this step, for both determinations, 5 mL of Carrez solutions (I and II) were added every 10 min followed by filtration. The obtained solution was titrated using Fehling's solution (A and B) and 1 % methylene blue (AOAC, 2000). The sugar content was calculated as $(f \times d)/(a - 0.1)$ where f is the factor due to the power of sugar (being 3.350 for reducing sugars and 5.150 for total sugars), d is the dilution factor, and a is the volume (ml) of the solution used during the titration.

3.3.4. In vitro fermentation

An *in vitro* gas production technique (IVGPT) was applied to evaluate the fermentation characteristics of pineapple by-products (Koura et al., 2022). Each sample of pineapple by-products was weighed (1.0025 ± 0.0011 g) in four replications in 120 mL bottles with 75 mL of medium in anaerobic condition. Two bottles containing only the medium were used as blank. To study the *in vitro* fermentation profile, two reference feedstuffs (corn grain, *Zea mays* L. and Sulla hay, *Hedysarum coronarium* L.) were also used. Sheep rumen liquor (10 mL) containing its micro-flora flushed under anaerobic conditions was added to the bottles. The rumen liquor was collected in pre-warmed thermos from three adult ewes (Comisana × Sarda breed, 45-50 kg of live weight and 8-10 years old) slaughtered according to EU legislation (EC Council Regulation 882/2004). In the laboratory, it was pooled, mixed, and filtered through a double-thickness cheesecloth. The sheep were raised on pasture and were supplemented with 600 g/head/d of concentrate. All procedures involving animals were approved by the Ethical Animal Care and Use Committee of the University of Napoli Federico II (Prot. 2019/0013729 of 8 February 2019). The bottles were sealed with an aluminum cover and then incubated at 39° C for 48 h. During the incubation period, the volume and the pressure of gas produced in the bottles were measured 22 times at 2-4 h intervals using a manual pressure transducer (Cole and Parmer Instrument Co., Vernon Hills, IL, USA). After each measurement, the bottles were shaken and replaced in the above-mentioned conditions. The fermentation was stopped by cooling (4°C), and pH was measured using a pH meter (model 720A+ Thermo Fisher Scientific, Rodano, MI, Italy). The supernatant liquors were sampled for the end-product analysis, and the remaining were filtered in pre-weight-sintered glass crucibles (Scott Duran, porosity #2). The crucibles were dried at 103°C and burnt at 55°C. Organic matter degradability at 48 h (OMD, %) was calculated as the difference between the incubated and residual OM.

3.3.5. End products analysis

Ten mL of supernatant liquor from each bottle was sampled at the end of incubation and centrifuged twice at 12,000 × g for 10 min at 4°C (Universal 32R centrifuge, Hettich FurnTech Division DIY, Melle-Neuenkirchen, Germany). Then, it was diluted (1:1) in 0.06 mol/l oxalic acid and injected into a gas chromatograph (Thermo-Quest 8000top Italia SpA, Rodano, Milan, Italy) equipped with a fused silica capillary column (30 × 0.25 mm, 0.25 μm film thickness, Supelco, Inc., Bellefonte, PA, USA) using pure acetic, propionic, butyric, iso-butyric, valeric, and iso-valeric acids as an external standard solution (Calabrò et al., 2022). The total volatile fatty acid (tVFA, mmol/g), acetate/propionate ratio (A/P), and branched-chain fatty acids [BCFA = (iso-butyrate + iso-valerate)/tVFA × 100] were also calculated.

3.3.6. Processing Data

For each bottle, the cumulative gas volume was related to the incubated organic matter (OMCV, mL/g) and to the degraded organic matter (Yield, mL/g). The metabolizable energy (ME, KJ/kg DM) content in pineapple by-products was estimated using the following formula (Menke and Steingass, 1988):

$$\mathrm{ME} = 2.43 + 0.1206 * \mathrm{G24} + 0.0069 * \mathrm{CP} + 0.0187 * \mathrm{EE} \quad \text{(Eqn. 1)}$$

where CP, EE, and GP are, respectively, crude protein, ether extract, and the volume of gas produced by 200 mg of dry matter after 24 h of incubation with the rumen liquor. Data of chemical composition, sugar content, estimated ME energy, and *in vitro* fermentation of pineapple by-products were statistically processed to verify the effect of varieties (Sugarloaf vs. Smooth Cayenne) and by-products (crown, bud end, peel, core, and pomace) according to the following model:

$$Y_{ijk} = \mu + V_i + P_j + (V * P)_{ij} + \varepsilon_{ijk} \quad \text{(Eqn. 2)}$$

Where Y is the experimental data, μ represents the general mean, V the variety effect (i = 1, 2), P is the effect of the by-product parts (j = 1, 2, 3, 4, 5), V × P is the interaction between the variety and by-products, and ε is the error term. GLM and Tukey's HSD test were used in JMP® software (Version 14 SW, SAS Institute Inc., Cary, NC, USA, 1989-2019). The distribution of data was checked by the Shapiro–Wilk test before any comparison, and the significant level was fixed at 5 %.

3.4.Results

3.4.1. Pineapple by-product production

The percentage of pineapple by-products generated during the fruit processing is presented in Figure 2. 2. The total by-products obtained during the processing represent around 83.81 % and 74.63 % of whole fruits in Smooth Cayenne and Sugarloaf varieties, respectively. There were no differences between the varieties for the crown, peel, and pomace, averaging 11.59, 30.60, and 24.04%, respectively. However, the core (8.91 vs. 7.47%) and bud end (5.71 vs. 3.88%) were higher ($p < 0.05$) for the SC variety compared to SL.

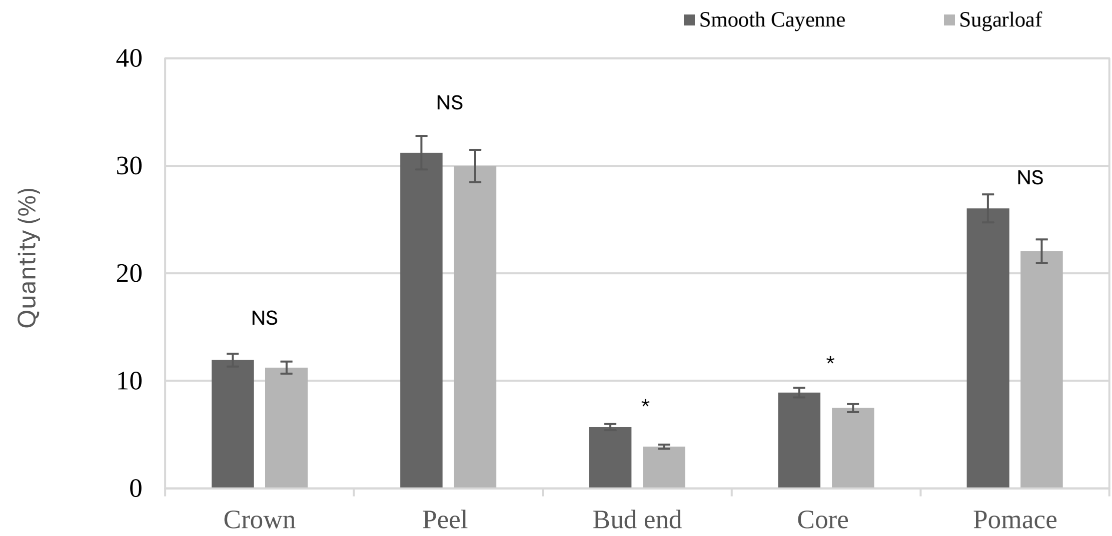

Figure 3. 2. The amount of the two varieties of pineapple by-products obtained by processing the whole fruit.

Between by-products: NS: non-significant, *: $p < 0.05$.

3.4.2. Chemical characteristics and estimated metabolizable energy

Table 3. 1. shows the chemical characteristics and estimated metabolizable energy value of the two pineapple by-product varieties. In general, highly significant differences ($p < 0.0001$) were found between the two varieties (except ME, $p = 0.0004$), and between the by-products for all parameters; the interaction Variety × By-product was highly significant as well. The DM content ranged between 14.24 % (core) to 20.38 % (pomace) in Smooth Cayenne and between 15.08 % (peel) to 20.98 % (pomace) in Sugarloaf. The

crown, peel, and pomace showed significantly higher CP content ($p < 0.0001$) than the bud end and core in both varieties. Ash content was high in the crown (6.08 vs. 4.81 % DM), bud end (6.22 vs. 4.59 % DM), and peel (5.62 vs. 4.93 % DM) compared to the core and pomace (2.48 vs. 1.58 and 3.00 vs. 2.78 % DM) in SC and SL, respectively, even if in Smooth Cayenne, the values were always higher. In both pineapple varieties, the highest ($p < 0.001$) EE and structural carbohydrate (NDF and ADF) values were found in the crown and the lowest in the core. Regarding ADL, the highest values were found in the Smooth Cayenne crown (3.09 % DM; $p < 0.0001$), in the Sugarloaf bud end and crown (2.19 and 2.08 % DM, respectively), and the lowest values were found in the pomace of both varieties.

Regarding sugars, the Smooth Cayenne crown showed the lowest content of TS and FS, whereas the core and pomace showed the highest ($p < 0.0001$) TS and FS content, respectively. On the other hand, the Sugarloaf pomace and crown showed the lowest TS and FS content, respectively whereas the core showed the highest values for both parameters.

The estimated ME content was significantly ($p < 0.001$) higher in the core and pomace and lower in the crown and bud end, while the estimated ME of the peel was intermediate, even if it was not significantly different from the core and pomace in the SL variety.

Table 3. 1. Chemical characteristics and estimated energy value of the two varieties of pineapple by-products

Items	**DM**	**CP**	**Ash**	**EE**	**NDF**	**ADF**	**ADL**	**TS**	**FS**	**ME**
	%	*% DM*						*%*	*% TS*	*MJ/kgDM*
Smooth Cayenne variety										
Crown	17.63^{B}	7.55^{A}	6.08^{A}	1.06^{A}	47.62^{A}	26.03^{A}	3.09^{A}	41.20^{D}	26.28^{D}	5.16^{C}
Bud end	15.79^{C}	5.41^{B}	6.22^{A}	0.48^{B}	45.60^{B}	21.28^{B}	2.34^{B}	57.23^{B}	28.92^{D}	5.11^{C}
Peel	15.63^{C}	7.51^{A}	5.62^{B}	0.45^{B}	33.85^{C}	16.61^{C}	1.77^{C}	52.15^{C}	35.40^{C}	6.30^{B}
Core	14.24^{C}	6.43^{B}	2.48^{D}	0.20^{C}	13.46^{E}	7.05^{E}	1.83^{BC}	70.43^{A}	43.70^{B}	7.82^{A}
Pomace	20.38^{A}	8.10^{A}	3.00^{C}	0.24^{C}	19.58^{D}	9.40^{D}	1.55^{C}	68.10^{A}	62.04^{A}	7.99^{A}
Sugarloaf variety										
Crown	20.62^{A}	8.48^{A}	4.81^{B}	0.68^{A}	39.01^{A}	20.31^{A}	2.08^{AB}	54.94^{C}	24.63^{B}	5.62^{B}
Bud end	17.07^{B}	6.54^{B}	4.59^{B}	0.37^{B}	33.27^{B}	15.19^{B}	2.19^{A}	58.87^{B}	36.91^{A}	5.94^{B}
Peel	15.08^{C}	8.01^{A}	4.93^{A}	0.35^{B}	26.48^{C}	11.97^{C}	1.60^{B}	58.44^{B}	27.79^{B}	7.34^{A}
Core	15.29^{C}	5.18^{B}	1.58^{D}	0.19^{C}	10.80^{E}	6.85^{E}	1.59^{BC}	84.96^{A}	40.40^{A}	7.95^{A}
Pomace	20.98^{A}	8.81^{A}	2.78^{C}	0.30^{BC}	21.53^{D}	9.75^{D}	1.27^{C}	40.81^{D}	36.58^{A}	7.36^{A}
p-value										
Var	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0004
BP	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Var x BP	0.0004	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
MSE	0.9033	0.2758	0.0053	0.0004	0.1440	0.0423	0.0171	0.8630	0.9850	0.0777

DM: dry matter, CP: crude protein, EE: ether extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, TS: total sugar, FS: free sugar, ME: metabolizable energy, Var: variety, BP: by-products. Along the column, for each variety, different letters indicate the statistical difference ($p < 0.001$). MSE: mean square error.

3.4.3. In vitro degradability, gas production, and VFA profiles

The *in vitro* fermentation characteristics of the two varieties of pineapple by-products are reported in Table 3. 2. The by-products significantly affected all parameters (p<0.0001), while the volume of gas related to the degraded organic matter (Yield) was significantly affected by both factors (variety and by-products).

Table 3. 2. *In vitro* fermentation characteristics in the two varieties of pineapple by-products

Items	pH	OMD	OMCV	Yield
		%	ml/g	ml/g
Smooth Cayenne variety				
Crown	6.46^{A}	56.14^{D}	146.51^{C}	259.45^{C}
Bud end	6.34^{B}	62.83^{C}	154.98^{C}	242.07^{D}
Peel	6.31^{B}	75.95^{B}	205.91^{B}	271.72^{B}
Core	5.98^{D}	84.82^{A}	244.72^{A}	314.25^{A}
Pomace	6.19^{C}	85.35^{A}	238.75^{A}	285.26^{B}
Sugarloaf variety				
Crown	6.44^{A}	62.61^{D}	158.52^{C}	253.21^{D}
Bud end	6.40^{A}	65.17^{D}	182.60^{B}	281.96^{C}
Peel	6.34^{B}	75.91^{C}	236.71^{A}	304.62^{A}
Core	5.92^{D}	86.55^{A}	255.02^{A}	284.87^{BC}
Pomace	6.18^{C}	81.41^{B}	240.03^{A}	296.35^{AB}
p-value				
Variety	0.8941	0.0135	<.0001	<0.0001
By-product	<0.0001	<0.0001	<0.0001	<0.0001
Var x BP	<0.0054	<0.0001	0.0141	<0.0001
MSE	0.0010	2.5070	45.2111	15.1512

OMD: organic matter degradability (% of incubated OM), OMCV: cumulative volume of gas related to incubated OM, Yield: cumulative volume of gas related to degraded OM, Var: variety, BP: by-products. Along the column for each variety, different letters indicate the statistical difference (p < 0.001). MSE: mean square error.

The pH was significantly (p < 0.0001) different in the by-products of each variety; in any case, it ranged from 5.92 to 6.46, indicating a correct fermentation process. After 48 h of incubation, organic matter degradability (OMD) ranged from 56.14 % (in the crown) to 85.35 % (in the pomace) in the Smooth Cayenne variety and from 62.61 % (in the crown) to 86.55 % (in the core) in the Sugarloaf variety. Large variations were also found in the cumulative volume of gas between the pineapple varieties and among the five by-products within the variety. In particular, OMCV varied from 146.51 to 244.72 mL/g for Smooth Cayenne and from 158.52 to 255.02 mL/g for Sugarloaf. Regarding the Yield parameter, a high variability was also found in the Smooth Cayenne variety (from 242.07 to 314.25 mL/g), but a low variability was observed in Sugarloaf (from 253.21 to 304.62 mL/g).

The core and pomace showed the lowest pH values with the highest OMD, OMCV, and Yield ($p < 0.001$) in Smooth Cayenne by-products; the same result was observed in Sugarloaf by-products, except for Yield.

The *in vitro* fermentation profile in pineapple by-products within 48 h of incubation is pictured in Figure 3. 3. The curve of corn grain and Sulla hay, incubated as reference feedstuffs, were also represented. Three groups characterized by low, medium, and high fermentation can be distinguished. The least fermentable, lower than Sulla hay, were the crowns of both varieties and the SC bud end, while the most fermentable were the cores and pomaces for both varieties and the SL peel, which were similar to corn grain. In the middle, the SC peel and SL bud end with a fermentation profile superimposable on Sulla hay.

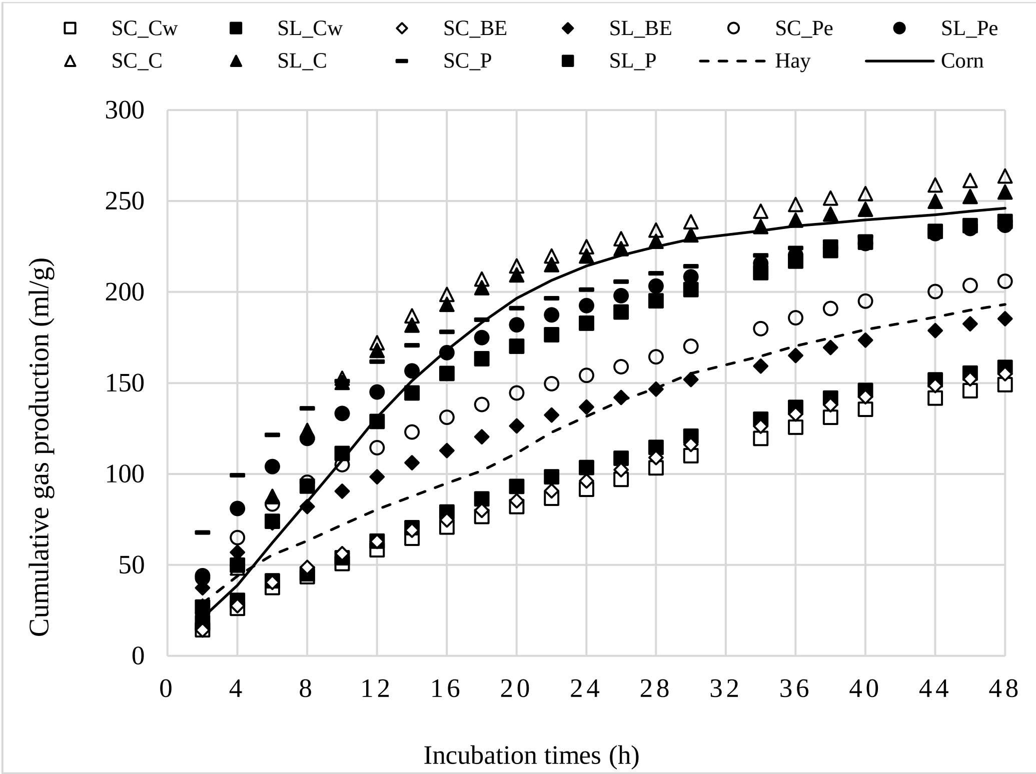

Figure 3. 3. *In vitro* fermentation profile in the two varieties of pineapple by-products along 48 h of incubation.

SL: Smooth Cayenne, SL: Sugarloaf, Cw: Crown, BE: Bud end, Pe: Peel, C: Core, and P: Pomace.

Table 3. 3. shows the *in vitro* fermentation-end products in the two varieties of pineapple by-products obtained after 48 h of incubation. All factors showed a significant effect ($p < 0.005$), even if the same trend was not always observed in both varieties, as demonstrated by the statistical significance of the interaction values presented in the table. In Smooth Cayenne, the acetate and acetate/propionate ratio were the highest in the crown, whereas propionate and butyrate were the highest in the core and pomace, respectively. Regarding Sugarloaf, the highest ($p<0.001$) amount of acetate, propionate, and butyrate was found in the core, while the acetate/propionate ratio was the highest ($p<0.0001$) in the crown. The highest ($p<0.0001$) amount of valeric acid was observed in the crown and core of

Smooth Cayenne and the core and pomace of Sugarloaf, while the lowest was obtained in the bud end for both varieties. For Smooth Cayenne and Sugarloaf varieties, the percentage of BCFA was significantly ($p<0.001$) higher in the crown and lower in the core. However, the core always showed the highest ($p<0.0001$) total VFA production.

Table 3. 3. *In vitro* fermentation end-products in the two varieties of pineapple by-products

Items	Ace	Pro	Iso-But	But	Iso-Val	Val	tVFA	BCFA	Ace/Pro
				mmol/g				*%*	
				Smooth Cayenne variety					
Crown	33.51^{A}	25.44^{C}	0.46^{A}	19.36^{C}	0.66^{A}	0.67^{A}	80.10^{C}	1.61^{A}	1.51^{A}
Bud end	29.29^{B}	24.22^{C}	0.31^{B}	21.26^{C}	0.39^{B}	0.43^{B}	75.90^{C}	1.06^{B}	1.38^{B}
Peel	25.20^{C}	23.05^{C}	0.38^{B}	24.88^{B}	0.46^{B}	0.45^{B}	74.43^{C}	1.30^{B}	1.24^{C}
Core	27.04^{BC}	50.05^{A}	0.17^{C}	25.26^{B}	0.22^{C}	0.70^{A}	103.4^{A}	0.43^{C}	0.62^{E}
Pomace	19.96^{D}	31.77^{B}	0.38^{B}	37.02^{A}	0.62^{A}	0.53^{B}	90.28^{B}	1.37^{A}	0.78^{D}
				Sugarloaf variety					
Crown	25.59^{BC}	21.05^{D}	0.38^{A}	17.15^{C}	0.59^{A}	0.48^{C}	65.25^{C}	1.67^{A}	1.36^{A}
Bud end	24.50^{C}	22.57^{CD}	0.33^{AB}	23.63^{B}	0.48^{AB}	0.39^{C}	71.92^{B}	1.26^{B}	1.22^{B}
Peel	27.08^{B}	24.23^{C}	0.26^{BC}	24.24^{B}	0.46^{B}	0.66^{B}	77.93^{B}	1.05^{BC}	1.23^{B}
Core	30.24^{A}	50.79^{A}	0.17^{D}	27.17^{A}	0.22^{C}	0.92^{A}	109.5^{A}	0.39^{D}	0.66^{C}
Pomace	21.89^{D}	36.09^{B}	0.21^{C}	12.24^{D}	0.25^{C}	1.02^{A}	71.70^{B}	0.77^{C}	0.72^{C}
p-value									
Var	0.0010	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	0.0034	<0.0001
BP	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Var x BP	<0.0001	0.0004	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0001
MSE	0.9510	1.4491	0.0008	0.7540	0.0021	0.0020	6.0990	1.3470	0.0020

Ace: acetate, Pro: propionate, Iso-But: iso-butyrate, But: Butyrate, Iso-Val: iso-valerate, Val: valerate, tVFA: total volatile fatty acid, BCFA: branched chain fatty acid, Ace/Pro: acetate to propionate ratio, Var: variety, BP: by-products. Along the column for each variety, different letters indicate the statistical difference ($p < 0.001$). MSE: mean square error.

The correlation between chemical composition and the IVGPT parameters of pineapple by-products is presented in Table 3. 4. The results showed that the OMD, OMCV, and Yield were significantly and negatively correlated with EE, Ash, NDF, and ADF, while pH was positively correlated with the same parameters.

Table 3. 4. Correlation between chemical composition and the in vitro fermentation characteristics of pineapple by-products (n = 20)

	pH	OMD	OMCV	Yield	tVFA
CP	0.40	-0.03	0.01	0.14	-0.56
	NS	NS	NS	NS	NS
EE	0.75	-0.85	-0.83	-0.65	-0.48
	*	**	**	*	NS
Ash	0.87	-0.84	-0.83	-0.67	-0.70
	**	**	**	*	*
NDF	0.90	-0.95	-0.95	-0.81	-0.72
	***	***	***	**	*
ADF	0.86	-0.95	-0.96	-0.83	-0.63
	**	***	***	**	NS
ADL	0.61	-0.85	-0.85	-0.63	-0.22
	NS	**	**	NS	NS
FS	-0.56	0.70	0.59	0.41	0.53
	NS	*	NS	NS	NS
TS	-0.72	0.59	0.54	0.31	0.80
	*	NS	NS	NS	**
ME	-0.83	0.96	0.98	0.83	0.67
	**	***	***	**	*

CP: crude protein, EE: ether extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, FS: free sugar, TS: total sugar, ME: metabolizable energy. OMD: organic matter degradability, OMCV: cumulative volume of gas related to incubated OM, Yield: cumulative volume of gas related to degraded OM, tVFA: total volatile fatty acid. NS: non-significant, *, **, ***: indicate $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively.

In addition, OMD and OMCV were positively ($p<0.001$) correlated with ME and negatively ($p<0.01$) with ADL. Regarding sugar, FS was correlated with OMD ($p<0.05$), and TS was correlated with pH and tVFA ($p<0.05$ and $p<0.01$, respectively). Any fermentation parameters were correlated with CP content.

3.5. Discussion

In this study, the individual by-products of two varieties of pineapple (Sugarloaf and Smooth Cayenne) cultivated in West Africa were characterized in terms of chemical composition, estimated ME, and *in vitro* fermentation parameters for their possible use in ruminant nutrition. The hypothesis is that some differences exist between the by-products of the fruits obtained during the processing.

3.5.1. Chemical characteristics: comparing by-products and variety

In our study, the amounts of pineapple by-products obtained after fruit processing nearly double those (skin, shoot tip, core, stems, crowns) reported by other investigations except for the peel (Hemung et al., 2022; Idayanti et al., 2022). In terms of chemical composition, other studies on pineapple by-products reported that CP, EE, NDF, and ADL range from 4.4-5.7, 0.2-1.2, 20.5-44.69, and 23.04-39.40 % DM, respectively. (Hemung et al., 2022; Melesse et al., 2018; Idayanti et al., 2022; Bulkaini, 2022). The same authors also quantified organic matter (OM), non-fiber carbohydrate (NSC), and water-soluble carbohydrate (WSC), resulting, respectively, in 95.54, 48.8, and 5.4 % DM. In our investigation, a low DM content was found in all by-products (14.24-20.98%). Ash, ether extract, and structural carbohydrates were present in decreasing quantities from the crown, bud end, peel, and pomace to the core in both varieties. The EE and ADL were low in all pineapple by-products for both varieties (0.20-1.06, 1.27-3.09 % DM). Regarding the categories, the crown and bud end pineapple by-product showed the highest lignin content compared to the peel, core, and pomace, whereas a low variation in crude protein was found between by-products. As expected, the range observed in the chemical composition is due to the parts of the by-products, which are different in terms of texture. The processing technique also influences the difference in terms of chemical composition between the by-products generated. The range of variation in the chemical composition found in this current study is in line with other research on several kinds of agro-industrial by-products (Mirzaei and Maheri-Sis, 2008; García-Rodríguez et al., 2019). An average fat content of 3.08 % has been reported in previous studies (Suksathit et al., 2011; Asaolu et al., 2016; Oduguwa et al., 2020). This is 0.43 % higher than the fat determined in this study. However, compared with the cited studies, the ADL content was similar. On the other hand, the structural carbohydrate (NDF and ADF) of the crown, bud end, and peel reported in this study for both varieties were comparable to the results already published on the same by-products (Págan et al., 2014; Choi et al., 2021). The NDF and ADF of the core and pomace were low and not much different from other by-products such as sugar beet pulp, pepper core, and citrus pulp (García-Rodríguez et al., 2019). However, some studies highlight the high proportion of NDF and ADF in dehydrated pineapple by-products (Correia et al., 2006; Oduguwa et al., 2020), comparable to the grass grown in West African natural pasture (Koura et al., 2022). This discrepancy might be due to the method of collection (separately vs. pooled: bud end, peel, core, and pomace together), processing techniques (homemaking vs. industrial), and cultivation area conditions (soils, temperature, and rainfall differences) (Rogério et al., 2007).

In this study, for both varieties, the crude protein level was higher in the crown, peel, and pomace (7.51 - 8.81 % DM) compared to the bud end and core (5.18 - 6.54 % DM). The crude protein content of pooled pineapple by-products (peel, bud end, core, and pomace) investigated in South America was higher than those obtained individually in the bud end and core but similar to the CP of the crown, peel, and pomace (Correia et al., 2006;

Rogério et al., 2007; Azevêdo et al., 2011). The crude protein reported in these papers is low (for the bud end and core) and similar (for the crown, peel, and pomace) compared to the range of 7 - 8 % recommended for the efficient functioning of rumen microorganisms (Van Soest et al., 1991). This means that there is a necessity to introduce protein source ingredients in the diet of ruminants when pineapple by-products are used.

Regarding the estimated metabolizable energy content, the highest values were obtained in the core and pomace, which also showed the highest sugar content (TS and FS) for the Smooth Cayenne and Sugarloaf varieties. As revealed previously, the feedstuffs that are rich in sugar as some pineapple by-products (e.g., core, pomace) could be useful as an energy source in ruminant nutrition (Siti Roha et al., 2013; Vastolo et al., 2022). Our findings were in accordance with previous research on pineapple by-products in terms of sugar content (Idayanti et al., 2022; Müller, 1978). They observed that pineapple by-products contain high levels of sugar (40-75%), mainly constituted by sucrose (70%) and then by glucose (20%) and fructose (10%), which are primary energy sources when they are present in animals' diet. The ME that we estimated was close to the results obtained by Correia et al., 2006 in Brazil and fell into the interval (10.7-14.5 MJ/kg DM) observed by Melesse et al., 2018 in the classification of fruit by-products rich in energy. However, some authors (Asaolu et al., 2016; Chaudhary et al., 2019) have observed the lowest sugar content (26.28%) in pineapple by-products compared to our data. These results suggested separating the pineapple by-products into two groups, ME and CP sources, before their inclusion in ruminants' diets.

3.5.2. In Vitro fermentation characteristics: comparing by-products and varieties

Regarding the *in vitro* fermentation characteristics, data showed that the IVGPT is a suitable method to study pineapple by-products when incubated for 48 h with sheep rumen fluid. The pH values (ranging from 5.92 to 6.46) varied within the normal interval of rumen pH value. Moreover, as reported in previous studies (Vastolo et al., 2023), significant correlations were observed underline the accordance between chemical composition and *in vitro* parameters: the increase in structural carbohydrates, ether extract, and ash reduced gas, VFA production, and OM degradability, whereas the increase in sugar and energy increased these parameters.

The results that emerged from *in vitro* fermentation show that the by-products with the highest amount of sugar and energy (core and pomace for both varieties) also had the highest organic matter degradability, cumulative gas production related to incubated OM (OMCV), and cumulative gas production related to degraded organic matter (Yield). These findings support the fact that microorganisms utilized energy from by-products during the fermentation for their own growth, converting the structural carbohydrate to end products (i.e., volatile fatty acid, carbon dioxide, and methane) (INRA, 2018). Regarding the end products, the acetate/propionate ratio are higher for the crown, bud end, and peel in both varieties (Ace/Pro > 1.0), while their total volatile fatty acid production is lower compared to the core and pomace. Regarding OM degradability, our results for the crown and bud end are consistent with those reported in the literature (Raseel et al., 2018). The higher OMD observed for core and pomace as well as for peel in this study are comparable to data published on *in vitro* trials carried out incubating pineapple waste with cow rumen fluid (Melesse et al., 2018; Idayanti et al., 2022).

The volatile fatty acid produced by microorganisms in the rumen represents the energy source for ruminants. High-level of volatile fatty acid means high energy available to satisfy requirements for maintenance and production (meat or milk). The total VFA recorded in this study was revealed to be higher than those reported in other investigations on pineapple by-products (Suksathit et al., 2011 Hattakum et al., 2019), as well as on other by-products such as banana peels, papaya peels, mango seed kernels, and tomato pomaces (Melesse et al., 2018; Marcos et al., 2019). Several factors such as sampling and chemical composition might affect volatile fatty acid production, which affects pH and microorganism development in the rumen. Surprisingly, previous research focused on natural pasture and cultivated grass in the pineapple growing area of West Africa reported similar results regarding the total VFA (Koura et al., 2022). These results might encourage further research to replace limited feedstuffs during the dry season with alternative resources, such as pineapple by-products, to help the farmers sustain their productivity through the dry season and maintain animal productivity throughout the year.

To better discuss the fermentation profile of the different pineapple by-products, the *in vitro* cumulative gas production recorded during the 48-h period of incubation is represented in Figure 3. 3., together with the corn grain and Sulla hay incubated as reference feedstuffs for energy and fiber sources, respectively. Three different groups of feedstuffs, in terms of fermentation patterns, are evidenced. The first group is composed of the crowns of both varieties and the bud end of the Smooth Cayenne variety, characterized by slow and low fermentability and more comparable to a forage of less quality. The second group is composed of pineapple by-products, which had fermentation characteristics similar to Sulla hay and concern the Sugarloaf bud end and Smooth Cayenne peel. The third group contains by-products (Smooth Cayenne: pomace and core; Sugarloaf: peel, pomace, and core) like corn grain, with a high fermentation rate in the first hours of incubation. However, the main differences are clearer in the first six hours of incubation for the first group, whereas the differences are clearer around 14 h and 48 h for the second and the third group, respectively. Moreover, the profile of the first and second groups of by-products was quite linear, meaning that an asymptote had not been achieved at this time of incubation (48 h) due to the high NDF content. Possibly, to better observe the characteristics of fermentation, more hours of incubation would have been necessary for this group of pineapple by-products.

3.5.3. Problems and opportunities in ruminant diets

The low DM content and high sugar level found in all by-products (as reported before) suggest a risk of rapid decomposition after the processing. This finding notes the long-term conservation problem and the difficulty of using them in animal feeding plans. Pineapple by-products, as fruit waste in general, were already noted to be problematic in terms of preservation (Upadhyay et al., 2010). However, the presence of easily fermentable sugars (free sugars) in these by-products justifies the availability of energy that would be useful by the microorganism in the rumen and, as reported (Mordenti et al., 2021; Palmonari et al., 2021; Plascencia et al., 2022), could increase dry matter intake, palatability, and animal performance by improving microbial activity and nitrogen utilization. Consequently, high microbial activity would contribute to enhancing the amount of digestible protein in the gut via microbial protein contribution.

The low content of EE (>5 % DM) and ADL in the by-products of both varieties is interesting because when these parameters are high in a ruminant's diet can depreciate the diet by affecting nutrient intake and digestibility. Consequently, the utilization of pineapple by-products as ingredients could reduce the amount of EE and ADL in the final diet, making it efficient for ruminants. A decrease in nutrient intake (DM, OM, CP, and NDF) was observed with an increase in EE up to 7.9 % (Haddad and Younis, 2004), while the DM and OM degradability of grape seed, dried tomato pulp, and pepper core by-products were low because of their high level of ADL (García-Rodríguez et al., 2019).

Regarding the CP content, it was lower than the minimum concentration (7 % DM) for the normal functionality of the rumen in some by-products (bud end and core). The lowest CP content (3.5 % DM) was reported when pineapple waste was ensiled with cassava root meal or cassava peel (4:1 ratio) for preservation issues (Oduguwa et al., 2020). These by-products cannot be utilized alone as protein sources in ruminant diets. On the other hand, interestingly, they showed energy content similar to the natural pastures of West Africa, which become scarce during the dry season (Koura et al., 2022). Pineapple by-products could be useful in this case to replace the grass (e.g., *Poaceae* Fam.) during the dry season, a critical period for grassing animals in the West African region. However, as mentioned above, the maturity status of pineapples, the processing technology applied, and environmental parameters can fluctuate the amount of sugar and ME in these by-products.

In addition, the utilization of pineapple peels up to 20 % in ruminants' diets formulated with 10 % milled corn and 70 % bran was revealed to improve feed intake and organic matter digestibility. The ability of pineapple by-products to enhance the intake and digestibility might be due to their sweetness and the presence of the bromelain enzyme, which is known to improve digestibility (Suksathit et al., 2011; Choi et al., 2021). Some authors also reported an improvement in the quality of animal products when pineapple by-products were used in the ruminants' diet as feed ingredients (Raseel et al., 2020; Liu et al., 2021). In 2022, Bulkaini reported that the addition of pineapple by-products fermented with lactic acid bacteria at levels of 20 % can improve the production performance and carcass quality of male Bali cattle. Based on this aspect, pineapple by-products can substitute a part of conventional feedstuffs in West African ruminants' diets as already reported for other fruit waste (Piquer et al., 2009; Elias et al., 2017).

3.6. Conclusion

Our results suggested that pineapple by-products have the potential to be used as feed ingredients in ruminants' feeding plans according to their chemical composition and *in vitro* fermentation parameters if they are properly and separately collected during fruit processing. The results suggested considering the crown, bud end, and peel as fiber sources (e.g., roughage), while the core and pomace could be substitutes for the concentrate feedstuffs in ruminants' diets. However, due to the high amount of sugar still present in pineapple by-products, their use at high levels in a diet could cause digestive disturbances. The results evidence the possibility of using pineapple by-products in animal nutrition; however, further studies must be conducted to determine the way it would be preserved for a long time. Additional studies are necessary to determine the right amount of pineapple by-products to include in West African ruminants' diets without affecting animal health as well as the performance, welfare, and quality of products. Further research must focus on some points: ensiling pineapple by-products with hay to enhance self-life and testing the *in vivo* effect of pineapple by-products to evaluate the performance, health status, and quality of the products (meat and milk). It is also important to standardize the techniques in collecting and processing pineapple fruits. Moreover, further data relating to by-product transportation costs and carbon emissions would be helpful to demonstrate the contribution to the circular economy that the use of these feedstuffs can make.

3.7. Acknowledgments

Thanks to AGRITECH National Research Center the European Union Next-Generation (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 June 17, 2022, CN00000022) for financial support. The manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

3.8. Reference

1. Agbangba, C.E. Réponses Agronomiques de l'ananas (*Ananas Comosus*) à La Fertilisation Minérale Au Bénin : Croissance, Rendement et Qualité Du Fruit. Ph.D. Thesis, Université Cheikh Anta Diop de Dakar (UCAD), Dakar, Senegal, 2016.

2. Amole, T.; Augustine, A.; Balehegn, M.; Adesogoan, A.T. Livestock Feed Resources in the West African Sahel. *Agron. J.* 2019, 114, 26–45.

3. AOAC. Official Method 925.35 Sucrose in Fruit and Fruit Product; Association of Official Analytical Chemists: Arlington, VA, USA, 2000.

4. AOAC. Official Methods of Analysis, 12 Vol, 18th ed.; Association of Official Analytical Chemists: Arlington, VA, USA, 2005.

5. Asaolu, V.O.; Binuomote, R.T.; Oyelami, O.S. Assessment of Feeding Value of Vegetable-Carried Pineapple FruitWastes to Red Sokoto Goats in Ogbomoso, Oyo State of Nigeria. *Afr. J. Biotechnol*. 2016, 15, 1648–1660.

6. Azevêdo, J.A.G.; Filho, S.D.C.V.; Pina, D.D.S.; Detmann, E.; Valadares, R.F.D.; Pereira, L.G.R.; Souza, N.K.D.P.; Silva, L.F.C. Intake, Total Digestibility, Microbial Protein Production and the Nitrogen Balance in Diets with Fruit By-Products for Ruminants. *Rev. Brasil. Zootec*. 2011, 40, 1052–1060.

7. Bulkaini, B. Production Performance and Carcass Quality of Male Bali Cattle by Feeding Fermented Pineapple Peel. *Asian J. Appl. Res. Commu Dev. Empower*. 2022, 6, 29–34.

8. Calabrò, S.; Oteri, M.; Vastolo, A.; Cutrignelli, M.I.; Todaro, M.; Chiofalo, B.; Gresta, F. Amaranthus Grain as a New Ingredient in Diets for Dairy Cows: Productive, Qualitative, and *in Vitro* Fermentation Traits. *J. Sci. Food Agric*. 2022, 102, 4121–4130.

9. Chaudhary, V.; Kumar, V.; Singh, K.; Kumar, R.; Kumar, V. Pineapple (Ananas Cosmosus) Product Processing: A Review. *J. Pharmacogn. Phytochem*. 2019, 8, 4642–4652.

10. Choi, Y.; Park, K.; Lee, S.; Na, Y. Determination of in Situ Degradation Parameters and Feeding Level of Pineapple (*Ananas Comosus L.*) Cannery By-Product to Hanwoo Steers. *Anim. Biosci.* 2021, 34, 85–92.

11. Correia, M.X.C.; Costa, R.G.; Da Silva, J.H.V.; De Carvalho, F.F.R.; De Medeiros, A.N. Use of Dehydrated Pineapple By-Product in Diets for Growing Goats: Digestibility and Performance. *Rev. Brasil. Zootec*. 2006, 35, 1822–1828.

12. EC Council. Regulation 882/2004 on Official controls performed to ensure verification of compliance with feed and food law, animal health and animal; EC Council: UE, 2004.

13. Elias, A.K.S.; Alves, K.S.; Oliveira, L.R.S.; Cutrim, D.O.; Pontes, V.P.; Melo, W.O.; Gomes, D.I. Carcass Yield, Cuts and Body Components in Lambs Fed a Pineapple By-Product Silage Diet. *Afr. J. Agric. Res*. 2017, 12, 2351–2357.

14. Faostat. New Food Balances; FAO: United Nation, 2021. Available online: http://www.fao.org/faostat/en/#data/FBS.

15. Firatoiu, A.R.; Chiurciu, I.A.; Marcuta, L.; Chereji, A.I.; Soare, E.; Voicu, V.; Marcuta, A. Study on the Production and Marketing of Pineapples Worldwide. In Proceedings of the 37th International Business Information Management Association (IBIMA), Cordoba, Spain, 1–2 April 2021; pp. 4973–4985, ISBN 978-0-9998551-6-4. Available online: http://www.incda-fundulea.ro/rar/nr37/rar37.27.pdf (accessed on 2 April 2021).

16. García-Rodríguez, J.; Ranilla, M. J.; France, J.; Alaiz-Moretón, H.; Carro, M. D.; López, S. Fermentation kinetics of agro-industrial by-products. *Animals* 2019, *9*, 861.

17. Gowda, N.K.S.; Vallesha, N.C.; Awachat, V.B.; Anandan, S.; Pal, D.T.; Prasad, C.S. Study on Evaluation of Silage from Pineapple (*Ananas Comosus*) Fruit Residue as Livestock Feed. *Trop. Anim. Health Prod.* 2015, 47, 557–561.

18. Haddad, S.G.; Younis, H.M. The effect of adding ruminally protected fat in fattening diets on nutrient intake, digestibility and growth performance of Awassi lambs. *Anim. Feed Sci. Technol*. 2004, 113, 61–69.

19. Hattakum, C.; Kanjanapruthipong, J.; Nakthong, S.;Wongchawalit, J.; Piamya, P.; Sawanon, S. Pineapple Stem By-Product as a Feed Source for Growth Performance, Ruminal Fermentation, Carcass and Meat Quality of Holstein Steers. *S. Afr. J. Anim. Sci.* 2019, 49, 147–155.

20. Hemung, B.O.; Sompholkrang, M.;Wongchai, A.; Chanshotikul, N.; Ueasin, N. A Study of the Potential of By-Products from Pineapple Processing in Thailand: Review Article. *Int. J. Health Sci.* 2022, 6, 12605–12615.

21. Idayanti, R.W.; Arifin, M.; Purbowati, E.; Purnomoadi, A. Utilization of PineappleWaste as a Roughage Source Diets for Ruminant: A Review. *Adv. Biol. Sci. Res*. 2022, 20, 123–130.

22. INRA. Feeding System for Ruminants; Wagningen Academic: Wagningen, Netherlands, 2018.

23. INSAE. Cahier Des Villages et Quartiers de Ville Département de l'Atlantique: Ministère Chargé du Plan, de La Prospective et du Développement; INSAE: Cotonou, Bénin, 2004; pp. 1–34.

24. Koura, B.I.; Vastolo, A.; Kiatti, D.D.; Cutrignelli, M.I.; Houinato, M.; Calabrò, S. Nutritional Value of Climate-Resilient Forage Species Sustaining Peri-Urban Dairy Cow Production in the Coastal Grasslands of Benin (West Africa). *Animals* 2022, 12, 3550.

25. Kumar, A. Utilization of Bioactive Components Present in Pineapple Waste: A Review. *J. Pharm. Innov.* 2021, 10, 954–961.

26. Liu, C.; Asano, S.; Ogata, H.; Ito, S.; Nakase, T.; Takeda, S.; Miyoshi, K.; Numata, Y.; Takahashi, K.; Kajikawa, H. Digestive, Fermentative, and Physical Properties of Pineapple Residue as a Feed for Cattle. *Anim. Sci. J*. 2021, 92, e13535.

27. Marcos, C.N.; de Evan, T.; Molina-Alcaide, E.; Carro, M.D. Nutritive Value of Tomato Pomace for Ruminants and Its Influence on *in Vitro* Methane Production. *Animals* 2019, 9, 343.

28. Melesse, A.; Steingass, H.; Schollenberger, M.; Rodehutscord, M. Component Composition, *in Vitro* Gas and Methane Production Profiles of Fruit By-Products and Leaves of Root Crops. *J. Agric. Sci*. 2018, 156, 949–958.

29. Menke, K.H.; Steingass, H. Estimation of the Energetic Feed Value Obtained from Chemical Analysis and *in Vitro* Gas Production Using Rumen Fluid. *Anim. Res. Dev*. 1988, 28, 7–55.

30. Mirzaei, A.; Maheri-Sis, N. Nutritive Value of Some Agro-Industrial By-Products for Ruminants—A Review. *World J. Zool* 2008, 3, 40–46.

31. Mordenti, A.L.; Giaretta, E.; Campidonico, L.; Parazza, P.; Formigoni, A. A Review Regarding the Use of Molasses in Animal Nutrition. *Animals* 2021, 11, 115.

32. Müller, Z.O. Feeding Potential of Pineapple Waste for Cattle. *Rev. Mond. Zootec*. 1978, 25, 25-29.

33. Oduguwa, B.O.; Sanusi, G.O.; Fasae, O.A.; Oni, O.A.; Arigbede, O.M. Nutritive value, growth performance and haematological parameters of West African dwarf sheep fed preserved pineapple fruit waste and cassava by-products. *Niger. J. Anim. Prod.* 2020, 40, 123–132.

34. Págan, R.S.; Rodríguez, C.A.; Valencia, C.E.M.; Randel, P.F. Pineapple and Citrus Silage as Potential Feed for Small Ruminant Diets: Fermentation Characteristics, Intake, Nutrient Digestibility, and Aerobic Stability. Rev. Colomb. *De Cienc. Pecu*. 2014, 27, 37–46.

35. Palmonari, A.; Cavallini, D.; Sniffen, C.J.; Fernandes, L.; Holder, P.; Fusaro, I.; Giammarco, M.; Formigoni, A.; Mammi, L.M.E. *In Vitro* Evaluation of Sugar Digestibility in Molasses. *Ital. J. Anim. Sci.* 2021, 20, 571–577.

36. Pinotti, L.; Luciano, A.; Ottoboni, M.; Manoni, M.; Ferrari, L.; Marchis, D.; Tretola, M. Recycling Food Leftovers in Feed as Opportunity to Increase the Sustainability of Livestock Production. *J. Clean. Prod*. 2021, 294, 126290.

37. Piquer, O.; Ródenas, L.C.; Casado Blas, E.; Pascual, J.J. Whole Citrus Fruits as an Alternative to Wheat Grain or Citrus Pulp in Sheep Diet: Effect on the Evolution of Ruminal Parameters. *Small Rumin. Res*. 2009, 83, 14–21.

38. Plascencia, A.; Barreras, A.; Valdés-García, Y.S.; Zinn, R.A. Granulated Cane Sugar as a Partial Replacement for Steam-Flaked Corn in Diets for Feedlot Cattle: Ruminal Fermentation and Microbial Protein Synthesis. *Fermentation* 2022, 8, 555.

39. Prado, I.N.; Lallo, F.H.; Zeoula, L.M.; Caldas Neto, S.F.; Nascimento, W.G.; Marques, J.A. Bulls Performance in Feedlot with Levels of Substituting Corn Silage by Pineapple By-Products Silage. *Rev. Brasil. Zootec*. 2003, 32, 737–744.

40. Raseel, K.; Chacko, B.; Shyama, K.; Sunanda, C.; Gangadevi, P.; Abraham, J. Evaluation of Pineapple Waste Based TMR on Performance of Crossbred Dairy Cows in Early Lactation. *Anim. Nutr. Feed Technol*. 2020, 20, 155–163.

41. Raseel, K.; Chacko, B.; Sunanda, C.; Dildeep, V.; Abraham, J. Nutrient Evaluation of Energy Rich Unconventional Feeds Available in Wayanad. *IJSN* 2018, 9, 117–118.

42. Roda, A.; Lambri, M. Food Uses of Pineapple Waste and By-Products: A Review. *Int. J. Food Sci. Technol.* 2019, 54, 1009–1017.

43. Rogério, M.C.P.; Borges, I.; Neiva, J.N.M.; Rodriguez, N.M.; Pimentel, J.C.M.; Martins, G.A.; Ribeiro, T.P.; Costa, J.B.; Santos, S.F.; Carvalho, F.C. Nutritive Value of Pineapple By-Product (*Ananas Comosus L.*) in Diets for Sheep: Intake, Apparent Digestibility, Energetic and Nitrogenous Balance. *Arq. Brasil. Med. Vet. Zootec*. 2007, 59, 773–781.

44. Siti Roha, A.M.; Zainal, S.; Noriham, A.; Nadzirah, K.Z. Determination of Sugar Content in PineappleWaste Variety N36. *Int. Food Res. J.* 2013, 20, 1941–1943.

45. Sodjinou, M.K.B.; Assouma-Imorou, A.; Olounlade, A.O. Technical Efficiency of Pineapple Production and Challenges in Southern Benin. *Afr. J. Agric. Res*. 2022, 18, 522–534.

46. Sruamisri, S. Agricultural Wastes as Dairy Feed in Chiang Mai. *Anim. Sci. J.* 2007, 78, 335-341.

47. Suksathit, S.;Wachirapakorn, C.; Opatpatanakit, Y. Effects of Levels of Ensiled PineappleWaste and Pangola Hay Fed as Roughage Sources on Feed Intake, Nutrient Digestibility and Ruminal Fermentation of Southern Thai Native Cattle. *Songklanakarin J. Sci.Technol.* 2011, 33, 281–289.

48. Upadhyay, A.; Lama, J.P.; Tawata, S. Utilization of PineappleWaste: A Review. *J. Food Sci. Technol. Nepal.* 2010, 6, 10–18.

49. Usman, M.; Nichol, J.E. Changes in Agricultural and Grazing Land, and Insights for Mitigating Farmer-Herder Conflict in West Africa. *Landsc. Urban Plan*. 2022, 222, 104–383.

50. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for Dietary Fiber, Neutral Detergent Fiber, and Non-Starch Polysaccharides in Relation to Animal Nutrition. *J. Dairy Sci*. 1991, 74, 3583–3597.

51. Vastolo, A.; Calabrò, S.; Carotenuto, D.; Cutrignelli, M.I.; Kiatti, D.d.; Tafuri, S.; Ciani, F. Maca (Lepidium Meyenii): *In Vitro* Evaluation of Rumen Fermentation and Oxidative Stress. *Fermentation* 2023, 9, 568.

52. Vastolo, A.; Calabrò, S.; Cutrignelli, M.I. A Review on the Use of Agro-Industrial CO-Products in Animals' Diets. *Ital. J. Anim. Sci.* 2022, 21, 577–594.

53. Vastolo, A.; Matera, R.; Serrapica, F.; Cutrignelli, M.I.; Neglia, G.; Kiatti, D.D.; Calabrò, S. Improvement of Rumen Fermentation Efficiency Using Different Energy Sources: *In Vitro* Comparison between Buffalo and Cow. *Fermentation* 2022, 8, 351.

54. Vieira, I.M.M.; Santos, B.L.P.; Santos, C.V.M.; Ruzene, D.S.; Silva, D.P. Valorization of PineappleWaste: A Review on How the Fruit's Potential Can Reduce Residue Generation. *Bioenergy Res.* 2022, 15, 924–934.

Chapter 4.

Pineapple By-Products In The West African Dwarf Lamb Diet Improve Palatability, Nutrient Intake And Digestibility

Article under review in *Food Science and Nutrition*:

FSN3-2024-11-3176.

4.1. Abstract

The animal production system in tropic and sub-tropic areas faces the challenge of feeding the animal year-round due to the importance of natural pasture and its scarcity or high lignin content during the dry season. Supplementing animal diets with feed resources like agro-industrial by-products will be a valid alternative. Pineapple (*Ananas comosus* L.) is the most produced fruit in tropical and subtropical areas. It is processed into different products for human consumption, leading to waste being thrown into the environment and causing pollution. The current study aims to evaluate the effect of industrial pineapple by-products (IPBP) substituting conventional feedstuff in the diet of West African Dwarf (WAD) lambs by *in vitro* and *in vivo* experiments to support the farmers and contribute to environmental protection. To achieve this aim, the IPBP were collected from a pineapple processing factory, dried, and included in the WAD diet (Test 1: 14%; Test 2: 28%) to be compared to the control (CTR). The diets were chemically analyzed and tested *in vitro* using the gas production technique. For the *in vivo* experiment, 24 WAD lambs (initial weight: 15.98±1.5 kg; aged: 6-7 months) were allotted to three experimental treatments in a completely randomized design to be fed by CTR, Test 1, and Test 2 diets. The experiment lasted for 64 days, including 14 days of adaptation and following data collection on intake, apparent digestibility, growth performance, and feeding behavior. As *in vitro* results, the inclusion of IPBP improved metabolizable energy (8.32 and 9.13 vs. 7.68 MJ/kg, for Test 1, Test 2, and CTR, respectively), fermentation rate, and branched chain fatty acid production. Organic matter degradability (OMD) varied from 85.1 to 86.7 % and was not significantly affected. Regarding the *in vivo* experiment, IPBP in the WAD lamb diet increased nutrient intake and apparent digestibility (except OM). The OM intake and apparent digestibility varied from 504-597 g/day and 81.6-84.3%, respectively. The animals' growth performances (daily weight gain, DWG) ranged between 61.3 to 75.5 g/day and were slightly higher in the Test 2 group without significant differences. The feeding time and number of ruminating chews per bolus increased while chewing time per ruminated bolus decreased when IPBP was introduced into the WAD lamb diet. These results confirm that IPBP can be included in the WAD sheep diet up to 28 % without detrimental effects.

Keywords: *in vitro* fermentation, *in vivo*, small ruminant, alternative feedstuff, West Africa.

4.2. Introduction

The demand for crop and animal products is increasing due to the world population's continuous growth, which is estimated to reach 9.8 billion and 11.2 billion by 2050 and 2100 respectively (UN, 2017). The land that supports crop cultivation and animal feeding (natural pasture or cultivated grass) is increasingly reduced by human occupation and the advancement of the oceans. In addition, some crops (e.g., maize grain and soybean) are used in animal feeding (Rajtar et al., 2020; Karlsson et al., 2021) to guarantee high performances (meat, milk, and eggs), whereas some crops processed for human consumption, lead to waste production which is continuously thrown in the environment

(Sharma et al., 2021) and contribute to the pollution. It is the case of pineapple (*Ananas comosus* L.), cultivated in tropical and sub-tropical areas, representing the third most consumed fruit in the world after oranges and bananas, which leads to around 18 million tons of waste every year (Kiatti et al., 2023). This waste can play a fundamental role in extensive ruminant production systems in tropical and sub-tropical areas, like the West Africa region, characterized by the long dry season and scarcity of natural pastures in the dry season period. Notwithstanding, the West African Dwarf (WAD) sheep and goat are well adapted to West Africa's adverse pedo-climatic conditions, showing resistance to trypanosomiasis, prolificacy, and suitability for year-round breeding, but the growth performances are still low in efficiency due to the genetic (not subject to improvement) and feeding strategy (reliance to natural pasture). To contribute to finding a solution to this problem, many investigations have been focused on alternative feed resources during the dry season and supplements formulated feed to natural pasture or cultivated guinea grass (*Panicum maximum*) (Ayandiran et al., 2019, Koura et al., 2022). For example, cotton seed meal, palm kernel meal, rice bran, copra meal, and cocoa husk were validated for ruminants' nutrition in West Africa (Amole et al., 2022; Idrissou et al., 2020). Like other agro-industrial by-products (AIBPs), pineapple waste can be utilized in ruminants' nutrition. Using pineapple by-products in ruminant nutrition could alleviate environmental pollution, and human and animal competition for cereals and legumes. However, the effect on animal performances during the dry season still needs to be tested.

Our previous investigation related to chemical characterization and *in vitro* evaluation showed that pineapple by-products (i.e., crown, bud end, peel, core, and pomace) present differences in terms of sugar and structural carbohydrates content, as well as fermentation kinetic compared to the conventional feedstuffs (Kiatti et al., 2023). Siti Roha et al., (2013) revealed that the energy source of pineapple by-products is mainly soluble carbohydrates, such as glucose, fructose, and sucrose, which are easily fermentable in the rumen. However, the pineapple by-products can vary between the processing technique applied (home vs. industrial) and the type of product obtained (juice, jelly, etc.) (Kiatti et al. 2023). The industrial pineapple by-products result from the squeezing of the fruit without the crown part while the bud end, peel, core, pomace, and crown are separately generated in the home processing technique. The experiment conducted by Prado et al. (2003) concluded that pineapple by-product silage can substitute corn silage up to 50 % in bulls' diet. In Portugal, the dehydrated pineapple by-products were added to the small ruminants' diet and contributed to improving energy intake and apparent digestibility of organic matter (OM), cellulose, and acid detergent fiber (ADF), as well as body weight gain (Correia et al., 2006; Rogério et al., 2007).

In West Africa, the *in vivo* with Red Sokoto goat revealed that they opted for conventional concentrate if presented with a different combination of wheat offal and pineapple waste (Asaolu et al., 2016). Other studies on pineapple by-products have considered whether it could be fed to ruminants in combination with other not well-known fruit or vegetable waste (Akinkunmi and Abegunde, 2024; Okoruwa et al., 2013). However, there is still a need to perform the *in vivo* experiment with pineapple by-products in combination with the well-known feedstuff to understand better its effect on intake and nutrient digestibility, animal performances, and their feeding behavior. Our objective was to evaluate the effect of industrial pineapple by-products (IPBP) inclusion in the diet of West African Dwarf (WAD, Djallonke) lambs through *in vitro* and *in vivo* studies.

4.3. Material and Methods

Three diets (CTR, Test 1, and Test 2) for West African Dwarf (WAD) lambs were formulated with industrial pineapple by-products (IPBP) inclusion at different levels (0 %, 14 %, and 28 %; respectively). The IPBP were collected from a local juice processing factory and dried before being used as feedstuff. The diets were analyzed for chemical composition and tested *in vitro*, using the gas production technique (experiment 1), and administered to 24 male WAD lambs to evaluate intake, apparent digestibility, growth performance, and feeding behavior (experiment 2).

4.3.1. Experiment 1. In vitro fermentation

The three diets were previously tested *in vitro* using the gas production technique (Koura et al., 2022) by incubating 1.00 ± 0.0027g (3 replications) in 120 mL capacity serum bottles. The bottles were incubated at 39°C under anaerobic conditions containing buffer solution (74 mL), reducing agent (4 mL), and three mixed sheep rumens fluids (10 mL). The rumen fluid was collected from three slaughtered adult sheep (*Merinizzata italiana* breed fed on natural pasture) respecting EU legislation (EC Council Regulation 882/2004) and transported to the laboratory in a pre-warmed thermos. The rumen fluid was treated following the method previously described by Kiatti et al. (2024). To ensure the fermentation process and correct the errors due to the solutions, three bottles were incubated without diet (blanks). During the incubation (120 h), the volume and pressure of the gas produced in the bottle were recorded (21 times) using a manual pressure transducer (Cole and Palmer Instrument Co., Illinois, USA) to determine the cumulative volume of gas related to incubated organic matter (OMCV, mL/g). At the end of incubation, the pH of each bottle was measured with a pH-meter (ThermoOrion 720 A+, Fort Collins, CO, USA), and the supernatants were sampled for volatile fatty acid determination, whereas the remaining in the bottles were filtered in their corresponding pre-weighed crucibles (porosity #2). The crucibles were dried (103°C, 24 h) and burned (550°C, 3 h) to determine the organic matter degradability (OMD, %). In addition, the cumulative volume of gas related to the degraded organic matter was calculated as Yield (mL/g). The volatile fatty acids (acetate, propionate, butyrate, iso butyrate, valerate, and iso-valerate) were determined by gas chromatography (ThermoQuest 8000top Italia SpA, Rodano, Milan, Italy) using an external standard solution composed of pure acetic, propionic, butyric, iso-butyric, valeric, and iso-valeric acids (Vastolo et al., 2022).The total volatile fatty acids (tVFA, Mmol/g iOM), acetate to propionate ratio, and branched-chain fatty acid proportion (BCFA), as [(iso-butyric acid + iso-valeric acid) / tVFA*100], were also calculated.

4.3.2. Experiment 2. In vivo trial

4.3.2.1. Study area

The *in vivo* experiment was carried out in a livestock farm located in Betecoucou (department of Collines, municipality of Dassa-Zoume, Republic of Benin), situated between 2°20’ and 2°28’ East longitude and 7°45’ and 7°52’ North latitude. The Dassa-Zoume is in the Sudano-Guinean area and is characterized by two rainy seasons (largest: March to July, lowest: October to November) and two dry seasons (lowest: August to September, largest: December to March) with total rainfall varying between 685 to 1702 mm and annual temperature mean of 27.5°C (Idrissou et al., 2015).

4.3.2.2. Ingredients and diets

The diets (CTR, Test 1, and Test 2) were formulated to be similar in terms of crude protein and neutral detergent fiber meeting the animals’ requirement with 100 g predicted daily weight gain (DWG; NRC; 2007; Umutoni et al., 2021). The animals were fed at 4.5 % of body weight (Kearl, 1982). In the diets, the amount of ingredients such as maize bran, rice bran, cotton seed meal, and palm kernel meal were fixed. Panicum C1 hay and maize, the most used forage and concentrate in sheep feeding plan, were progressively reduced from CTR diet to experimental diets and replaced by IPBP in two concentrations (14 and 28 % in Test 1 and Test 2, respectively). The percentage of ingredients and the chemical composition of diets are reported in Table 4. 1.

4.3.2.3. Experimental design

A total of 24 male West African Dwarf (WAD, Djallonke) lambs aged 6 to 7 months with 15.98 ± 1.5 kg of initial live weight means were allotted to three experimental treatments in a completely randomized design to evaluate the feed intake, apparent digestibility, growth performance, and feeding behavior.

Twenty-one days before the feeding trial, the animals received Albendazole® 500 (10 kg/bolus) against gastro-intestinal parasites and Stress Vitam® by intra-muscular injection against stress. The experiment lasted after 64 days including 14 days of adaptation to the new diet. At the beginning of the experiment, the animals were weighed individually using an electronic balance (50 kg ± 10g), and three groups of eight animals were constituted to be fed on different diets (CTR, Test 1, and Test 2). The animals were individually housed in 1.20 x 1.20 m boxes equipped with two fixed bowls each for feeding and watering.

The daily diet quantities were divided into two and served twice a day, administered at 08:00 a.m. and 02:00 p.m. The daily feed amounts were adjusted weekly according to weight gain, whereas water was provided *ad libitum* in the stalls. After 14 days of adaptation, the daily diets and refusals were weighed using an electronic scale of 10 kg ± 1.0 g. The refusals were then pooled per animal per week, and 500 g was sampled. The samples collected each week per animal were mixed at the end of the experiment and then 250 g were taken for laboratory analysis.

4.3.2.4. Determination of digestibility

Three animals per group were selected, and their total feces were collected during the last five days using the collection panties. The adaptation for panties wearing was observed seven days before feces collection. During the collection, the panties were emptied in the morning and in the evening and weighed. The daily fresh feces per animal were mixed and 20 % was taken and directly dried at 65°C. The feces of five consecutive days were also pooled per animal and 250 g were sampled for laboratory analysis.

4.3.2.5. Feeding behavior

The animals' behaviors were observed after 30 days for a period of 24 h (11:00 p.m. to 11:00 p.m. of the next day) per group of animals (Bateson and Martin, 1993) and ended for three consecutive days (one day per group of animals). The observations were made by three people who served the diets to the animals and were previously trained to observe the animals' behavior. The animals were under fluorescent light during the night to facilitate the observations. The technique consists of observing the animals every 5 minutes and noting their behavior, such as feeding, watering, resting, and ruminating. The absence of mandibular movement was considered as resting (Costa et al., 2013). In addition, the number and the time of chewing per ruminated bolus and total ruminated boli per day were recorded using a digital stopwatch (Bürger et al., 2000).

4.3.3. Chemical analysis

The samples of experimental diets (CTR, Test 1, and Test 2), refusals, and feces samples were analyzed for dry matter (DM, ID: 934.01, 103°C), crude protein (CP, ID: 2001.11), ether extract (EE, ID: 920.39), and ash (ID: 942.05) following the official methods (AOAC, 2005). The fiber content (neutral detergent fiber, NDF_{om}; acid detergent fiber, ADF_{om}; acid detergent lignin, ADL) were also determined (Van Soest et al., 1991) in all samples using an Ankom[220] Analyzer unit (Ankom Technology Corporation, Fairport, NY, USA) and burning the bags at 550°C to correct the fiber content as ash free.

4.3.4. Data processing

Metabolizable energy (ME, MJ/kg) was estimated using the equation proposed by Menke and Steingass (1988):

$$ME = 2.2 + 0.1357GP + 0.0057CP + 0.0002859CP^2 \qquad \text{(Eqn. 1)}$$

where CP is the crude protein content (g/kg DM) and GP the gas produced *in vitro* after 24 hours of fermentation (mL/200 mg). The *in vitro* fermentation kinetics were performed per bottle and reported as average for each experimental diet using Groots' model (Groot et al., 1996).

$$G = \frac{A}{(1+\left(\frac{B}{t}\right)^{C})} \qquad \text{(Eqn. 2)}$$

where G is the total gas produced at the end of incubation time (ml/g of incubated OM) at time t (h), A is the asymptotic gas production (ml/g), B is the time at which 1/2 of A is reached (h), and C is the curve switch. The fermentation pattern regarding the maximum fermentation rate (R_{max}, mL/h) and the time it occurred (T_{max}, h) was calculated as (Bauer et al., 2001):

$$R_{max} = \frac{A*B^{C}*C*T_{max}^{(-C-1)}}{(1+B^{C}*T_{max}^{-C})^2} \qquad \text{(Eqn. 3)}$$

$$T_{max} = B * (\frac{C-1}{C+1})^{\frac{1}{C}} \quad \text{(Eqn. 4)}$$

Diets, refusal, and feces chemical analysis were used to calculate the apparent digestibility as: Apparent digestibility (%) = [(Nutrient in the diet – Nutrient in feces)/Nutrient in diet] x 100. Regarding the growth performances, the animals were weighed every week using the scale (50 kg ± 10 g) then the daily weight gain (DWG) was calculated as:

$$DWG = \frac{LW(n+1) - LWn}{7} \quad \text{(Eqn. 5)}$$

where LW_n is the live weight of the previous week and $LW_{(n+1)}$ the live weight of the current week. The feeding efficiency (FE, g DM intake/h) and feed conversion ratio (FCR) were calculated: FE = DMI/FT and FCR = DMI/DWG, where DMI is the dry matter intake (g/day), FT the feeding time (h/day) and DWG is the daily weight gain (g/day).

4.3.5. Statistical analysis

The data collected from *in vivo* experiments on intake, digestibility, and animal growth performance were applied to the Shapiro-Wilk test to validate the normal distribution. After this, all the data, including *in vitro* parameters, were submitted for analysis of variance with all pairs' comparisons using Tukey SHD. The following model was used:

$$Yi = \mu + Di + \varepsilon i \quad \text{(Eqn. 6)}$$

where Y is experimental data, μ is the general mean, D is the diet effect (i = 1, 2, 3), and ε is the error term. All the statistical analyses were made using JMP software (Version 14 SW, SAS Institute Inc., Cary, NC, USA, 1989-2019), and a significant level was fixed at 5 %. Correlation between chemical composition, *in vitro,* and *in vivo* data were also made using JMP® software (Version 14 SW, SAS Institute Inc., Cary, NC, USA, 1989–2019).

4.4. Results

4.4.1. Chemical composition of the diets

The chemical composition of the diets is reported in Table 4. 1. The metabolizable energy (ME, MJ/kg) estimated based on crude protein and the gas produced after 24 h *in vitro* fermentation showed a little increase with industrial pineapple by-product (IPBP) inclusion (7.68 vs. 8.32; 9.13 MJ/kg for CTR, Test 1 and Test 2; respectively). In addition, the inclusion of IPBP in the sheep diet tends to reduce acid detergent fiber (ADF) and acid detergent lignin (ADL).

Table 4. 1. Ingredients (%) and chemical composition of the diets

Diets ingredients	**Treatment**		
	CTR	**Test 1**	**Test 2**
Panicum C1 hay	40	30	19
IPBP	0	14	28
Maize grain	24	20	17
Maize bran	5.0	5.0	5.0
Rice bran	18	18	18
Cotton seed meal	9.0	9.0	9.0
Palm kernel meal	3.0	3.0	3.0
Commercial mineral	1.0	1.0	1.0
Chemical composition			
DM, %	89.04	89.85	90.02
CP, % DM	8.86	8.84	9.04
Ash, % DM	9.84	9.25	9.63
EE, % DM	3.26	3.19	3.11
NDF_{om}, % DM	43.93	43.03	42.96
ADF_{om}, % DM	30.14	28.04	27.02
ADL, % DM	13.39	12.36	11.00
ME, MJ/kg	7.68	8.32	9.13

CTR: control diet; Test 1: experimental diet with 14 % of IPBP; Test 2: experimental diet with 28 % of IPBP; IPBP: industrial pineapple by-products; DM: dry matter; CP: crude protein; OM: organic matter; EE: ether extract; NDF_{om}: ash free neutral detergent fiber; ADF_{om}: ash free acid detergent fiber; ADL: acid detergent lignin; ME: metabolizable energy estimated by Menke and Steingass (1988) method.

4.4.2. *In vitro fermentation*

The *in vitro* fermentation characteristics of experimented diets are presented in Table 4. 2. The Test 2 diet showed a higher significant cumulative volume of gas related to incubated OM (OMCV) and maximum fermentation rate (R_{max}), it also reached the R_{max} earlier ($p<0.05$) in terms of time (T_{max}), compared to the CTR and Test 1 diets. The same trend was observed for the gas production profile (Figure 4. 1.) and fermentation rate (Figure 4. 2.) over the time of incubation (120 h). However, pH, organic matter degradability (OMD), Yield, potential gas production (A), and the time to reach A/2 (B) were not significantly different ($p>0.05$) among the diets.

Table 4. 2. *In vitro* fermentation characteristic of experimental diets

Items	CTR	Test 1	Test 2	P_value	MSE
pH	6.51	6.48	6.47	0.577	0.002
OMD, %	85.1	85.6	86.7	0.288	2.210
OMCV, mL/g	249^{C}	256^{B}	267^{A}	0.002	1.643
Yield, mL/g	423	421	431	0.658	131.4
A, mL/g	304	297	312	0.161	275.9
B, h	33.5	23.2	23.1	0.094	11.39
T_{max}, h	7.57^{A}	8.24^{A}	3.65^{B}	0.036	1.011
R_{max}, mL/h	5.79^{B}	6.82^{AB}	9.00^{A}	0.022	0.303

CTR: control diet, Test 1: experimental diet with 14 % of IPBP, Test 2: experimental diet with 28 % of IPBP, OMD: organic matter degradability; OMCV: cumulative volume of gas related to incubated OM; Yield: cumulative volume of gas related to degraded OM; A: potential gas production; B: time at which A/2 was reached; R_{max}: maximum fermentation rate; Tmax: time at which maximum rate was reached. MSE: mean square error. Along the row, for each diet, letters indicate statistically significant differences ($p < 0.05$).

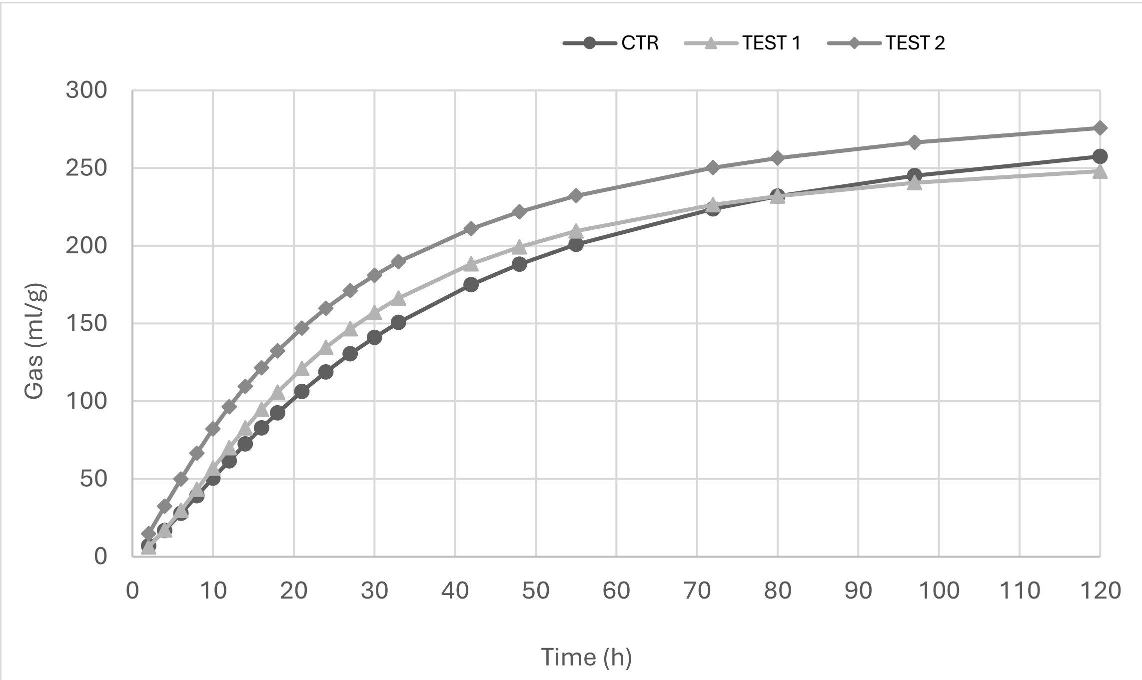

Figure 4. 1. *In vitro* gas production profile over time (120 h) of experimental diets.

CTR: control diet; Test 1: experimental diet with 14 % IPBP; Test 2: experimental diet with 28 % IPBP; IPBP: industrial pineapple by-products.

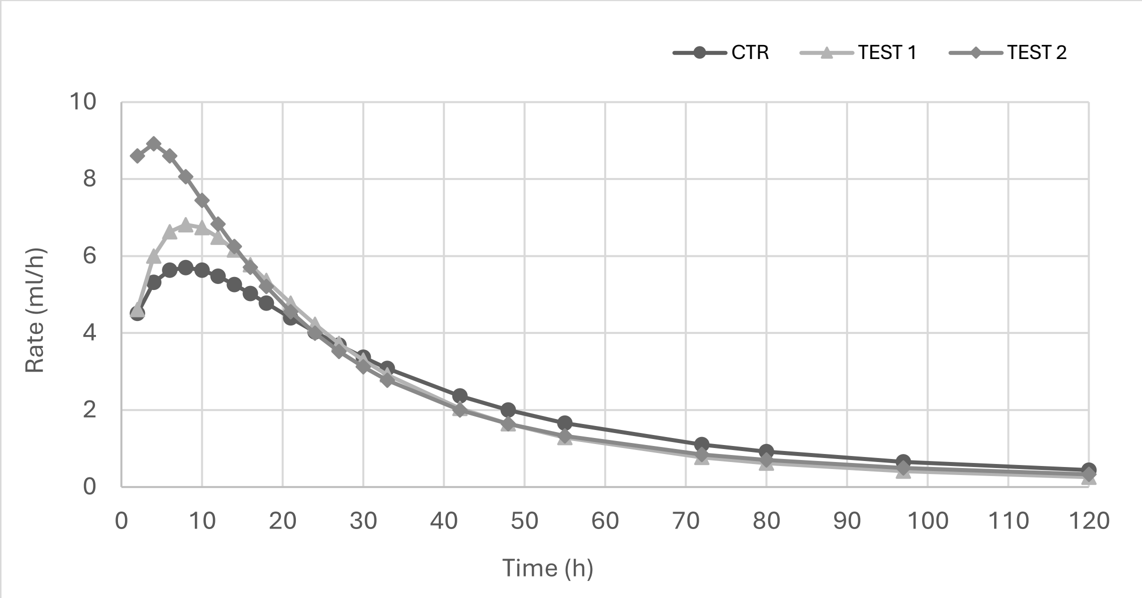

Figure 4. 2. *In vitro* fermentation rate over time (120 h) of experimental diets.

CTR: control diet; Test 1: experimental diet with 14 % IPBP; Test 2: experimental diet with 28 % IPBP; IPBP: industrial pineapple by-products.

In vitro fermentation, and end-products of experimental diets are reported in Table 4. 3. All the parameters determined were significantly different except the iso-butyrate, valerate, and acetate to propionate ratio. The CTR diet showed the highest significant total volatile fatty acid (tVFA: 52.72 Mmol/g iOM) compared to Test 1 (46.4 Mmol/g iOM) and Test 2 (46.3 Mmol/g iOM) diets. The individual volatile fatty acids, reported as % tVFA, showed significantly ($p<0.05$) higher acetate and propionate for the CTR diet, whereas significantly ($p<0.05$) higher butyrate, iso-valerate, and branched chain fatty acid were observed for Test 1 and Test 2 diets.

Table 4. 3. *In vitro* fermentation end-products of experimental diets

Items	CTR	Test 1	Test 2	P_value	MSE
tVFA, Mmol/g iOM	52.7^{A}	46.4^{B}	46.3^{B}	0.002	0.856
Ace, % tVFA	59.3^{A}	55.9^{B}	56.9^{B}	0.004	0.591
Pro, % tVFA	24.5^{A}	23.7^{B}	23.3^{B}	0.001	0.044
Iso-But, % tVFA	1.53	2.05	1.81	0.052	0.035
But, % tVFA	9.74^{B}	12.0^{A}	11.8^{A}	0.008	0.385
Iso-Val, % tVFA	2.89^{B}	4.20^{A}	4.19^{A}	0.005	0.047
Val, % tVFA	1.95	2.13	1.97	0.287	0.018
BCFA	0.08^{B}	0.13^{A}	0.13^{A}	0.003	0.006
Ace/Pro	2.42	2.36	2.44	0.182	0.002

CTR: control diet; Test 1: experimental diet with 14 % of IPBP; Test 2: experimental diet with 28 % of IPBP; IPBP: industrial pineapple by-products; tVFA: total volatile fatty acids; Ace: acetate; Pro: propionate; Iso-But: iso-butyrate; But: butyrate; Iso-Val: iso-valerate; Val: valerate; BCFA: branched-chain fatty acid proportion; Ace/Pro: acetate to propionate ratio. MSE: mean square error. Along the row, for each diet, letters indicate statistically significant differences ($p< 0.05$).

4.4.3. In vivo experiment

The *in vivo* parameters, i.e., intake and apparent digestibility of the tested diets and growth performances of West African Dwarf (WAD) lambs, are presented in Table 4. 4. The nutrient intake (DM, OM, CP, NDF_{om}) was significantly different (p<0.05) among diets, except for DM intake per body weight (BW), showing always the highest values in the group of animals fed with Test 2 diets followed by Test 1 and CTR diets. The DM and CP intake varied from 559 to 660 g and 49.5 to 63.0 g/kg, respectively. In addition, the same significant trend of feed intake was observed for the apparent digestibility, only the CTR and Test 1 diets for CP digestibility were similar. The apparent digestibility of OM, CP, and NDF were 84.3, 81.1, and 75.8 % respectively for the Test 2 diet. The diets did not significantly affect the WAD growth performances, even if the highest values were always recorded in WAD fed with the Test 2 diet. However, the average daily weight gain (DWG) was 61.3, 69.6, and 75.5 g/day, with 3.25, 3.69, and 4.00 kg as total weight gain during the experiment for CTR, Test 1, and Test 2 diets, respectively.

Table 4. 4. *In vivo* performance of West African Dwarf lambs

Items	CTR	Test 1	Test 2	P_value	MSE
	Nutrients' intake				
DM, g/kg BW	30.5	30.3	32.7	0.285	8.076
DM, g	559^{C}	600^{B}	660^{A}	0.001	8.774
OM, g	504^{C}	544^{B}	597^{A}	<0.001	0.180
CP, g	49.5^{C}	53.0^{B}	63.0^{A}	0.005	0.429
NDF_{om}, g	246^{B}	258^{B}	284^{A}	0.003	9.711
	Apparent digestibility				
OM, %	84.3^{A}	81.6^{B}	84.3^{A}	0.001	1.224
CP, %	75.4^{B}	75.9^{B}	81.1^{A}	<0.001	2.770
NDF_{om}, %	61.1^{C}	72.9^{B}	75.8^{A}	<0.001	2.085
	Growth performance				
Initial weight, kg	15.9	15.9	16.1	0.983	2.460
Final weight, kg	18.5	19.9	20.3	0.277	3.394
Total gain, kg	3.25	3.69	4.00	0.101	0.254
DWG, g/day	61.3	69.6	75.5	0.101	9.039

CTR: control diet; Test 1: experimental diet with 14 % of IPBP; Test 2: experimental diet with 28 % of IPBP; IPBP: industrial pineapple by-products; BW: body weight; NDF_{om}: ash free neutral detergent fiber; DM: dry matter; CP: crude protein; OM: organic matter; NDF_{om}: ash free neutral detergent fiber; BW: body weight; DWG: daily weight gain. MSE: mean square error. Along the row, for each diet, letters indicate statistically significant differences (p< 0.05).

The feeding behavior and chewing activities of WAD lambs fed with control and an experimental diet containing IPBP were reported in Table 4. 5. Significant differences were observed for feeding time with the highest being in Test 2 (3.58 h/day, p = 0.008). In addition, the number of ruminated boli per day was not affected but IPBP inclusion decreased and increased significantly chewing time per ruminated bolus and number of ruminating chews per bolus, respectively. The feeding behavior showed that the animals spent more time resting (12.6 to 14.0 h/day) compared to the time reserved for feeding and watering in both groups of animals. However, the ruminating time was about two times higher and two times lower than the feeding and resting time, respectively, for all groups. However, the diet containing 28 % of IPBP (Test 2) showed higher feeding efficiency (FE: 0.230 g/h, $p<0.001$) without effect on the feed conversion ratio ($p = 0.062$).

Table 4. 5. Feeding behavior and chewing activities in West African Dwarf lambs

Items	CTR	Test 1	Test 2	P_value	MSE
Feeding time, h/day	2.71^{B}	3.14^{B}	3.58^{A}	0.008	0.019
Watering time, h/day	0.63	0.83	0.91	0.093	0.030
Resting time, h/day	13.7	12.6	13.5	0.080	0.341
Ruminating time, h/day	6.67	6.58	5.85	0.312	0.561
No. ruminating chews/bolus	45.8^{C}	53.7^{A}	49.3^{B}	<0.001	3.831
Chewing time/ruminated bolus, s	43.5^{A}	41.1^{AB}	37.9^{B}	0.005	3.869
No. ruminated *boli*/day	507	492	480	0.253	61.753
FE g/h	0.167^{C}	0.206^{A}	0.230^{A}	<0.001	0.001
FCR	9.11	8.61	8.75	0.062	2.514

CTR: control diet; Test 1: experimental diet with 14 % of IPBP; Test 2: experimental diet with 28 % of IPBP; IPBP: industrial pineapple by-products; FE (DM intake/FT): feeding efficiency. FCR (DM intake/DWG): feed conversion ratio; MSE: mean square error. Along the row, letters indicate statistically significant differences ($p< 0.05$).

The correlation between chemical composition and some *in vitro* and *in vivo* parameters of WAD lambs diet containing pineapple by-products is reported in Table 4. 6. Regarding the *in vitro* parameters, ADF_{om} and ADL were negatively correlated with fermentation rate (R_{max}) and estimated ME, whereas they were positively correlated with tVFA and propionate production. The structural carbohydrates (NDF_{om}, ADF_{om}, and ADL) and EE tend to reduce *in vitro* OM degradability without significant differences. Moreover, significant correlations were observed between diet chemical composition and the parameters registered *in vivo*, where structural carbohydrates and EE reduce nutrient digestibility (CP, NDF, and OM), DM intake, and feeding time. The same parameters were positively correlated with ruminating time and bolus chewing time. The opposite trend was observed for CP content in the diets with the *in vivo* experiment parameters.

Table 4. 6. Diets chemical composition correlation with *in vitro* and in vivo parameters

Items	EE	Ash	CP	NDF_{om}	ADF_{om}	ADL
			Chemical composition vs. *in vitro* parameters (n = 6)			
OMD	-0.711	-0.201	0.542	-0.732	-0.777	-0.803
R_{max}	-0.025	-0.186	0.883*	-0.458	-0.847*	-0.894*
ME	-0.211	-0.299	0.823*	-0.556	-0.937**	-0.964**
tVFA	0.405	0.681	-0.486	0.811	0.956**	0.839*
Ace	-0.007	0.885*	-0.108	0.563	0.660	0.433
Pro	0.478	0.345	-0.764	0.850*	0.964**	0.947**
Iso-But	-0.461	-0.706	0.328	-0.753	-0.861*	-0.709
But	0.051	-0.892*	0.185	-0.584	-0.716	-0.505
Iso-Val	-0.262	-0.728	0.457	-0.829*	-0.901*	-0.738
Val	-0.026	-0.328	-0.319	0.070	0.027	0.184
BCFA	-0.362	-0.712	0.449	-0.830*	-0.922**	-0.772
			Chemical composition vs. *in vivo* parameters (n = 347)			
DM intake	-0.317*	-0.117*	0.543***	-0.390***	-0.576***	-0.588***
CP dig	-0.108*	0.055	0.573***	-0.270***	-0.501***	-0.553***
NDF dig	-0.172**	-0.376***	0.566***	-0.559***	-0.746***	-0.703***
OM dig	-0.211***	-0.354***	0.752***	-0.659***	-0.905***	-0.878***
FT	-0.096	-0.936***	0.071	-0.568***	-0.599***	-0.406***
Rum time	0.214***	-0.049	-0.975***	0.555***	0.879***	0.951***
chew time	0.223***	0.195***	-0.920***	0.660***	0.967***	0.985***

EE: ether extract; CP: crude protein; NDF_{om}: neutral detergent fiber; ADF_{om}: acid detergent fiber; ADL: acid detergent lignin; OMD: organic matter degradability; R_{max}: maximum fermentation rate; ME: estimated metabolizable energy; tVFA: total volatile fatty acid; Ace: acetate; Pro: propionate, Iso-But: iso-butyrate, But: butyrate, Iso-Val: iso-valerate, Val: valerate, BCFA: branched chain fatty acid; FT: feeding time; Rum time: ruminating time; Shew time: ruminated bolus chewing time. *, **, ***: p<0.05, 0.01, 0.001, respectively.

4.5.Discussion

4.5.1. In vitro fermentation

The first step of the study, including the *in vitro* experiment, suggested that the inclusion of IPBP in the diet reduces the structural carbohydrate specifically ADF and ADL, in both experimental diets whereas the metabolizable energy (ME), estimated with *in vitro* parameters, improved. However, the degradability of *in vitro* organic matter (OMD) did not vary significantly. Still, the IPBP increases the fermentation rate, gas production profiles, and branched chain fatty acid (BCFA) production during the incubation process. According to other authors, the presence of easy fermentable energy sources mainly represented by glucose, fructose, and sucrose in IPBP may justify these findings (Siti Roha et al., 2013). The values of *in vitro* OMD obtained in this study were higher than the interval of (73 – 75%) reported for cattle, sheep, and goats (Idayanti et al., 2021), but similar to those found in an analogous study on pineapple core and pomace which are the parts of pineapple richer in sugars (Kiatti et al., 2023). Similarly, the addition of a mix of sugars and acids in a total mixed ratio incubated with bovine and buffalo rumen revealed low *in vitro* OMD and high total volatile fatty acid production (Vastolo et al., 2022). The inclusion of IPBP and easily fermentable ingredients in ruminants' diets had similar results in improving gas production and fermentation rate. The same outcome was observed using molasse in the ruminants' diet (Palmonari et al., 2021). As sugar plus or molasse, industrial pineapple by-products can be preserved and used in ruminant nutrition to improve fermentation and feed palatability. However, the rumen liquor donors and chemical composition of the diet can significantly affect the degradability and the *in vitro* end product production as shown in the correlation in Table 4. 6. In West Africa, *Panicum maximum* and *Pennisetum purpureum* are considered resilient grass and are widely used in ruminant farming in the pasture or preserved as hay. The *in vitro* fermentation characteristics of these forages showed low OMD (49-66 %), estimated ME (4 - 9 MJ/kg DM), and fermentation kinetics parameters (Koura et al., 2022; Babayemi, 2007). The combination of *Panicum maximum* or *Pennisetum purpureum* hay with IPBP could improve the nutritive value of the diet, reflecting better performances in the animals and economic benefit for the farmers. Moreover, the diets containing IPBP in this study resulted in higher BCFA production compared to the control diet. The IPBP may contain some secondary compounds that could protect the protein of the diet from rumen microorganism attack. This action is beneficial for the absorption of proteins in the gut of the digestive tract. Indeed, some authors mentioned the presence of polyphenol compounds in pineapple by-products and their benefits in ruminants' nutrition (Lubaina et al., 2020; Sukri et al., 2023).

4.5.2. In vivo experiment

The second part of this study evaluated the *in vivo* effect of IPBP substituting conventional feedstuff in the diet of West African Dwarf (WAD) lambs. The results suggest that nutrient intake and apparent digestibility improved with the inclusion of both levels of IPBP (14 % and 28 %) in the WAD lamb diet, even if no significant effect on growth performance was observed.

Moreover, the control groups' animals spent less feeding time, with a higher chewing time per ruminated bolus while the nutrient intake increased in the group of animals fed with the Test 1 and Test 2 diets containing pineapple by-products. This result might be due to the decreasing of ADF and ADL, and the palatability improvement of tested diet by pineapple by-products. The OM, CP, and NDF_{om} intakes were related to the DM intake. All the DM intake values we recorded are higher than those reported in WAD lambs fed with cotton seed cake replacement soybean pulp in the diet (Idrissou et al., 2020). The feed intake in WAD goats was increased by wheat offal-carried pineapple waste inclusion in the diet (Ayandiran et al., 2019) but it was always low compared to the intake we observed for WAD lambs. Sowande et al. (2007) found similar DM intake with the same breed of sheep when the protein content of experimental diets was improved with ground nut cake and palm kernel cake addition. The intake of the diet containing agro-industrial by-products can vary according to their origin and the amount included. The animal species, breed, and physiological stage must be considered to optimize diet ingestion. In this study, the apparent nutrient digestibility (CP and NDF_{om}) was improved by including 28 % IPBP in the WAD lamb diet, even if the apparent OM digestibility was similar in the CTR and Test 2 diets. These findings are in line with the data obtained *in vitro* and with the literature (Suksathit et al., 2011). The same authors also observed the improvement of DM, OM, and ADF digestibility when pineapple by-products were added to ruminants' diets. In addition, no adverse effects were reported on nutrient utilization (DM, CP, NDF, and ADF) when sheep were fed with pineapple by-product silage (Cordeiro et al., 2022). Okoruwa et al. (2013) found that a combination of pineapple by-products and orange pulp can be administered to WAD sheep, improving rumen indices (pH, temperature, VFA, and NH_3-N), nutrient digestibility, and growth performance.

Despite the differences observed in feed intake and apparent digestibility, the growth performances were not significantly affected, even though the values for the animals fed IPBP were slightly improved. The DWG of both groups varied from 61.3 - 75.5 g/day. The formulation of the Test 2 diet reduces by around 30 % the amount of maize grain used in the CTR diet, with similar growth performance in both groups. The utilization of agro-industrial by-products as alternative feed sources will reduce cereals input in animal nutrition and minimize feeding costs without lowering productivity (Amole et al., 2021). In our study, a higher DWG was recorded compared to other research using preserved cassava root meal and pineapple waste (1:4) or dried cassava peel and cassava root meal (4:1) (Oduguwa et al., 2013). Ouedraogo et al. (2022) observed 78.0, 61.0, and 56.0 g of DWG in growing sheep supplemented with flour of *Mucuna deeringiana*, pods of *Piliostigma thonningii* and cotton cake, respectively, which were not so far to our findings. Moreover, the growth of 65.0 to 102 g/day published as West African sheep performances was in line with the result of the current study (Idrissou et al., 2020; Umutoni et al., 2021).

Observing feeding behavior is a crucial issue when researching a new ingredient in animal feed because it helps to understand the acceptability of the new ingredient. Industrial pineapple by-product inclusion in the diet incites the animals to spend more feeding time when it is introduced at 14 or 28 % level. Feeding, resting, and ruminating times observed in the animals fed with IPBP were similar to the feeding behavior previously reported in the lambs fed with diets containing different sources of fat (Alba et al., 2021). The higher feeding time (5 h) and lower resting time (10 h) were found during an *in vivo* experiment using plant extracts as phytogenic additive in Brazilian sheep (Da Silva et al., 2017). In addition, the number of ruminating chews per bolus was similar to other reports, while the number of ruminated boli per day was higher (Costa et al., 2013). Both cited authors found that ruminants spend more time resting and ruminating than feeding. Regarding the FCR parameter, expressing the quantity of diet that the animal needs to produce a unit of meat, was lower for diets containing IPBP compared to the control, indicating how more efficiently animals converted diets' nutrients into meat. The clear effect on the intake and the slight growth of the animals fed with IPBP compared to the control approve the interesting aspect of this by-product. However, the by-product characteristics (nutrient content, origin, and processing technique), the animal species and the breed can considerably influence the feeding behavior and performance of the animals.

4.6. Conclusion

The industrial pineapple by-products inclusion as a substitution to the conventional feedstuffs (i.e., maize grain, panicum hay) in small ruminants' nutrition improves the *in vitro* fermentation rate, estimated metabolizable energy, and branched chain fatty acid production. Tested *in vivo*, on West African Dwarf lambs, the pineapple by-products improve the diet palatability, increasing nutrient intake and apparent digestibility without significantly affecting the growth performance. The diets containing pineapple by-products were efficient and reduced around 30 % of maize grain utilization compared to the control. Due to the palatability, pineapple by-products increase feeding time and the number of ruminating chews per bolus while the chewing time per ruminated bolus decreases. The evidence from this study suggests that pineapple by-products can be included in up to 28 % of WAD sheep diet. The current study was limited by not analyzing the meat quality of sheep fed IPBP. This aspect, which could further encourage the use of pineapple by-products, should be investigated in future studies.

4.7. Acknowledgments

Thanks to AGRITECH National Research Center the European Union Next-Generation (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 June 17, 2022, CN00000022) for financial support. The manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

The authors also thank the International Agreement for Co-Operation between the University of Naples, Federico II (Department of Veterinary Medicine and Animal Production), Italy and the National University of Agriculture (School of Animal Production and Health), Republic of Benin. DR 2632 del 27/6/22 (Resp. Prof. Serena Calabrò).

4.8. Reference

1. Akinkunmi, A.O.; Abegunde, T.O. Organic Acids And Antinutritional Contents Of Cabbage Leaves Waste And Pineapple Peels Silage Fed To West African Dwarf (WAD) Goats. *Nigerian J. Anim. Prod.* 2024, 1654-1658.
2. Alba, H.D.R.; Freitas Júnior, J.E.d.; Leite, L.C.; Azevêdo, J.A.G.; Santos, S.A.; Pina, D.S.; Cirne, L.G.A.; Rodrigues, C.S.; Silva, W.P.; Lima, V.G.O.; Tosto, M.S.L.; De Carvalho, G.G.P. Protected or Unprotected Fat Addition for Feedlot Lambs: Feeding Behavior, Carcass Traits, and Meat Quality. *Animals* 2021, 11, 328. https://doi.org/10.3390/ani11020328.
3. Amole, T.; Augustine, A.; Balehegn, M.; Adesogoan, A. T. Livestock feed resources in the West African Sahel. *Agron. J.* 2022, 114(1), 26-45.

4. AOAC. Official Methods of Analysis, 12 Vol, 18th ed.; Association of Official Analytical Chemists: Arlington, VA, USA, 2005.
5. Asaolu, V.O.; Binuomote, R.T.; Oyelami, O.S. Assessment of feeding value of vegetable-carried pineapple fruit wastes to Red Sokoto goats in Ogbomoso, Oyo State of Nigeria. *AJB.* 2016, 15(31), 1648-1660.
6. Ayandiran, S.K.; Odeyinka, S.M.; Oyebanji, B.O. Hematological and Biochemical Parameters of West African Dwarf (WAD) Goats Fed Wheat Offal- Carried Pineapple Waste (WCPW). *Int J Anim Sci.* 2019, 3(2), 1046s.
7. Babayemi, O.J. *In vitro* fermentation characteristics and acceptability by West African dwarf goats of some dry season forages. *AJB.* 2007, 6(10).
8. Bateson, M.; and Martin P. Measuring behavior: an introductory guide; *Cambridge University Press*, 2021.
9. Bauer, E.; Williams, B.A.; Voigt, C.; Mosenthin, R.; Verstegen, M.W.A. Microbial activities of faeces from unweaned and adult pigs, in relation to selected fermentable carbohydrates. *J. Anim. Sci.* 2001, 73, 313–332.
10. Bürger, P.J.; Pereira, J.C.; Queiroz, A.C.D.; Coelho da Silva, J.F.; Valadares Filho, S.D. C.; Cecon, P.R.; Casali, A.D.P. Ingestive behavior in Holstein calves fed diets with different concentrate levels. *Rev. Bras. Zootec* 2000, 29, 236-242.
11. Cordeiro, C.D.C.; Fernandes, A.M.; Oliveira, T.S.D.; Camilo, M.G.; Baffa, D.F.; Glória, L.S.; Bernardo, S.E.E. Intake, total apparent digestibility, and microbial efficiency of sheep fed pineapple waste silage in different planes of nutrition. *Rev. Bras. Zootec.* 2022, 51.
12. Correia, M.X.D.C.; Costa, R.G.; Silva, J.H.V.D.; Carvalho, F.F.R.D.; Medeiros, A.N.D. Use of dehydrated pineapple by-product in diets for growing goats: digestibility and performance. *Rev. Bras. Zootec.* 2006, 35, 1822-1828.
13. Costa, R.G.; Treviño, I.H.; Medeiros, G.R.D.; Medeiros, A.N.D.; Gonzaga Neto, S.; Azevedo, P.S.D.; Pinto, T.F. Feeding behavior and performance of sheep fed cactus pear in substitution of corn. *Rev. Bras. Zootec.* 2013, 42, 785-791.
14. Da Silva, C.S.; de Souza, E.J.O.; Pereira, G.F.C.; Cavalcante, E.O.; de Lima, E.I.M.; Torres, T.R.; ... da Silva, D. C. Plant extracts as phytogenic additives considering intake, digestibility, and feeding behavior of sheep. *Trop. Anim. Health Prod. 2017*, 49, 353-359.
15. EC Council. Regulation 882/2004 on Official controls performed to ensure verification of compliance with feed and food law, animal health and animal; EC Council: UE, 2004.
16. Groot, J.C.; Cone, J.W.; Williams, B.A.; Debersaques, F.M.; Lantinga, E.A. Multiphasic analysis of gas production kinetics for *in vitro* fermentation of ruminant feedstuff. *Anim. Feed Sci. Technol.* 1996, 64, 77–89, https://doi.org/10.1016/S0377-8401(96)01012-7.

17. Idayanti, R.W.; Arifin, M.; Purbowati, E.; Purnomoadi, A. Utilization of PineappleWaste as a Roughage Source Diets for Ruminant: A Review. *Adv. Biol. Sci. Res*. 2022, 20, 123–130.

18. Idrissou, Y. Performance d'embouche des ovins Djallonké complémentés avec les fourrages de Gliricidia sepium et de Leucaena leucocephala au Centre du Bénin. Mémoire de Master, FA Université de Parakou, Bénin; 2015.

19. Idrissou, Y.; Worogo, H.S.S.; Assani, A.S.; Ayena, J.A.; Assogba, B.G.C.; Traoré, I.A. Cottonseed cake replacement by soybean pulp in the diet of West African Dwarf lambs in Benin: zootechnical and economic performances. Rev. Elev. Med. Vet. Pays Trop. 2020, 73(2), 107-111.

20. Karlsson, J.O.; Parodi, A.; van Zanten, H.H.; Hansson, P.A.; Röös, E. Halting European Union soybean feed imports favors ruminants over pigs and poultry. *Nat Food* 2021, **2**, 38–46 https://doi.org/10.1038/s43016-020-00203-7

21. Kiatti, D.d.; Vastolo, A.; Koura, B.I.; Vitaglione, P.; Cutrignelli, M.I.; Calabrò, S. The Chemical Characteristics and *In Vitro* Degradability of Pineapple By-Products as Potential Feed for Ruminants. Animals 2023, 13, 3238.

22. Kiatti, D.d; Koura, B.I.; Vastolo, A.; Chiacchio, M.F.; Vitaglione, P.; Dossa, L.H.; Cutrignelli, M.I.; Calabrò, S. Sustainable ruminant nutrition in West Africa by *in vitro* characterization of cashew apple by-products. *Heliyon* 2024, 10(18).

23. Koura, B.I.; Vastolo, A.; Kiatti, D.d.; Cutrignelli, M.I.; Houinato, M.; Calabrò, S. Nutritional Value of Climate-Resilient Forage Species Sustaining Peri-Urban Dairy Cow Production in the Coastal Grasslands of Benin (West Africa). *Animals* 2022, 12, 3550.

24. Lubaina, A.S.; Renjith, P.R.; Roshni, A.S.; Thompson, J. Identification and quantification of polyphenols from pineapple peel by high performance liquid chromatography analysis. *Adv. Zool. Bot* 2020, 8, 431-438.

25. Menke, K.H.; Steingass, H. Estimation of the Energetic Feed Value Obtained from Chemical Analysis and *in Vitro* Gas Production Using Rumen Fluid. *Anim. Res. Dev*. 1988, 28, 7–55.

26. NRC (National Research Council). Nutrient requirements of sheep; Washington (DC): National Academy Press, 1985. p.99.

27. Okoruwa, M.I.; Obiku, A.; Agbonlahor, I. Rumen indices and performance response of West African dwarf (WAD) sheep as influenced by orange with pineapple pulps. *Int. J. Agrisci. 2013, 3(11), 862-870.*

28. Ouedraogo, S.; Sanou, L.; Savadogo, P.; Kabore-Zoungrana, C.Y. Comparative nutritional evaluation of the two leguminous fodder trees Prosopis africana and Piliostigma thonningii: effects of different levels of pod-based supplementation on the growth performance of Djallonke sheep. *Int. J. Biol. Chem. Sci.* 2022, 16(5), 1831-1846.

29. Palmonari, A.; Cavallini, D.; Sniffen, C.J.; Fernandes, L.; Holder, P.; Fusaro, I.; Giammarco, M.; Formigoni, A. Mammi, L.M.E. *In vitro* evaluation of sugar digestibility in molasses. *Ital. J. Anim. Sci.* 2021, 20, 571–577.

30. Prado, I.N.D.; Lallo, F.H.; Zeoula, L.M.; Caldas Neto, S.F.; Nascimento, W.G.D.; Marques, J.D.A. Bulls performance in feedlot with levels of substituting corn silage by pineapple by-products silage. *Rev. Bras. Zootec.* 2003, 32, 737-744.

31. Rajtar, P.; Górka, P.; Śliwiński, B.; Wieczorek, J.; Boros, D.; Micek, P. Effect of rye grain derived from different cultivars or maize grain use in the diet on ruminal fermentation parameters and nutrient digestibility in sheep. Ann. Anim. Sci. 2020, DOI: 10.2478/aoas-2020-0118.

32. Rogério, M.C.P.; Borges, I.; Neiva, J.N.M.; Rodriguez, N.M.; Pimentel, J.C.M.; Martins, G.A.; Carvalho, F.C. Nutritive value of pineapple by-product (*Ananas comosus L.*) in diets for sheep. 1. Intake, apparent digestibility, energetic and nitrogenous balance. *Arq. Bras. Med. Vet. Zootec.* 2007, 59, 773-781.

33. Sharma, P.; Gaur, V. K.; Sirohi, R.; Varjani, S.; Kim, S. H.; Wong, J. W. Sustainable processing of food waste for production of bio-based products for circular bioeconomy. *Bioresour. Technol.* 2021*,* 325, 124684.

34. Siti Roha, A.M.; Zainal, S.; Noriham, A.; Nadzirah, K.Z. Determination of Sugar Content in PineappleWaste Variety N36. *Int. Food Res. J.* 2013, 20, 1941–1943.

35. Sowande, O.S.; Olayode, B.A.A.; Egbeyale, L.T. Effect of diet quality on feeding time and performance of West African dwarf sheep. *Arch. Zootec.* 2007, 56(216), 943-946.

36. Sukri, S.A.M.; Andu, Y.; Sarijan, S.; Khalid, H.N.M.; Kari, Z.A.; Harun, H.C.; ... Van Doan, H. Pineapple waste in animal feed: A review of nutritional potential, impact and prospects. *Ann. Anim. Sci.* 2023, 23(2), 339-352.

37. Suksathit, S.; Wachirapakorn, C.; Opatpatanakit, Y. Effects of levels of ensiled pineapple waste and pangola hay fed as roughage sources on feed intake, nutrient digestibility and ruminal fermentation of Southern Thai native cattle. *Songklanakarin J. Sci. Technol* 2011, 33, 281−289.

38. Umutoni, C.; Bado, V.; Whitbread, A.; Ayantunde, A.; Gangashetty, P. Evaluation of chemical composition and *in vitro* digestibility of stovers of different pearl millet varieties and their effect on the performance of sheep in the West African Sahel. Acta Agriculturae Scandinavica. *Section A-Anim. Sci.* 2021, 70(2), 91-99.

39. UN (United Nation). World Population Prospects, United Nations: New York, NY, USA, 2019.

40. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for Dietary Fiber, Neutral Detergent Fiber, and Non-Starch Polysaccharides in Relation to Animal Nutrition. *J. Dairy Sci*. 1991, 74, 3583–3597.

41. Vastolo, A.; Matera, R.; Serrapica, F.; Cutrignelli, M.I.; Neglia, G.; Kiatti, D.d.; Calabrò, S. Improvement of Rumen Fermentation Efficiency Using Different Energy Sources: *In Vitro* Comparison Between Buffalo and Cow. *Fermentation* 2022, 8, 351. https://doi.org/10.3390/ fermentation8080351.

Chapter 5.

Potential Use Of Tropical And Subtropical Fruit By-Products In Pig Diet: *In Vitro* Two-Step Evaluation

Article under review in *Anim. Feed Sci. Technol.*

ANIFEE-D-24-01210

5.1. Abstract

Pineapple (*Ananas comosus* L.), cashew (*Anacardium occidentale* L.), and mango (*Mangifera indica* L.) are among the main cultivated plants in tropical and subtropical regions due to the high demand for their products around the world. From harvesting and processing, the fruits of these plants generate a huge amount of waste, generally thrown into the environment causing pollution. At the same time, farmers in these areas face scarcity, feed costs, and competition between humans and animals. The current study, in this context, aims to propose alternative feeds for pigs by characterization of cashew, pineapple, and mango fruit by-products through an *in vitro* two-step (simulating gastro-intestinal and caecum digestion) study to provide feeds not competing with humans and promoting eco-sustainable livestock. To achieve this aim, No. 10 by-products [i.e., pineapple peel and pomace; cashew nut testa, cashew (var. yellow) whole fruit and pomace; cashew (var. red) whole fruit and pomace; mango peel, kernel, and testa] were sampled in Benin. Corn meal, soybean meal, and beet pulp were also considered as referenced feed. The samples were analyzed for proximate chemical composition and *in vitro* two-step digestion method (enzymatic + microbial digestibility). The result reports a low dry matter (DM) content specifically in the pomace, peel, and whole apple (13.0 - 27.2 %) while higher lipids were observed for cashew nut testa (26.4 % DM) and mango kernel (11.2 % DM). The by-products investigated fall within the interval of referenced feeds for structural carbohydrates (NDF: 7.6 - 47.1 % DM) and protein (7.65 - 51.2 % DM) except mango by-products with low protein content (2.51 - 4.69 % DM). The total dry matter digestibility, short chain fatty acid, and gas production were low for cashew by-products stopped after 48 h of incubation while the same parameters for other by-products stopped at 72 h were not so far compared to the referenced feeds. Specifically, the results suggest that pineapple pomace, cashew whole apple, pomace, and testa can be considered as feedstuff in fattening pigs, presenting characteristics partly similar to beet pulp. To feed pigs, mango peels and kernels should be combined with a protein feed source, such as soybean meal. However, in the future it will be necessary to investigate the issue of by-product conservation, such as pelleting, to carry out *in vivo* studies and propose using cashew, pineapple, and mango by-products in pig diets.

Keywords: mango, cashew, pineapple, gas production, enzymatic digestion, monogastric nutrition.

5.2. Introduction

According to the Food and Agriculture Organization of the United Nations, approximately 1.3 billion tons of food are wasted worldwide every year from harvesting to processing, passing by fresh consumption. Fruits, vegetables, roots, and tubers account for around 80 % of total yearly waste (Porter et al., 2016; Omre et al., 2018) of total yearly waste due to their perishability. In tropical and subtropical regions, pineapple, cashew, and mango cultivation contribute significantly to waste generation (Kiatti et al., 2023 and 2024).

Pineapple (*Ananas comosus* L.) is native to tropical and subtropical areas (Asia, South and North America, Africa, and Oceania), with Cayenne, Sugarloaf, Spanish, Queen, Pernambuco, and Perolera the main cultivated varieties (Agbangba, 2016; Vieira et al., 2022). In 2021, global pineapple production was estimated at 28,179,348 tons. Costa Rica, Philippines, and Brazil are the main contributors, whereas in West Africa, Nigeria, and Benin are ranked 8^{th} and 17^{th}, with 1,671,440 and 386,906 tons produced, respectively (Sodjinou et al., 2022). Considering the amount of globally produced pineapple, more than 18 million tons of waste are generated each year. Generally, because of the short shelf-life high moisture and sugar content.

Cashew tree (*Anacardium occidentale* L.), native to tropical South America, has also spread to other tropical and subtropical areas (Asia, Africa, and Central America) due to the demand for its products, mainly nuts (Das and Arora, 2017). It includes about 60 genera and 400 species and two principal varieties (red and yellow) in accordance with peduncle (apple or false fruit) color. The nut is the true fruit and does not present a difference between variety and representing the first interest product of cashew plantations (Kiatti et al., 2024). Nut represents 10 % of total fruit while the apple represents 90 % and it is less processed due to its perishability and astringent taste (Das and Arora, 2017). The world cashew nut production was estimated at 5,535,510 tones with around one-third coming from West African regions (FAOSTAT, 2022). The cashew apples processed generate the pomace whereas the nuts generate shell and testa (Da Silva et al., 2023).

Mango (*Mangifera indica* L.) is one of the most important tropical fruits in the world and currently ranked 5^{th} among the major fruit crops due to its succulence, exotic flavor, delicious taste, and the nutrients it contains (Muchiri et al., 2012; Ravani and Joshi, 2013). Mango production is continuously increasing (from $35.7x10^6$ tons in 2009 to $55.9x10^6$ tons in 2019) with African countries accounting for about 40 %, mainly supported by the West African region (Ajila et al., 2013; Timbilfou et al., 2023). However, mangos are consumed freshly (80 %) or processed (20 %) into puree, nectar, leather, pickles, canned slices, and chutney generating 50-65 % of fruits as waste composed of seed (endocarp: kernel + testa + shell) and peel (Ravani and Joshi, 2013; Sanon et al., 2013).

In general, fruit by-products, e.g. cashews, pineapple, and mango, are thrown into the environment, contributing to global warming, water pollution, and human health problems. These mentioned problems will be reduced by recycling waste in other fields creating a circular economy. In West Africa conditions, the promising field should be in the animal production sector, which faces the challenges of feeding the animals according to their requirement due to the high feeding cost (60-85 %) and cereals competition (Lawrence et al., 2008; Kiatti et al., 2023). The selection of the most appropriate raw materials and the formulation of a feeding plan are two factors that can influence the efficiency indicators in livestock farms (Pinotti et al., 2019). Strategies and solutions, such as a “food recovery hierarchy”, are needed to reduce feed production’s environmental impact and use of natural resources by increasing their reuse (Mourad, 2016). Moreover, many factors are limiting the use of unconventional feed, such as limited knowledge and high variability of their chemical composition, high polyphenols, and moisture content (Ravindran and Blair, 1992; Vastolo et al., 2022). The previous study, even limited, reported that cashew, pineapple, and mango by-products are useful as alternative feedstuff in West African livestock for small ruminants (Kiatti et al., 2023 and 2024), growing and finishing pigs (Kiendrebeogo et al., 2019), laying hens (Barry et al., 2019) and dairy cows (Milogo et al., 2022). However, there is still a need to provide more knowledge of these by-products for their wide adoption in animal nutrition. In this context, the present research aims to contribute to the characterization of tropical and subtropical fruit by-products (cashew, pineapple, and mango) in pig nutrition through an *in vitro* two-step (gastro-intestinal and caecum) study to provide feeds not competing with humans and promoting eco-sustainable livestock.

5.3. Material and Methods

5.3.1. Experimental design

The study was performed in 2024 collecting No. 10 by-products derived from pineapple processing (No. 2), cashew (No. 5), and mango (No. 3). Different areas of Benin were chosen for sampling based on their greatest suitability for growing the fruits. All the samples were oven-dried at 65°C and milled at 1.1 mm screen to be analyzed for chemical composition and *in vitro* two-step evaluation using caecal content from growing pigs. In addition, three feeds commonly used in the pig diet [corn meal (CM), soybean meal (SBM), and beet pulp (BP)] were also selected as reference feeds, for their already known nutritional suitability in pigs.

5.3.2. Samples collection

Pineapple samples (var. Smooth Cayenne) were collected in Atlantic district, located in the sub-humid zone of Benin (6°18′- 6°58′ N and 1°56′ - 2°30′ E) and characterized by two rainy seasons and two dry seasons (average annual rainfall: 1200 mm; temperature ranging from 27 to 31 °C; relative humidity ranging from 69 to 97 %) (INSAE, 2004). A total of 40 fruits were collected randomly from three different farms. Following local traditions, the fruits were cleaned by removing the crowns and buds using a steel knife. Then, the peel and core were separated from the pulp, which was pressed using a hydraulic press to remove the juice and retain the pomace. Two samples were selected from pineapple waste generated: pineapple peel (P_Peel) and pomace (P_Pom).

Cashew apple samples (var. yellow and red) were collected in the municipality of Parakou in North-East Benin (8.55' - 10.53' N and 2°00' - 3°50' E), in the Sudanian Zone, characterized by one dry and one rainy season (annual rainfall ranging from 900 to 1000 mm; mean relative temperature and humidity 27.5°C and 54.9 %, respectively) (Gnanglè et al., 2011). Cashew apples were directly collected after nuts were harvested, and the variety (red and yellow) was separated. The apples were processed to juice using a hydraulic press machine (Tianyu Youdo Machine, model UDZL-W33, dimensions 330 x 330 x 600 mm, Kaifeng, China) generating pomace as waste. In addition to the pomace, the whole apples per variety were also collected due to their availability as well in the cashew plantation as nuts harvesting waste. So, four samples from cashew apples: cashew yellow whole fruit (CY_WF), cashew yellow pomace (CY_Pom), cashew red whole fruit (CR_WF) and cashew red pomace (CR_Pom). Cashew nut testa (CNT), waste from nuts processing was sampled at the factory and added to the cashew apple sample for the study.

Mango samples were collected in the municipality of Natitingou in the North-Ouest of Benin (10°18′ N and 1°22′ E), in the Sudanian Zone characterized by one dry and one rainy season (annual rainfall ranging from 900 to 1000 mm) (Gnanglè et al., 2011). About 50 kg of fresh fruits were collected from five different mango plantations and processed by peeling and moving the endocarp. The wastes generated from mango fruit processing were peel, kernel, taste, and husk. The mango peel (M_Peel), kernel (M_Ker), and testa (M_Te) were sampled for the study.

5.3.3. Chemical composition

The samples were analyzed according to the official procedures of the Association of Chemists (AOAC, 2005) to determine dry matter (DM, ID 934.01), ether extract (EE, ID 920.39), crude protein (CP, ID 2001.11) and ash (ID 942.05). The structural carbohydrate fractions, such as neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were also determined (Van Soest et al., 1994) and expressed excluding the residual ash.

5.3.4. In vitro two-step digestibility evaluation

To determine the digestibility in monogastric animals, a two-step *in vitro* method was used, where the first phase (Step 1) simulates the digestive processes occurring in the stomach and small intestine by pepsin and pancreatin enzymes action (Boisen and Fernandez, 1997) and the second phase (Step 2) reproduce the intestinal microbial activity happening in the large intestine by symbiotic bacteria (Musco et al., 2017).

Step 1: approximately 2 ± 0.0024 g of each substrate was weighed, in 4 replicates, into 300 ml flasks; a blank was included in each series; 50 ml of phosphate buffer (0.1 M, pH 6.0) and 20 ml of HCl (0.2 M) were added to each flask, keeping the flasks under a continuous agitation, after having adjusted the pH of each flask to 2.0 using 1 M HCl and 1 M NaOH. Finally, 2.0 ml of a freshly prepared solution containing 10 mg of pepsin (Sigma, P7000-100g) was added. The flasks were closed with rubber stoppers and then incubated under continuous agitation for 2 h in a thermostatic bath at 39°C (Incubator SI60; Stuart Scientific, Stone, Staffordshire, UK). At the end of the incubation, the flasks were cooled, and 20 ml of phosphate buffer (0.2 m, pH 6.8) and 10 ml of 0.6 M NaOH buffer solution were added. The pH was again adjusted to 6.8 with a solution of 1M HCl and 1M NaOH; then, 2 ml of a freshly prepared pancreatin solution containing 200 mg of powdered pancreatin (Sigma, P1225-100g) was added to each flask. The flask content was mixed and placed in a thermostatic bath to be incubated at 39°C for 4 h with continuous shaking. The undigested residue was collected in pre-weighed Whatman 934-AH filters. The undigested substrates filtered were dried overnight at 65 °C and weighed. The substrates were collected for the *in vitro* fermentation (Step 2).

Step 2: the residual material obtained in Step 1 was incubated with swine caecal content. For this purpose, approximately 0.5 ± 0.0026 g of Step 1 predigested material of each sample was weighed, in three replications, into 120 ml serum bottles. Samples of caecum content were collected at slaughter in an EU-authorized slaughterhouse (EC Council, 2004) from four 12-month-old, 100 kg fattening pigs (Landrace x Large White crosses) fed commercial diet (CP: 14.8 %; gross energy: 15.0 MJ/kg). The material collected in heated thermoses was rapidly transported to the laboratory, where it was pooled, filtered through a double layer of gauze, diluted (1:6) in NaCl solution, and added to each bottle (5.0 ml) already containing the medium (79 ml) under continuous flow of CO_2. The bottles were incubated at 39 °C. Depending on the chemical composition, the incubation was stopped at different times: *i)* at 48 hours: CNT, CY_WF CY_Pom, CR_WF, CR_Pom, and CM; *ii)* at 72 hours: P_Peel, P_Pom, M_Peel, M_Ker, M_Te, SBM, and BP. During the incubation, gas pressure and volume were manually measured at 3-24 h intervals using a pressure transducer (Cole and Parmer Instrument Co., Vernon Hills, IL, USA). Dry matter digestibility (DMD) was determined by filtering the contents of the bottles through glass crucibles (Scott Duran porosity #2) and drying them for 24 h at 103°C. The fermentation liquid was collected from each bottle to determine the pH using pH-meter (model 3030 Alessandrini Instrument glass electrode, Jenway, Dunmow, UK) and the production of short-chain fatty acids (SCFA: acetic, propionic, butyric, iso-butyric, iso-valeric, and valeric) using gas chromatograph (Thermo Fisher Scientific, Rodano, MI, Italy; model 1310 trace).

5.3.5. Data processing and statistical analysis

Non-structural carbohydrates (NSC, % DM, 103°C) were calculated as follows:

$$NSC = DM - NDF - CP - EE - ash \qquad \text{(Eqn. 1)}$$

Total dry matter digestibility (DMDt, %) was calculated based on the data obtained in Step 1 (DMDe, enzymatic digestibility, %) and Step 2 (DMDm, microbial digestibility, %) as follows:

$$DMDt = [(100 - DMDe) \times (DMDm / 100)] + (DMDe) \qquad \text{(Eqn. 2)}$$

Gas and short-chain fatty acid production (at 48 and 72 h) were plotted against grams of incubated organic matter (OMCV, ml/g and SCFA, mmol/g, respectively). The proportion of branched-chain fatty acids (BCFA) was calculated as follows:

$$BCFA = [(\text{iso-valeric} + \text{iso-butyric})/SCFA] \qquad \text{(Eqn. 3)}$$

The software JMP® (Version 14 SW, SAS Institute Inc., Cary, NC, USA, 1989–2019) was utilized to analyze the data. Digestibility data and *in vitro* fermentation data separately for the two incubation times, were analyzed using Student's t-test to test the effect of the Feedstuff as a single factor, according to the model:

$$y_{ij} = \mu + F_i + \varepsilon_{ij} \qquad \text{(Eqn. 4)}$$

where y is the tested parameters, μ the general mean, F feedstuffs factor (i = 1-10), and ε is the error term.

5.4. Results

5.4.1. Chemical composition

The chemical composition of the by-products is reported in Table 5. 1. The average dry matter content – excluding CNT, CM, and SBM (91.4, 89.3, and 86.9 %, respectively) – was low (24.8 ± 13.7 %). The ash content among the by-products was slightly higher only in P_Peel (5.95 % DM), even if it was lower than SBM (7.77 % DM). As regards the fractions of structural carbohydrates, a group of by-products with a high content of cell wall (NDF higher than 25 % DM) was identifiable (P_Peel, CY_Pom, CR_Pom, M_Te, BP), where the lignin content was highly variable (ADL range: from 1.83 to 11.56 % DM). In all the other feedstuffs NDF values fall between 10 and 20 % DM, but the ADL content was in some cases rather high; as expected, only CM appears particularly poor in all fiber components (NDF, ADF, ADL: 7.58, 2.83, 2.40 % DM, respectively). On the other hand, the average values of non-structural carbohydrates (NSC), excluding SBM (14.6 % DM), were high (54.3 ± 13.1 % DM). Regarding lipids, only CNT, followed by M_Ker, showed high content of EE (26.4 and 11.2 % DM, respectively), and excluding the above mentioned, they vary between 0.27 and 4.73 % DM. The protein content, as known, was very high in SBM (51.2 % DM), was fair in pineapple and cashew by-products (average value: 8.55 ± 1.60 % DM), but very low in mango by-products (M_Peel, M_Ker and M_Te: 2.51, 4.69 and 3.97 % DM, respectively).

5.4.2. In vitro digestibility

Table 5. 2 shows the percentage values of *in vitro* DM digestibility, reported as enzymatic, microbial, and total. In both incubation times considered, 48 and 72 hours, the values of the three parameters differ significantly ($p < 0.001$) between substrates and as expected, overall highest in the longer incubation time. Among those stopped at 48 hours, total digestibility was, as expected, significantly higher in corn meal and lower in cashew testa (88.3 and 28.5 %, CM and CNT, respectively; $p < 0.001$); the same trend was observed in enzymatic and microbial digestibility. It was also observed that in some by-products (CY_WF, CR_WF, CR_Pom) the enzymatic digestibility was more consistently higher than the microbial one, while in others (CNT, CY_Pom, CM) the difference was minimal. Among the samples stopped at 72 hours, total digestibility is particularly high in SBM (94.2 %; $p < 0.001$) and significantly lower in M_Te (33.6 %, $p < 0.001$); the same trend was observed in enzymatic and microbial digestibility. In addition, in some feeds (P_Peel, P_Pom) the enzymatic digestibility was similar to the microbial one, while in most of these (M_Peel, M_Ker, M_Te, SBM, and BP) the enzymatic digestibility is lower than the microbial one.

Table 5. 1. Chemical composition of cashew, pineapple and mango by-products

Items	DM	Ash	NDF	ADF	ADL	EE	CP	NSC
	%				*% DM*			
P_Peel	14.5	5.95	35.0	17.2	1.83	0.47	7.77	44.3
P_Pom	17.6	3.91	20.3	9.82	1.70	0.27	9.18	50.2
CNT	91.4	2.25	22.5	17.6	6.79	26.4	11.5	35.6
CY_WF	13.0	3.00	16.1	15.9	7.67	2.19	7.54	61.3
CY_Pom	21.6	2.33	33.8	28.5	11.6	4.73	10.2	43.2
CR_WF	13.3	3.54	19.7	18.4	9.69	1.51	6.21	59.6
CR_Pom	27.2	2.16	33.5	31.8	11.5	3.17	7.73	47.6
M_Peel	22.2	4.45	12.4	11.3	1.52	1.46	2.51	75.5
M_Ker	42.1	2.40	10.9	4.18	0.14	11.2	4.69	68.7
M_Te	52.0	3.82	25.3	24.1	8.68	3.66	3.97	59.9
CM	86.7	1.48	7.6	2.83	2.40	1.72	7.65	68.2
SBM	89.3	7.77	14.9	5.07	0.40	0.99	51.2	14.6
BP	24.3	1.50	47.1	23.8	2.70	0.83	9.32	33.0

P_Peel: pineapple peel; P_Pom: pineapple pomace; CNT: cashew nut testa; CY_WF: cashew (var. yellow) whole fruit; CY_Pom: cashew (var. yellow) pomace; CR_WF: cashew (var. red) whole fruit; CR_Pom: cashew (var. red) pomace; M_Peel: mango peel; M_Ker: mango kernel; M_Te: mango testa. CM: corn meal; SBM: soybean meal; BP: beet pulp. DM: dry matter; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre; ADL: Lignin detergent fibre; EE: ether extract; CP: crude protein; NSC: non-structural carbohydrates.

5.4.3. In vitro fermentation characteristics

Table 5. 3 shows the *in vitro* fermentation characteristics obtained after 48 and 72 hours of incubation. In both fermentation times, the values of all the parameters considered are highly and significantly different ($p < 0.001$) between the substrates and overall higher in the longer incubation. The pH values (ranging from 5.60 up to 6.32) for all substrates tested fall in the interval considered physiological in pig cecum-colon (5.2 - 7.8). This indicates that the *in vitro* buffer system had guaranteed an environment suitable for microbial fermentation. At 48 hours, CM (OMCV: 142 ml/g; $p < 0.001$) was the substrate that produced the highest gas, and CNT (OMCV: 27.3 ml/g; $p < 0.001$) was the one that produced the least. The same trend was observed for SCFA where acetate and propionate are the most represented. Butyric acid was the highest in CM (26.0 mmol/g, $p < 0.001$). The branched chain fatty acids (BCFA) were significantly higher ($p < 0.001$) in cashew apple by-products (10.6, 9.66, and 11.4, 6.84, for CY_WF, CY_Pom, CR_WF, and CR_Pom, respectively). After 72 h of fermentation, the substrates that produced the highest gas were P_Pom, M_Ker, and BP ($p < 0.001$) and the one that produced the least was P_Peel ($p < 0.001$). The trend was similar for SCFA even if no statistical significance emerged. Butyric acid produced was significantly higher quantity in M_Ker (25.7 mmol/g; $p < 0.001$). The proportion of BCFA was significantly higher in M_Te and lower in BP (6.23 and 3.29, respectively; $p < 0.00$).

5.4.4. In vitro fermentation kinetics

Figures 5. 1. and 5. 2. represent the gas production profiles of the studied samples incubated for 48 and 72 hours. The by-products incubated for a shorter time, 48 hours, [cashew nut testa, cashew (var. yellow) whole fruit, cashew (var. yellow) pomace, cashew (var. red) whole fruit; cashew (var. red) pomace; corn meal] showed similar and lower curves than corn meal incubated as a reference feed (Figure 5. 1.) indicating that, compared to the most used cereal in pig diets, the fruit by-products incubated are poor of residual nutrients. For the by-product gas profiles, it was also possible to note that the curves were characterized by a biphasic shape, indicating the presence of nutrients that were fermented in two different phases: a faster one, which was exhausted around 24 hours, and a slower one which probably goes beyond 48 hours. The by-products incubated for a longer period (72 hours) (mango peel, kernel and testa, pineapple peel, and pomace) showed intermediate curves compared with soybean meal and beet pulp ones, incubated as reference feeds (Figure 5. 2.) and could be classified into three groups depending on the fermentation speed: *i)* slow fermentation process with low gas production (M_Te); *ii)* medium fermentation rate with high gas production (M_Peel, P_Peels, P_Pom, SBM and BP); *iii)* rapid fermentation speed after the first 12 hours of incubation and high gas production (M_Ker). Even at 72 hours, it was possible to note that all the curves, except that of the M_Ker, showed a biphasic shape due to the presence of both fractions with fast and slow fermentability, even if the timing is less clear in this case.

Table 5. 2. *In vitro* two-step DM digestibility (%) of cashew, pineapple and mango by-products

Items	**DMDe**	**DMDm**	**DMDt**
	Incubation at 48 hours		
CNT	18.8^{E}	12.0^{D}	28.5^{E}
CY_WF	60.0^{B}	24.3^{B}	69.7^{B}
CY_Pom	30.0^{D}	24.7^{B}	47.3^{C}
CR_WF	61.4^{B}	20.4^{C}	69.3^{B}
CR_Pom	38.0^{C}	10.6^{D}	44.6^{D}
CM	67.3^{A}	64.1^{A}	88.3^{A}
p-value	<0.0001	<0.0001	<0.0001
MSE	2.742	0.530	0.837
	Incubation at 72 hours		
P_Peel	50.3^{B}	59.0^{E}	79.6^{D}
P_Pom	68.3^{A}	68.8^{CD}	90.1^{B}
M_Peel	48.4^{B}	70.1^{C}	84.6^{C}
M_Ker	10.0^{C}	67.4^{D}	70.7^{E}
M_Te	11.8^{C}	24.7^{F}	33.6^{F}
SBM	65.2^{A}	83.2^{A}	94.2^{A}
BP	64.0^{A}	78.0^{B}	92.1^{B}
p-value	<0.0001	<0.0001	<0.0001
MSE	8.07	0.907	0.752

P_Peel: pineapple peel; P_Pom: pineapple pomace; CNT: cashew nut testa; CY_WF: cashew (var. yellow) whole fruit; CY_Pom: cashew (var. yellow) pomace; CR_WF: cashew (var. red) whole fruit; CR_P: cashew (var. red) pomace; M_P: mango peel; M_K: mango kernel; M_Te: mango testa; CM: corn meal; SBM: soybean meal; BP: beet pulp. DMDe: enzymatic dry matter digestibility; DMDm: microbial dry matter digestibility; DMDt: total dry matter digestibility. MSE: mean square error. Along the column, different letters indicate statistically significant differences $p < 0.05$.

Table 5. 3. *In vitro* fermentation characteristics after 48 h and 72 h of cashew, pineapple and mango by-products

Items	pH	OMCV	Ace	Pro	Iso-but	But	Iso-val	Val	SCFA	BCFA
		ml/g				*mmol/g*				
					Incubation at 48 hours					
CNT	6.25^{AB}	27.3^{D}	11.6^{D}	3.04^{E}	0.22^{D}	2.55^{B}	0.54^{D}	0.51^{D}	18.5^{D}	4.12^{D}
CY_WF	6.25^{AB}	45.0^{C}	20.4^{B}	7.23^{BC}	1.34^{B}	3.85^{B}	2.50^{A}	1.04^{C}	36.4^{BC}	10.6^{AB}
CY_Pom	6.24^{B}	42.0^{C}	17.4^{C}	6.20^{C}	0.97^{C}	3.40^{B}	2.11^{B}	1.74^{B}	31.7^{BC}	9.66^{B}
CR_WF	6.32^{A}	52.0^{BC}	17.4^{C}	5.34^{D}	1.23^{B}	3.09^{B}	2.25^{B}	1.28^{C}	30.6^{C}	11.4^{A}
CR_Pom	6.20^{B}	58.0^{B}	20.7^{B}	7.54^{B}	0.96^{C}	3.91^{B}	1.54^{C}	1.96^{B}	36.7^{B}	6.84^{C}
CM	5.75^{C}	142^{A}	38.5^{A}	13.1^{A}	1.90^{A}	26.0^{A}	2.47^{A}	5.86A	87.9^{A}	4.98^{D}
p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
MSE	0.003	28.52	2.569	0.387	0.015	1.273	0.05	0.033	11.02	0.467
					Incubation at 72 hours					
P_Peel	5.90^{B}	226^{C}	59.4^{AB}	17.8^{BC}	1.57	15.0^{B}	2.20	2.58	98.5	3.84^{C}
P_Pom	6.06^{A}	285^{A}	64.8^{A}	26.7^{A}	1.73	12.4^{B}	2.34	3.02	111	2.67^{CD}
M_Peel	5.75^{C}	221^{C}	55.1^{B}	20.1^{B}	1.65	9.02^{B}	2.33	2.39	90.6	4.38^{B}
M_Ker	5.70^{CD}	281^{A}	43.7^{CD}	26.6^{A}	1.58	25.7^{A}	2.26	2.13	102	3.73^{C}
M_Te	6.12^{A}	136^{D}	32.0^{D}	12.8^{C}	1.45	12.7^{AB}	2.89	2.99	69.9	6.23^{A}
SBM	5.84^{BC}	253^{B}	49.6^{BC}	28.1^{A}	1.87	13.4^{B}	2.36	3.09	98.4	4.30^{B}
BP	5.61^{D}	276^{A}	60.3^{AB}	24.2^{AB}	1.43	16.8^{AB}	2.10	2.86	108	3.29^{D}
p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.147	<.0001
MSE	0.007	47.37	46.19	22.56	0.100	33.10	0.223	0.143	290	0.061

P_Peel: pineapple peel; P_Pom: pineapple pomace; CNT: cashew nut testa; CY_WF: cashew (var. yellow) whole fruit; CY_Pom: cashew (var. yellow) pomace; CR_WF: cashew (var. red) whole fruit; CR_P: cashew (var. red) pomace; M_P: mango peel; M_K: mango kernel; M_Te: mango testa. CM: corn meal; SBM: soybean meal; BP: beet pulp. OMCV: cumulative volume of gas related to incubated OM; SCFA: short chain fatty acids; Ace: acetate; Pro: propionate; Iso-But: iso-butyrate; But: butyrate; Iso-Val: iso-valerate; Val: valerate; BCFA: branched chain fatty acids. MSE: mean square error. Along the column, different letters indicate statistically significant differences $p < 0.05$.

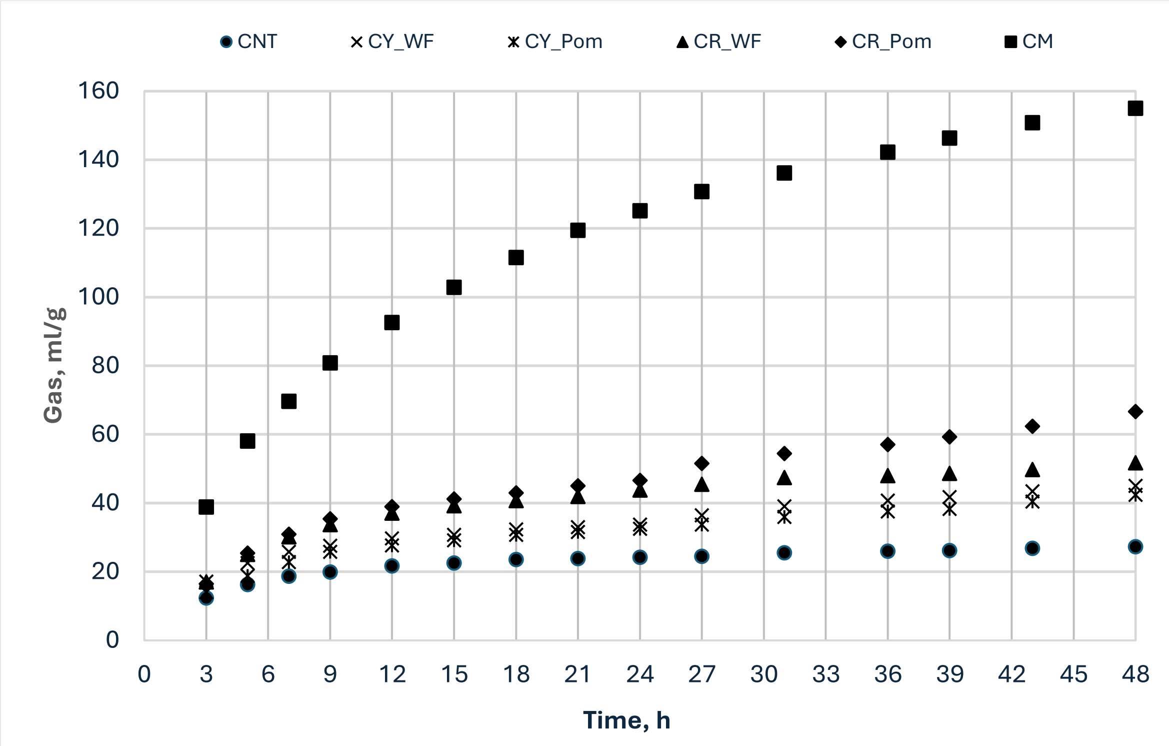

Figure 5. 1. *In vitro* gas production profiles of by-products incubated for 48 hours.

CNT: cashew nut testa; CY_WF: cashew (var. yellow) whole fruit; CY_Pom: cashew (var. yellow) pomace; CR_WF: cashew (var. red) whole fruit; CR_Pom: cashew (var. red) pomace; CM: corn meal.

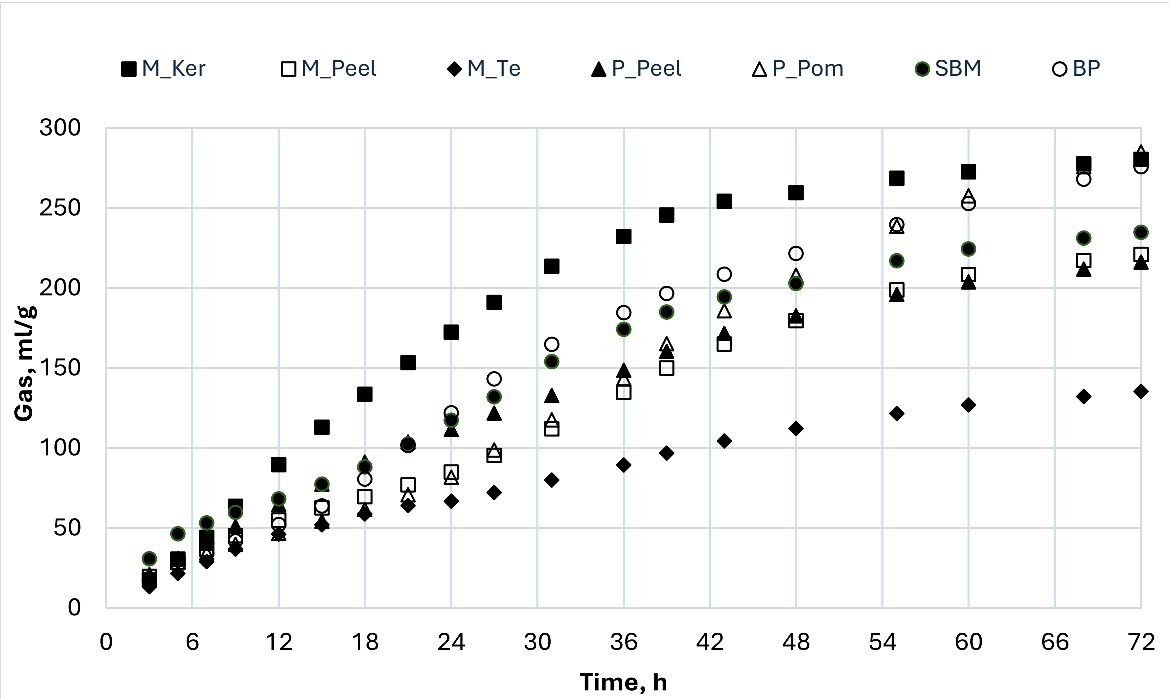

Figure 5. 2. *In vitro* gas production profiles of by-products incubated for 72 hours.

P_Peel: pineapple peel; P_Pom: pineapple pomace; M_P: mango peel; M_Ker: mango kernel; M_Te: mango testa; SBM: soybean meal; BP: beet pulp.

5.5.Discussion

Discuss the nutritional characteristics as well as the *in vitro* digestion and fermentation characteristics of the by-products derived from the three selected tropical and subtropical fruits (pineapple, cashew, and mango) as promising ingredients in the pig feeding plan was not easy due to the scarcity of data in the literature on similar studies. Furthermore, one of the major difficulties in comparing the data obtained with those of other authors lies in the great variation of processing methods to which they are subjected, often artisanal and linked to local processing methods. The referenced feeds such as corn meal (CM), soybean meal (SBM), and beet pulp (BP) showed the variation in terms of the chemical composition of feedstuff that pigs can accept. The by-products investigated fall within the interval of referenced feeds for NDF (7.6 - 47.1 % DM) and CP (7.65 - 51.2 % DM), except the by-products from mango fruits with low CP. However, only M_Peel, M_Ker, and pineapple by-products (P_Peel and P_Pom) have low ADL comparable to pig-referenced feeds. The negative effect of high fibers and their lignification on pig nutrient utilization and fermentation profile were previously reported (Vastolo et al., 2019; Vasconcelos et al., 2020). This effect justifies the high differences observed for total digestibility and fermentation kinetic of investigated by-products compared to CM both stopped at 48h of incubation. In addition, the by-products contain more nonstructural carbohydrates (NSC) than SBM and BP, whereas they were not so far from one of CM. A large portion of NSC is digested by the microflora of the large intestine in pigs, contributing up to 50 % of dietary energy (Choct and Kocher, 2000). This result is observed for the by-products stopped at 72 h of incubation showing greater DMDm, SCFA, and fermentation process as well.

5.5.1. Use of pineapple by-products in pig nutrition

Previous studies (Kiatti et al., 2023) indicated that the chemical composition of pineapple by-products is partially in accordance with previous publications, and this is probably because the production areas and the soil-climatic conditions, as well as the type of processing, are different. In the bibliography, studies that used pineapple by-products in the formulation of diets for pigs are few, very dated, and report unfavorable effects, especially in terms of low palatability and high fiber content (Göhl, 1982). The same author reported the improvement of body weight gain and feed conversion efficiency of older pigs when pineapple by-products were added up to 50 % of the diet but beyond this level, these parameters were depressed. Wadhwa and Bakshi (2013) noted that the pigs did not accept dried pineapple waste presented *ad libitum* and, due to the high crude fiber content (20 %) which limits its use in young pigs' diets. Moreover, the decrease in pigs' average daily gain (17 - 24 %) and feed intake (12 – 21 %) were observed with pineapple by-products inclusion up to 270 g/kg DM in the basal diet composed of corn and soybean meal while feed conversion ratio was not affected (Vasconcelos et al., 2020). The authors reported also an increase in insoluble and total dietary fiber digestibility and lean meat percentage whereas the carcass fat deposition decreased with pineapple by-products inclusion in pigs' diets. However, in this study, both pineapple by-products, specifically pomace, seem to be promising. In particular, in pomace, the nutrient content and *in vitro* fermentation characteristics are similar to beet pulp in terms of protein content, structural carbohydrates, digestibility, gas, and fatty acids production. So, this by-product could be a suitable ingredient for pigs' feeding plan. However, the low dry matter content is a critical issue to consider because preservation over time makes it difficult.

5.5.2. Use of cashew by-products in pig nutrition

A slight difference between yellow and red varieties of cashew apple by-products emerged, both in terms of chemical composition and *in vitro* parameters, whereas CNT contains more lipids (EE: 26.4 % DM), which, despite being an important energy source limits microbial activity resulting low total digestibility, SCFA and gas production. All the parameters related to cashew apple and testa by-products were lower compared to the CM which did not produce gas after 48 hours. However, the differences might be due to the chemical composition, particular ADL and EE content, and secondary compounds in cashew apple by-products. Previous studies reported a higher content of secondary compounds principally tannin in cashew whole apple and pomace (Da Silva et al., 2023, Kiatti et al., 2024) and cashew nut testa (Trox et al., 2011; Poommarin et al., 2017). Some *in vivo* studies on the use of cashew apples in pig diet report encouraging data. In the Philippines, fresh (20 %) or sun-dried (20 %) cashew wastes were used as a supplement to a mixed ration of rice bran, corn meal, and fish meal in fattening pigs. Animal growth performances were not significantly different from those obtained with the control diet, while feed costs were significantly reduced (Acero et al., 2013). In Brazil, dried cashew pomace fed to growing pigs up to 20 % in the diet, composed of soybean meal or sorghum, showed lower protein and energy digestibility (12 and 23 %, respectively), thus also reducing the metabolizable energy of the diet from 4125 to 3225 kcal/kg (Farias et al., 2008). In addition, it was proposed to soak CNT in distilled water (1:10, w/v) for 24 h to reduce tannin and include it in the finishing pig diet by up to 5 % to maintain the intake and nutrient digestibility (Poommarin et al., 2017). Overall, cashew by-products (especially pomace and whole fruit) can be an ingredient for pigs due to their protein content, not too high structural carbohydrate content, high digestibility, and BCFA production. However, due to their high-water content, it is necessary to find an adequate system to preserve them.

5.5.3. Use of mango by-products in pig nutrition

The three mango by-products showed different characteristics: *i)* the peel has a limited protein content and a low level of non-lignified structural carbohydrates, which led to a high digestibility, high gas, and short-chain fatty acids production after 72 hours of fermentation; *ii)* the testa has a low protein level and a fair content of lignified structural carbohydrates, which contributed to the low digestibility after 72 hours, accompanied by an equally low production of gas (slow kinetics) and short-chain fatty acids; *iii)* the kernel shows the best nutritional value with higher protein content, limited content of structural carbohydrates and a high level of crude lipids, which led to a high degradability, gas production (faster kinetics) and short-chain fatty acids, in particular butyric acid. This last aspect is particularly interesting in monogastric because this acid represents an energy source for the enterocyte. In the literature, most of the studies on the chemical composition refer to kernel and peel and the data reported are similar to those obtained in the present study for the kernel, but slightly different in the peel especially regarding NDF and CP (Heuzé et al., 2019). *In vivo* studies reported that dried mango peels can be included at 10 % in the diets for finishing pig without any negative effects on feed conversion index, animal performance, and providing economic advantages (Rao et al., 2003). Mango by-products (especially peel and kernel) could represent a raw material in pig feeding due to their digestibility and lipid content, but they need to be integrated with crude protein sources. However, Timbilfou et al. (2023) published that palm kernel meal, rice bran, maize bran, and wheat bran, in the following order according to an economic point of view can be used at 25 % as sorbent for mango peel (75 %) to produce feed suitable for monogastric and ruminant as well.

5.6. Conclusion

Due to their chemical composition, digestibility, and *in vitro* fermentation characteristics, pineapple pomace, cashew pomace, and whole fruit could be included in the diet for fattening pigs, presenting characteristics partly similar to beet pulp. Overall, also mango by-products (especially peel and kernel) could represent a raw material in pig feed, due to the high digestibility and lipid content, even if they have to be integrated with a crude protein source. In addition, as these by-products are available in tropical and subtropical areas, their recycling in pig nutrition will reduce feeding costs, protect the environment from their decomposition, and increase breeders' income. For all by-products, further studies are needed to find a solution for their preservation (i.e., drying, ensiling). Combining these by-products with others with higher DM as sorbent should be an option to preserve cashew apple, pineapple, and mango by-products for pig nutrition. Furthermore, *in vivo* tests should verify the appropriate level of integration in pig diet to not negatively influence palatability, ingestion, and adequate growth performance. Nowadays, the tendency is to consider the by-product as a co-product, i.e. a valuable material generated during a production cycle together with others that can be sold or reused profitably. The non-leader co-product must be treated with the same care and attention as the main co-product. Consequently, by-products become a resource, with added socio-economic value if the nutritional integrity is preserved. Therefore, the supply chain that generates them should be designed to support it by ensuring not only legal compliance with safety requirements but also the adaptation of processing techniques to improve nutritional quality.

5.7. Acknowledgements

Finanziamento della Ricerca di Ateneo 2023 (FRA): Pasta leftovers as animal feeds (PASLEFT) (Resp. Dr. Francesco Serrapica).

5.8. Reference

1. Acero, L. H., Lagan, C. G., & Padul, M. A. C. Growth performance of fattening hogs fed with fresh and dried cashew apples. *IPCBEE* 2013, 51, 23-27.

2. Agbangba, C.E. Réponses Agronomiques de l'ananas (Ananas Comosus) à La Fertilisation Minérale Au Bénin: Croissance, Rendement et Qualité Du Fruit. Ph.D. Thesis, Université Cheikh Anta Diop de Dakar (UCAD), Dakar, Senegal, 2016.

3. Ajila, C.M.; Brar, S.K.; Verma, M.; Tyagi, R.D.; Godbout, S.; Valéro, J.R.. Bioprocessing of agro-byproducts to animal feed. *Crit. Rev. Biotechnol*. 2012, 32(4), 382-400.

4. AOAC. Official Methods of Analysis, 12 Vol, 18th ed.; Association of Official Analytical Chemists: Arlington, VA, USA, 2005.

5. Barry, D.; Kiendrebeogo, T.; Sere, M.; Combari, A.; Logtene, Y.M.; Kabore-Zoungrana, C.Y. Effects of Mango Wastes-Based Diets on the Growing Parameters of Laying Hens and Biometric Parameters of the Eggs. *OALib* 2019, 6. e5868.https://doi.org/10.4236/oalib.1105868.

6. Boisen, S.; Fernández, J.A. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by *in vitro* analyses. *Anim. Feed Sci. Technol.* 1997, *68*(3-4), 277-286.

7. Choct, M.; Kocher, A. Non-starch carbohydrates: Digestion and its secondary effects in monogastric. In *Proceedings-Nutrition Society of Australia* 2000, 24, 31-38.

8. Da Silva, J.; De Brito, E.S.; Ferreira, S.R.S. Biorefinery of cashew by-products: Recovery of value-added compounds. *FBT*. 2023, *16*(5), 944-960.

9. Das, I.; Arora, A. Post-harvest processing technology for cashew apples - A review. *J. Food Eng.* 2017, 194, 87–98, https://doi.org/10.1016/j.jfoodeng.2016.09.011.

10. EC Council. Regulation 882/2004 on Official controls performed to ensure verification of compliance with feed and food law, animal health and animal; EC Council: UE, 2004.

11. FAOSTAT. Food and agriculture organization of the United Nations (FAO). https://www.fao. org/faostat/en/#home Accessed in November 2022.

12. Farias, L.A.; Lopes, J.B.; de Figueirêdo, A.V.; de Noronha Albuquerque, D.M.; de Araújo Neto, A.A.; Ramos, L.D.S.N. Cashew pulp meals (Anacardium occidentale l.) for growing pig: nutrient metabolism and performance. *Ci. Anim. bras.* 2008, 100-109

13. Gnanglè, C.P.; Glèlè Kakaï, R.; Assogbadjo, A.E.; Vodounnon, S.; Yabi, J.A.; Sokpon, N. Tendances Climatiques Passées, Modelisation, Perception et Adaptations Locale au Bénin. *Climatologie* 2011, 8, 27–40.

14. Göhl, B. Les aliments du bétail sous les tropiques; FAO, Division de Production et Santé Animale: Roma, Italy, 1982.

15. Heuzé, V.; Tran, G.; Archimède, H.. Mango (Mangifera indica) forage. Feedipedia 2019. https://feedipedia.org/node/129 Last updated on July 31, 2019.

16. INSAE. Cahier Des Villages et Quartiers de Ville D*é*partement de l'Atlantique: Minist*è*re Charg*é* du Plan, de La Prospective et du D*é*veloppement; INSAE: Cotonou, Bénin, 2004; pp. 1–34.

17. Kiatti, D.d.; Vastolo, A.; Koura, B.I.; Vitaglione, P.; Cutrignelli, M.I.; Calabrò, S. The Chemical Characteristics and *In Vitro* Degradability of Pineapple By-Products as Potential Feed for Ruminants. Animals 2023, 13, 3238.

18. Kiatti, D.d; Koura, B.I.; Vastolo, A.; Chiacchio, M.F.; Vitaglione, P.; Dossa, L.H.; Cutrignelli, M.I.; Calabrò, S. Sustainable ruminant nutrition in West Africa by *in vitro* characterization of cashew apple by-products. *Heliyon* 2024, 10(18).

19. Kiendrebeogo, T.; Logtene, Y.M.; Kabore-Zoungrana, C.Y. Effets de rations a base de dechets de mangue sur les performances ponderales et la qualite de la carcasse de porcs Korhogo en croissance au Burkina Faso. *J. Appl. Biosci.* 2019, 129, 13039-13049. https://doi.org/10.4314/jab.v129i1.7

20. Lawrence, J.D.; Mintert, J.R.; Anderson, J.D.; Anderson, D.P. Feed grains and livestock: impacts on meat supplies and prices. *Choices* 2008, *23*(2), 11-15.

21. Milogo, X.D.; Kiendrebeogo, T.; Ouedraogo, I.Z.; Koara, H.; Sawadogo, D.P.; Bougouma-Yameogo, V.M.C. Influence of Mango Feed-Based Diets on the Production and Economic Profitability of Montbeliarde Cows' Milk at the End of Lactation in Burkina Faso. *J. Anim. Sci.* 2022, 12, 239-250.

22. Mourad, M. Recycling, recovering and preventing food waste: Competing solutions for food systems sustainability in the United States and France. *J. Cleaner Prod.* 2016, 126, 461-477.

23. Muchiri, D.R.; Mahungu, S.M.; Gituanja, S.N. Studies on mango (Mangifera indica, L.) kernel fat of some Kenyan varieties in Meru. *JAOCS*. 2012, 89, 1567-1575.

24. Musco, N. Cutrignelli, M.I.; Calabrò, S. Tudisco, R.; Infascelli, F.; Grazioli, R.; Lo Presti, V.; Gresta, F.; Chiofalo, B. Comparison of nutritional and antinutritional traits among different species (*Lupinus albus L., Lupinus luteus L., Lupinus angustifolius L.*) and varieties of lupin seeds. *J. Anim. Physiol. Anim. Nutr.* 2017, 101, 1227-1241.

25. Omre, P.K.; Singh, S.; Shikha, S. Waste utilization of fruits and vegetables-A review. *South Asian J. Food Technol. Environ.* 2018, 4(1): 605-615

26. Pinotti, L.; Tretola, M.; Luciano, A.; Ottoboni, M. Feed design applying circular economy principles: The case of former food products. In *Book of Abstracts of the 70th Annual Meeting of the European Federation of Animal Science*: Wageningen Academic 2019, 247-247).

27. Poommarin, P.; Sulabo, R.C.; Sevilla, C.C. Feeding value of dried cashew nut testa in finishing pigs: Effects on nutrient digestibility and gut morphology. *Songklanakarin J. Sci. Technol.* 2018, *40*(6), 1300-1305.

28. Porter, S. D.; Reay, D. S.; Higgins, P.; Bomberg, E. A half-century of production-phase greenhouse gas emissions from food loss & waste in the global food supply chain. *Sci. Total Environ.* 2016, *571*, 721-729.

29. Rao, D.S.; Ravi, A.; Yedukondalu, R. Inclusion of dried mango (Mangifera indica) peels in finisher rations of pigs on their performance. *Indian J. Anim. Nut.* 2003, 20(1), 120-123.

30. Ravani, A.; Joshi, D. Mango and it's by product utilization–a review. *Trends Post Harvest Technol.* 2013, 1,1, 55-67.

31. Ravindran, V.; Blair, R. Feed resources for poultry production in Asia and the Pacific. II. Plant protein sources. *Worlds Poult. Sci. J.* 1992, 48,3, 205-231.

32. Sanon, H.O.; Kanwe, A.B.; Millogo, A.; Ledin, I. Chemical composition, digestibility, and voluntary feed intake of mango residues by sheep. *Trop. Anim. Health Prod.* 2013, *45*(2), 665-669.

33. Sodjinou, M.K.B.; Assouma-Imorou, A.; Olounlade, A.O. Technical Efficiency of Pineapple Production and Challenges in Southern Benin. Afr. J. Agric. Res. 2022, 18, 522–534.

34. Timbilfou, K.; Grace, K.M.; Etienne, S.; Drissa, B.; Isidor, O.Z.; Vianey, T. Influence of Sorbent Type on Drying Efficiency, Production Costs and Nutritional Values of Mango By-Products Feeds for Livestock. *Food Nut. Sci.* 2023, 14, 1221-1231. https://doi.org/10.4236/fns.2023.1412076.

35. Trox, J.; Vadivel, V.; Vetter, W.; Stuetz, W.; Kammerer, D.R.; Carle, R.; Scherbaum, V.; Gola, U.; Nohr D.; Biesalski H.K. Catechin and epicatechin in testa and their association with bioactive compounds in kernels of cashew nut (Anacardium occidentale L.). *Food Chem.* 2011, *128*(4), 1094–1099.

36. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for Dietary Fiber, Neutral Detergent Fiber, and Non-Starch Polysaccharides in Relation to Animal Nutrition. *J. Dairy Sci*. 1991, 74, 3583–3597.

37. Vasconcelos, T.S.; Thomaz, M.C.; Castelini, F.R.; Alvarenga, P.V.A.; De Oliveira, J.A.; Ramos, G.F.; Ono, R.K.; Milani, N.C.; dos Santos Ruiz, U. Evaluation of pineapple byproduct at increasing levels in heavy finishing pigs feeding. *Anim. Feed Sci. Technol.* 2020, *269*, 114664.

38. Vastolo, A.; Calabrò, S.; Cutrignelli, M.I. A review on the use of agro-industrial CO-products in animals' diets. *Ital. J. Anim. Sci.* 2022, *21*(1), 577-594.

39. Vastolo, A.; Calabró, S.; Liotta, L.; Musco, N.; Di Rosa, A.R.; Cutrignelli, M.I.; Chiofalo, B. *In vitro* fermentation and chemical characteristics of Mediterranean by-products for swine nutrition. *Animals* 2019, *9*(8), 556.

40. Vieira, I.M.M.; Santos, B.L.P.; Santos, C.V.M.; Ruzene, D.S.; Silva, D.P. Valorization of PineappleWaste: A Review on How the Fruit's Potential Can Reduce Residue Generation. *Bioene. Res.* 2022, 15, 924–934.

41. Wadhwa, M.; Bakshi, M.P.S. Utilization of fruit and vegetable wastes as livestock feed and as substrates for generation of other value-added products. *RAP pub*. 2013, *4*, 67.

Chapter 6.

Final Consideration And Future Perspectives

Through *in vitro* and *in vivo* methods, this thesis aimed to study the nutritional characteristics of some by-products deriving from the processing of three tropical and sub-tropical fruits (pineapple, cashew, and mango) widely cultivated in the West African region for their potential use in livestock feed.

The issue of livestock nutrition in West Africa is a complex challenge driven by several intertwined factors:

- *seasonal scarcity of pasture:* in West Africa, pasture availability fluctuates with the rainy and dry seasons. During the dry season, grazing resources dwindle significantly, leaving livestock without sufficient forage. The forage scarcity and low-quality lead to a drop in livestock productivity and can cause overgrazing, which degrades the soil further and reduces future pasture availability;
- *cost of energy sources*: supplementing livestock diets with alternative feeds, particularly energy-rich sources like grains, is expensive. The cost can rise sharply due to market fluctuations and limited supply, making it economically unfeasible for many farmers to provide enough supplementary feed. This can lead to reduced livestock weight gain, milk production, and farmers income;
- *competition with human consumption*: in some West African countries, there is significant competition between livestock and humans for key staple crops like maize, sorghum, and millet. These grains are essential both for human consumption and as energy sources in livestock diets, which places pressure on resources and drives up prices. As a result, small-scale livestock farmers struggle to afford adequate feed, especially during the dry season when pasture is scarce.

Addressing these challenges requires innovative strategies, such as developing resilient forage crops, promoting integrated crop-livestock systems, and exploring alternative feed sources (i.e., by-products such as pineapple, cashew, and mango) that don't compete directly with human food supplies. Moreover, using by-products in livestock feed aligns well with the goals of Agenda 2030 and could help reduce the environmental footprint of livestock production. This approach, for example, contributes to reducing waste and promoting resource efficiency (Goal #12: Responsible consumption and production). In fact, by incorporating agricultural and food industry by-products (like crop residues, fruit and vegetable wastes, brewers' grains, etc.) into livestock feed, we effectively "close the loop" by turning waste products into valuable inputs. This reduces food waste and supports a circular economy, maximizing resource use efficiency and decreasing the need for primary feed resources like grains and soy, which often compete with human food supplies. In addition, by replacing energy-intensive feed crops with by-products, livestock emissions related to feed production could be significantly reduced (Goal #13: Climate Action). For example, feed crops require inputs like fertilizers, pesticides, and significant water resources, all contributing to emissions. On the other hand, by-products often have a lower environmental footprint because they are secondary outputs from existing food production processes. Finally, by-products are usually more cost effective than traditional feeds, making them a more accessible option for smallholder farmers and improving economic resilience, as requested in Goal #12 by making sustainable practices more attainable across different socioeconomic levels in the agricultural sector.

Integrating by-products into livestock diets offers a highly feasible solution for more sustainable livestock production. However, the approach does require careful consideration of nutritional balance, as by-products vary widely in quality and nutrient content. Additionally, broader adoption would benefit from supportive policies, technical assistance, and awareness-building initiatives, ensuring these practices align with animal health and productivity goals.

Using by-products in livestock feed presents specific challenges related to analyzing their components and ensuring proper preservation. By-products from various food industries can contain bioactive components (e.g., sugar, polyphenols). High sugar levels in certain by-products (e.g., fruit and vegetable residues) can be beneficial for energy but may lead to fermentation and spoilage if not properly managed. Ensuring a balanced sugar content is essential to avoid metabolic issues in livestock. Polyphenols, found in by-products like cashew apples and pomace, grape pomace or olive cake, have antioxidant properties that could benefit animal health. However, high polyphenol content may sometimes reduce palatability or interfere with nutrient absorption. Proper analysis and adjustment are needed to balance these bioactive compounds. The by-products studied in this thesis revealed the presence of plant secondary compounds that attract nowadays researchers in the animal nutrition field. These metabolites, particularly tannins, were reported to positively affect animals' gastro-intestinal parasites and limit the pollutants gas produced during rumen fermentation (e.g., methane). Further investigation on the effect of cashew apple by-products, and mango kernel secondary compounds on parasite activity and methane production will be interesting. It could contribute to reducing antibiotic utilization in ruminants' production systems.

By-products are often highly perishable due to their high moisture content, making preservation crucial for maintaining quality and reducing spoilage. The most common preservation methods, drying, reduces water content, slowing down microbial growth and spoilage, but it can be energy intensive. Ensiling (anaerobic fermentation) is another approach that can preserve nutrients and even improve digestibility, but it requires careful control of pH and anaerobic conditions to prevent spoilage. In general, they can be applied to cashew apple, pineapple, and mango by-products, ensiling them especially with less palatable and less nutritional feed such as hay, straw, and crushed cereals stover. Other by-products such as palm kernel meal, soybean meal, rice bran, maize bran, and wheat bran might be investigated as a sorbent to cashew, pineapple, and mango by-products if they are both available in the same area.

Evaluating by-products for livestock feed using *in vitro* and *in vivo* techniques is essential for understanding their nutritional value, digestibility, and potential effects on animal health. Compared to *in vivo* studies, as advantages, the *in vitro* methods are less expensive (do not require large numbers of animals, housing, or long-term monitoring), time-efficient (can be conducted quickly, allowing for rapid screening), work on the controlled experimental environment (such as temperature, pH, microbial population, etc.). Also, they reduce the need for live animal use, aligning with ethical standards and the 3Rs (Replacement, Reduction, and Refinement) principle in animal research. However, the results obtained *in vitro* may not always translate to actual *in vivo* responses due to differences in animal physiology, microbiome composition, nutrient absorption, and metabolic processes. The *in vitro* techniques used in this thesis allowed us to obtain data regarding degradability and digestibility, fermentation kinetics, and final products of fermentation (volatile fatty acids), which are useful in better characterizing novel feedstuffs as by-products.

On the other hand, the *in vivo* techniques provide a complete picture of how by-products affect the animal's digestive system, metabolism, and overall health (i.e., growth performance, nutrient utilization, etc.). Considering the peculiarity of some by-products (i.e., secondary metabolites content) *in vivo* experiments should be necessary to understand how the animals interact with the diet containing raw, preserved or extracts from cashew, pineapple, and mango by-products. It will help to evaluate the ingestion, digestion, feeding behavior, animal products (quantity and quality) and methane and parasite mitigation. So, combining *in vitro* and *in vivo* methods provides the best approach. The first is valuable for initial screening and narrowing down by-products with potential benefits, while the second confirms or not the in vitro findings through assessments of the real impact on animal health and performance. Together, these methods help to maximize the sustainability and effectiveness of by-product use in livestock diets.

The principal information on the nutritional characteristics regarding the studied by-products – cashew (*Anacardium occidentale* L.), pineapple (*Ananas comosus* L.), and mango (*Mangifera indica* L.) – needs to be known to avoid any adverse effect through their collection, preservation and utilization in animal nutrition as guidelines:

- low dry matter and high easily fermentable sugar will cause rapid decomposition and growth of harmful bacteria in cashew apple and pineapple by-products. They must be stored properly and rapidly after processing;
- high content of easily fermentable sugar in cashew apple and pineapple by-products can cause acidosis to the ruminant if they are given *ad libitum* or in high proportion to the ruminants;
- the high amount of lignin and polyphenols in cashew by-products must be taken into consideration for the animal tolerance limit and to guarantee an adequate intake and digestibility;
- in pineapple, separate crowns, bud ends and peel to core, and pomace due to their high differences in terms of chemical composition and fermentation kinetics;
- utilized pineapple by-products to improve the palatability of less appreciated feed in livestock nutrition;
- in mango, separate the peel and kernel and utilize them in a pig diet supplemented with a protein source.

However, several factors, such as cultivation conditions (soil and climate), cultivation practices, ripeness, species, varieties, processing techniques, collection methods, and preservation techniques, were mentioned to influence the chemical nutrition and fermentation profiles of fruit by-products.

The future of using by-products as livestock feed in West Africa holds promising potential for:

- *sustainable livestock feeding systems:* integrating by-products into livestock feed could advance a circular economy, where agricultural and food industry wastes are repurposed rather than discarded. This approach can contribute to resource efficiency, minimize waste, and reduce environmental impact. By-products from local industries, such as fruit waste, could lessen the reliance on imported feeds, such as soybean and maize, which are often costly and subject to market fluctuations.
- *improving smallholder livelihoods and economic resilience*: by-products are often more affordable than traditional feed ingredients. This could make livestock farming more accessible to smallholder farmers, allowing them to improve their profit margins and reinvest in their operations. Farmers who generate by-products as waste from their primary crops (e.g., cashew, pineapple, mango) could create a secondary income stream by selling them as feed, fostering economic resilience in rural areas. The strategy in expanding the by-product value chain (collection, processing, distribution). could also create jobs in rural communities, contributing to poverty alleviation and local economic growth.
- *enhancing livestock productivity and food security*: by-products can provide essential nutrients, such as fiber, protein, and energy, as well as secondary metabolites, which can improve animal health and productivity. By-products could help address the seasonal feed shortages that challenge livestock farmers, particularly during the dry seasons. Properly preserved by-products can act as a buffer, ensuring continuous feed availability and improving livestock resilience to climate variability.
- *environmental benefits*: effective using by-products could reduce waste accumulation, lower pollution from agricultural residues, and decrease greenhouse gas emissions from waste decomposition. This aligns with climate goals and supports sustainable land use. By using by-products, which often have a lower water and land footprint compared to traditional feed crops, West Africa could reduce the environmental strain on its limited agricultural resources.
- *technological innovations and capacity building*: investing in technologies for drying or ensiling by-products could enhance their storage life and nutritional value, making them more viable for long-term livestock feeding. For instance, mobile and affordable drying units could allow smallholder farmers to preserve by-products without requiring extensive infrastructure. More research on local by-products will be essential, including nutritional profiling and best practices for inclusion in diets. Partnerships with research institutions, national and international universities, and extension services can facilitate knowledge-sharing. Educating farmers on the nutritional potential and safe use of by-products will be critical for widespread

adoption. Extension services and agricultural organizations can play a significant role in raising awareness and providing technical support.

- *policy and institutional support*: policies that support by-product utilization, such as subsidies for processing equipment or tax incentives for feed innovation, could promote the practice. Additionally, establishing quality standards for by-products would help ensure safety and efficacy. Governments and organizations could work on building efficient supply chains for by-products to ensure they are accessible and affordable for livestock farmers. Improved transport, storage facilities, and market linkages can facilitate this.

In conclusion, the studies conducted in this thesis demonstrate that some by-products from cashew apple, pineapple, and mango – typically discarded as waste – can serve as valuable alternative feedstuffs for livestock in West African region. These by-products can provide an essential feed source during the dry season, helping to alleviate the shortage of natural pasture, which is the primary roughage for ruminants in West Africa. Incorporating these by-products into livestock diets can reduce feeding costs and decrease the competition for cereals between humans and animals, while maintaining livestock growth performance throughout the year. Moreover, recycling these by-products into animal nutrition mitigates also the environmental impact of their decomposition and improves public health in surrounding areas of fruit processing facilities by reducing waste accumulation.

Therefore, this approach supports sustainable livestock production and contributes to a more resilient, cost-effective, and environmentally friendly agricultural system in West Africa. However, effective use of these resources requires proper collection and preservation and adherence to guidelines on chemical composition, fermentation profiles, and polyphenol content, as outlined in this thesis. In summary, the successful use of by-products requires a thorough understanding of their chemical composition and potential impacts on livestock, along with effective preservation strategies to maintain the quality and stability.

However, future investigations could continue using these by-products in animal nutrition through a multidisciplinary approach. More studies in vitro and in vivo need to be conducted to assess how animals respond to the inclusion of by-products in their diet, regarding palatability, feeding behavior, and potential stress indicators. In addition, the economic analysis could instead propose a model that includes the cost-effectiveness of incorporating by-products into animal feeding plans, considering factors such as purchase cost, potential savings, and impacts on production. A holistic assessment combining these findings will help to identify optimal strategies and guide the practical adoption of by-products that safeguard welfare and profitability.

Acknowledgments

I thank all the leaders of the University of Napoli Federico II (Italy), which offers opportunities to young international students to continue their studies in good conditions and contribute to the development of their home country and the world.

I thank Prof. Aniello Anastasio, the Head of the Department of Veterinary Medicine and Animal Production, Prof. Paolo De Girolamo, the Coordinator of the Doctoral School in Veterinary Science, and all administrative staff for the academic activities they proposed for the best quality of our training.

I am particularly grateful to Prof. Serena Calabrò, my supervisor, for the guidance, coaching, and advice during my PhD program. Together with you, I have implemented my PhD project. Thanks to you, I have been involved in laboratory work, data understanding, statistical analysis, writing articles, reviewing and submitting to journals, and presenting results at conferences. This achievement would not have been expected without your patience and your encouragement.

I express my gratitude to all the members of the Feed Analysis laboratory for their participation in the laboratory activities of the current thesis. To Prof. Monica I. Cutrignelli for her contribution to performing the analysis, manuscript revision, and her advice to improve the quality of the thesis. To Dr. Maria Ferrara, thanks for her support during the laboratory analysis, specifically the chromatographic analysis. To Dr. Alessandro Vastolo and Dr. Nadia Musco for their support during the laboratory, statistical analysis, and draft revisions. To the students Teresa Montefusco, Martina Cafaro, Giuseppe Cirillo, and Barbara Piccirillo for participating in the laboratory analysis and *in vitro* fermentation trials.

Thanks to Prof. Laura Rinaldi and Dr. Vincent Niderkorn, coordinators of MiCliFeed LIFE project in Italy and France, respectively, for the opportunity they gave me to stay at INRAe Clermont-Ferrand and participate in a sheep *in vivo* experiment using the metabolic chambers. I am grateful to the coordinators of the projects PSR-SAVEPEB, PNRR-Agritech, and FRA-PASLEFT that I participated in, thanks to my supervisor.

I am grateful to Prof. Felicia Masucci, Prof. Giuseppe De Rosa, Prof. Antonio Di Francia for hosting me at the laboratory of Animal feed at the Department of Agriculture in Portici, and especially to Dr. Francesco Serrapica for his collaboration and support in the laboratory analysis. I also thank Prof. Paola Vitaglione, Manuela F. Chiacchio, and Attilio Visconti of the Laboratory of Food and Health at the Department of Agriculture in Portici for their participation in the sugar and secondary compounds analysis of the samples.

I thank Dr. Bossima I. Koura from the Université Nationale d'Agriculture (Kétou, Benin) for his support in the thesis, particularly advice on methods and support for the by-product sampling and the *in vivo* experiment, as well as for data analysis, and draft revision. I also thank Dr. Assani S. A. Assani, Dr. Daouda Yaoussa, Sounou A. Alban, Maladé A. Nourou-Dine, and Houndagnon Félicité for their support in collecting the studied by-products and conducting the *in vivo* experiment at the "ferme d'Etat de Bétécoucou" in Benin. I thank Prof. Youssouf Toukourou and Pro. Luc Hippolyte Dossa, my previous supervisors for my Bachelor's and Master's degrees, respectively, for their teaching in the past, encouragement, and advice which helped me handle and plan the research activities.

I am particularly grateful to all the members of my family, specifically Toussaint, Catherine, Boniface, Sylvain, Tianga, and Marguerite, for their different support during my study.

I thank my lovely lady Monique A. Dari for her presence in my life, encouragement, and help in handling my emotions and feelings so that I could focus on my thesis project. Thank you very much for the family responsibilities you took on alone in my absence from home.

I would also like to thank Dr. Roberto Beneduce, Maria Laura Baldascini, and Marianna Vignola without forgetting Mounirou A. Atchakpari, who supported me during difficult times by allowing me to continue my thesis.

Finally, I thank all those who are not mentioned here but who have contributed in various ways to the realization and completion of this thesis; please accept my deep gratitude.

Curriculum Vitae

Kiatti Dieu donné is holder of a Bachelor in Agro-pastoralism and Meat Products Technology at Faculty of Agronomy, University of Parakou (Benin) in 2018 and a Master in Animal Production and Biotechnology at Faculty of Agricultural Sciences, University of Abomey-Calavi (Benin) in 2021. He was selected through an international PhD student call at the Department of Veterinary Medicine and Animal Production, University of Napoli Federico II (Italy). He worked on by-product characterization and an approach to climate change by fruits processing waste recycling in livestock nutrition as alternative feed. His PhD has led to 04 articles and to 05 abstracts in the conference's books. However, during his PhD, he participated actively in 05 training courses, 04 projects gaining knowledge and collaboration expertise, which led to 06 additional articles and 08 abstracts in the conference's books.

Project

MiCliFeed: Mitigating climate impact of small ruminants through innovative feeding approaches. Collaboration project between Italy, France, Belgium, and Greece, funded by the LIFE Program of the European Union LIFE20 CCM/GR/001703 (Resp. Laura Rinaldi).

SAVEPEB: Environmental protection and economic valorization of the Bagnolese Sheep. PSR Campania, Italy 2014/2020 Misura 19. (Resp. Vincenzo Peretti).

AGRITECH: Centro Nazionale per le Tecnologie dell'Agricoltura. Spoke 5: Sustainable productivity and mitigation of environmental impact in livestock systems (PNRR Cod. MIUR CN00000022) (Resp. Gianluca Neglia).

PASLEFT: Pasta leftovers as animal feed. FRA: University Research Funding, UNINA (Resp. Francesco Serrapica).

Training

- Training school "One health biotype diagnostic help", University of Napoli Federico II, Napoli, Italy, 11-16 November 2024.
- *In vivo* experiment testing the effect of Diet rich in polyphenols (Tanin) on sheep growth performance, digestibility, parasite (*Haemonchus contortus*), and CH_4 emission in metabolic chamber, INRAe, France, Clermont-Ferrand, 15 April – 15 July 2024.
- Workshop "Writing and Presenting Scientific Papers" organized by the European Association of Animal Production (EAAP), Florence, Italy, 31 August 2024.
- Summer school "Protein transition in animal feeding" organized by the University of Tuscia and ASPA, Trento, Italy, 1-6 August 2022.
- Course in "Scientific writing" organized by Department of Agricultural, University of Napoli Federico II, Portici, Italy, 6 September – 13 December 2022.

Research skills

- Grass sampling and species identification in natural and cultivated pasture
- By-products sampling and micro-silage making for their characterization
- *In vivo* ruminant samples collection: blood, faces, and rumen liquor
- *In vivo* ruminant fattening trial: diet formulation, feeding behavior, performance monitoring
- Chemical composition analysis using the AOAC method
- Structural carbohydrate quantification using the method of Van Soest et al. (1991)
- Animal feedstuffs characterization using *in vitro* gas production technique
- Coprological and hematocrit analyses
- Statistical analysis using Nonlin (SAS), JMP®, R-core software.

Awards

The 75th EAAP young researchers' Scholarship competition, Florence, Italy, 1st to 5th September 2024.

Revision in international journals

1) Stevens et al. 2024. The Effects of Harvest Maturity of Eragrostis tef 'Moxie'Hay and Supplemental Energy Source on Forage Utilization in Beef Heifers. *Animals*, *14* (2), 254.
2) Hu 2024. Proteomics Reveals the Obstruction of Cellular ATP Synthesis in the Ruminal Epithelium of Growth-Retarded Yaks. *Animals*, *14* (8), 1243.
3) Burhoop et al. 2024. Impact of Partial Oil Removal on Energy Content of Distillers Grains Plus Solubles for Finishing Cattle. *Animals*, *14* (16), 2329.
4) Punzalan et al. 2024. Copra Meal: A Review of Its Production, Properties, and Prospects. *Animals*, *14* (11), 1689.
5) Máximo et al. 2024. Nutritive Value and Degradation Kinetic Parameters of Three Plants for Feeding Bradypus variegatus Schinz, In Vitro Evaluation. *Animals*, *14* (18), 2645.
6) Alimi et al. 2024. Livestock feed resources used as alternatives during feed shortages and their impact on the environment and ruminant performance in West Africa: a systematic review. *Frontiers in Veterinary Science*, *11*, 1352235.

Publications in international journal

1) **Kiatti D.d.**, Koura B.I., Vastolo A., Chiacchio M.F., Vitaglione P., Dossa L.H., Cutrignelli M.I., Calabrò S. (2024) Sustainable ruminant nutrition in West Africa by in vitro characterization of cashew apple by-products. *Heliyon*, 10 (18) e37737 https://doi.org/10.1016/j.heliyon.2024.e37737.
2) Vastolo A., Sannino M., Masucci F., Di Francia A., Sarubbi F., Faugno S., **Kiatti D.d.**, Serrapica F. (2024) The use of small diameter nozzles in temperature-

controlled hemp oil extraction allows high oil yields and good quality residual hemp cake feed. *Front. Vet. Sci.* pp. 1-10. 10:1322637. https://doi.org/10.3389/fvets.2023.1322637.

3) Vastolo A., **Kiatti D.d.**, Cutrignelli M.I., Calabrò S. (2024) Mixed hay produced in Southern Italy: nutritive value and environmental impact. *Acta IMEKO*, 13 (1), pp. 1-5. https://doi.org/10.21014/actaimeko.v13i1.1665.
4) **Kiatti D.d.**, Vastolo A., Koura B.I., Vitaglione P., Cutrignelli M.I., Calabrò S. (2023) The chemical characteristics and in vitro degradability of pineapple by-products as potential feed for ruminants. Animal,13, 3238. https://doi.org/10.3390/ani13203238.
5) Vastolo A., Calabrò S., Carotenuto D., Cutrignelli M.I., **Kiatti D.d.**, Tafuri S., Ciani, F. (2023) Maca (Lepidium meyenii): In vitro evaluation of rumen fermentation and oxidative stress. *Fermentation*, 9, 568. https://doi.org/10.3390/fermentation9060568.
6) Vastolo A., Matera R., Serrapica F., Cutrignelli M.I., Neglia G., **Kiatti D.d.**, Calabrò S. (2022) Improvement of rumen fermentation efficiency using different energy sources: in vitro comparison between buffalo and cow. *Fermentation*, 8 (8), 351. https://doi.org/10.3390/fermentation8080351.
7) Vastolo A., Cutrignelli M.I., Serrapica F., **Kiatti D.d.**, Di Francia A., Masucci F., Calabrò S. (2022) Evaluation of in vitro starch digestibility and chemical composition in pasta former foods. *Front. Vet. Sci*. 9:1049087. https://doi.org/10.3389/fvets.2022.1049087.
8) Koura B.I., Vastolo A., **Kiatti D.d.**, Cutrignelli M.I., Houinato M., Calabrò S. (2022) Nutritional value of climate-resilient forage species sustaining peri-urban dairy cow production in the coastal grasslands of Benin (West Africa). *Animals*, 12, 3550. https://doi.org/10.3390/ani12243550.

Publication under revision

1) **Kiatti D.d.,** Koura B.I., Vastolo A., Cutrignelli M.I., Calabrò S. Pineapple by-products in West African Dwarf lamb diet improve palatability, nutrient intake and digestibility, *Food Sci. Nut.* FSN3-2024-11-3176.
2) **Kiatti D.d**, Serrapica S., Musco M., Cafaro M., Calabrò S. Potential use of tropical and subtropical fruits by-products in pig diet: in vitro two-step evaluation, *Anim. Feed Sci. Technol* ANIFEE-D-24-01210.

Conferences publications

1) **Kiatti D.d.**, Koura I.B., Vastolo A., Cutrignelli M.I., Calabrò S. (2024) Chemical and *in vitro* pineapple by-products characterization for sustainable small ruminant production. Book of Abstracts of the *75th EAAP*, ISBN: 979-12-210-6769-9, 1-5 September 2024, Florence, Italy, pp. 983.
2) Bosco A., Nappa A., Capezzuto G., Lucibelli S., Nocerino M., Di Donato L., Musella V., Vastolo A., **Kiatti D.d.**, Calabrò S., Cutrignelli M.I., Sotiraki S.,

Rinaldi L. (2024) Anthelmintic efficacy of agro-industrial by-products against gastrointestinal nematodes of sheep: in vitro tests. *26th FeMeSPRum Congress*, 20 - 23 June, Novi Sad, Serbia, ISBN 978- 86-7520-611-8, pp. 28-33.

3) **Kiatti D.d.**, Vastolo A., Koura B.I., Cutrignelli M.I., Vitaglione P., Calabrò S. (2023) Cashew (*Anacardium occidentale* L.) apple by-products: a promising feed in animal nutrition. Book of Abstracts: Animal Production Science: Innovations and sustainability for future generation, *ASPA 25th Congress,* 13–16 June, Bari, Italy, pp. 93.
4) Vastolo A., Ruggiero A., Calabrò S., **Kiatti D.d.**, Cutrignelli M.I. (2023) Influence of grainfree and cereal-based diets on postprandial glycemic response. Book of Abstracts: Animal Production Science: Innovations and sustainability for future generation, *ASPA 25th Congress,* 13–16 June, Bari, Italy, pp. 490.
5) Vastolo A., **Kiatti D.d.**, Calabrò S., Bosco A., Nocerino M., Cutrignelli M.I., Rinaldi L. (2023) Mapping the spatial distribution of agro-industrial co-products and sheep livestock farming in Italy. Book of Abstracts: Animal Production Science: Innovations and sustainability for future generation, *ASPA 25th Congress,* 13–16 June, Bari, Italy, pp. 177.
6) Calabrò S., Primi R., **Kiatti D.d.**, Manganello F., Tiberi C., Sestili F., Palombieri S., Ronchi B., Danieli P.P. (2023) Nutritional characteristics of innovative feed containing by-products from high-amylose wheat. Book of Abstracts: Animal Production Science: Innovations and sustainability for future generation, *ASPA 25th Congress,* 13–16 June, Bari, Italy, pp. 333.
7) Calabrò S., **Kiatti D.d.**, Vastolo A., Peretti V., Di Paolo M., Ciotola F. (2023) Management of Bagnolese sheep in Campania Region: animal and pasture characteristics. Book of Abstracts: Animal Production Science: Innovations and sustainability for future generation, *ASPA 25th Congress,* 13–16 June, Bari, Italy, pp. 173.
8) Vastolo A., Nocerino M., **Kiatti D.d.**, Bosco A., Calabrò S., Cutrignelli M.I., Rinaldi L. (2023) In vitro evaluation of grape pomace in the diet of adult sheep. *25th International FeMeSPRum Congress*,19 – 20 October, Eboli, SA, Italy.
9) **Kiatti D.d.**, Vastolo A., Koura B.I., Cutrignelli M.I., Calabrò S. (2023) Characterization of non-conventional feedstuff for ruminants' nutrition: case of cashew and pineapple by-products. *25th International FeMeSPRum Congress,* 19 – 20 October, Eboli, SA, Italy.
10) **Kiatti D.d.**, Vastolo A., Koura I.B., Cutrignelli M.I., Toukourou Y., Calabrò S. (2022) Growth performance of Sahelian x Djallonké crossbreed pre-weaning lambs in the subsaharan area of West Africa. Book of Abstract: Alternative feed resources and their management for transiting towards sustainable ruminant production. *Seminar FAO-CIHEAM Network*, 27-29 September, Catania, Italy. pp. 100.
11) Vastolo A., Calabrò S., **Kiatti D.d.**, Cutrignelli M.I. (2022) Could Hemp (*Cannabis sativa* L.) flower co-products be used in ruminant nutrition? Book of Abstract: Alternative feed resources and their management for transiting towards sustainable ruminant production. *Seminar FAO-CIHEAM Network*, 27-29 September, Catania, Italy. pp. 89.

12) Vastolo A., **Kiatti D.d.**, Cutrignelli M.I., Ciani F., Tafuri S., Calabrò S. (2022) Nutritional characteristics of different varieties of Maca (Lepidium meyenii Walp.). *Proc. 26th Congress of the European Society of Veterinary and Comparative Nutrition (ESVCN),* 6-8 Sept 2022, Basel, Switzerland, pp. 126.
13) **Kiatti D.d**, Koura B.I., Vastolo A., Cutrignelli M.I., Calabrò S. (2022) Pineapple (*Ananas Comosus*) By-Products as Feed for Ruminants. 1er CSI-UNA), Thème: Agriculture, entre crises économique, environnementale, sanitaire et politique : enjeux et réponses adaptatives en Afrique de l'Ouest 28-30 Novembre 2022, Sakété, Bénin. pp. 186.