R. Bras. Zootec., 55:e20250025, 2026
https://doi.org/10.37496/rbz5520250025

Non-ruminants
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Dietary supplementation of enzymatic complexes with and without *Yucca schidigera* extract and emulsifier for weaned piglets

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Received: February 6, 2025
Accepted: August 7, 2025

How to cite: Silva, W. A.; Pascoal, L. A. F.; Costa e Silva, L. F.; Guerra, R. R.; Givisiez, P. E. N.; Watanabe, P. H.; Almeida, J. L. S.; Medeiros, C. J.; Silva, M. B.; Azevedo, M. L.; Soares, P. C. and Araújo, W. J. 2026. Dietary supplementation of enzymatic complexes with and without *Yucca schidigera* extract and emulsifier for weaned piglets. Revista Brasileira de Zootecnia 55:e20250025. https://doi.org/10.37496/rbz5520250025

Editors:
Ines Andretta
Cesar Augusto Pospissil Garbossa

ABSTRACT - The effects of dietary supplementation with enzymatic complexes and plant extracts on weaned piglets were evaluated. Sixty piglets (28 days old) were assigned to six treatments: Control diet (CD); CD with 125 g/ton of *Yucca schidigera* extract (CDY); CD with 200 g/ton of enzymatic complex (CDE); CD with 400 g/ton of multi-enzymatic complex with emulsifier (CDME); CDE + 125 g/ton YSE (CDE+Y1) and CDE + 250 g/ton YSE (CDE+Y2). Piglets on the CDE+Y1 diet had better feed conversion ratio (P<0.05). CDE, CDME and CDE+Y1 improved digestibility of dry matter, organic matter, fiber, energy and phosphorus, reducing phosphorus excretion (P<0.05). CDME improved jejunum morphology, increasing villus height, mucosal thickness and absorptive area (P<0.05), while CDE+Y2 reduced goblet cells (P<0.05). YSE increased serum GGT levels; phosphorus levels were lower with CDE+Y2 and AST was reduced in the CD group (P<0.05). Exogenous enzymes combined with plant extracts lowered TNF-α mRNA expression and increased nutrient transporter expression (SGLT-1, NaPi-IIb, PEPT-1) (P<0.05). Overall, enzymatic additives, with or without plant extracts, improved feed efficiency, nutrient digestibility and intestinal health. However, further research is needed to elucidate the effects of the interaction between enzyme complexes and plant extracts.

Keywords: exogenous enzymes, intestinal health, phytogenics nutrition, swine

1. Introduction

The incorporation of enzymatic complexes into piglet diets is intended to enhance the digestion of plant-based ingredients, which constitute the major fraction of these diets, and to compensate for the

immature endogenous enzyme secretion capacity of piglets. Various dosages and combinations of exogenous enzymes, such as phytases, carbohydrases, proteases, and lipases, have been evaluated in piglet nutrition (Aranda-Aguirre et al., 2021).

In parallel, growing concerns regarding antimicrobial resistance associated with the use of performance-enhancing antibiotics and the environmental impacts of intensive swine production have prompted the exploration of alternative dietary strategies. Among these, phytogenic products have emerged as promising candidates (Adegbeye et al., 2019; Zhu et al., 2022). The plant extract from *Yucca schidigera* (YSE) has gained attention as a dietary additive due to its anti-inflammatory effects potential to mitigate environmental pollution by reducing nitrogen excretion (Zúñiga-Serrano et al., 2022).

Moreover, due to the limited bile secretion capacity in young piglets, which limits effective lipid emulsification, research has also focused on dietary emulsifiers (Mendoza and van Heugten, 2014). One such emulsifier, polyethylene glycol ricinoleate glycerol (PGRG), synthesized from castor oil (*Ricinus communis* L.), has been proposed to improve lipid availability (Bontempo et al., 2016), although studies on its use in swine diets remain limited (Sun and Kim, 2019).

Controlling feed costs remains a critical factor in achieving economically viable and environmentally sustainable animal production. The use of exogenous enzymes is widely accepted as a strategy to optimize feed utilization and reduce environmental impact (Sampath et al., 2023). Likewise, plant-derived secondary metabolites have demonstrated benefits for enhancing production efficiency in non-ruminants (Zúñiga-Serrano et al., 2022).

Notably, Sampath et al. (2023) reported promising performance improvements in pigs from weaning to finishing when supplementing diets with a combination of β-mannanase, *Yucca schidigera* extract, and amino-zinc. However, to date, no studies have specifically examined the combined effects of enzymatic complexes and *Yucca schidigera* extract.

Given these considerations, the combined use of enzymatic complexes and YSE could represent a synergistic strategy to enhance productive efficiency, nutrient digestibility, and absorption. This may occur via improvements in intestinal morphometry and health, ultimately contributing to more sustainable swine production through reduced environmental emissions.

2. Material and methods

2.1. Animal care

The experimental trial was conducted at the Swine Laboratory of the Department of Animal Science, Center for Human, Social, and Agrarian Sciences (CCHSA) of the Universidade Federal da Paraíba (UFPB), Campus III, in Bananeiras, Paraíba, Brazil (6°45'12" S and 35°38'57" W). All procedures in this research were approved by the Ethics Committee on Animal Use of the Universidade Federal da Paraíba (CEUA-UFPB), under protocol number 5476260521.

2.2. Animals, housing, and experimental diets

Sixty weaned piglets at 28±1 days of age were used, comprising 30 castrated males and 30 females of the same commercial lineage, sourced from a commercial farm, with an initial average weight of 6.43 ± 0.25 kg. The number of animals used followed the determinations of the CEUA/UFPB, and the sample size calculation was carried out as described by Sakomura and Rostagno (2016). The animals were housed in suspended nursery cages with slatted plastic floors, equipped with nipple drinkers and semi-automatic feeders.

The animals were distributed in a randomized block design (RBD) to control for differences in initial weight. The piglets were divided into six treatments (experimental diets), with five replicates, and each experimental unit consisted of two animals (one male and one female).

The experimental diets (Table 1) were formulated to meet the minimum nutritional requirements of the animals, according to Rostagno et al. (2017). The treatments evaluated were enzymatic complexes in combination with *Yucca schidigera* extract, where the additives replaced a fraction of the inert component of the diets. The treatments were: CD: control diet; CDY: CD with 125 g ton^{-1} of *Yucca schidigera* extract (YSE) (De-Odorase®; guarantee level: 65 mg kg^{-1} of sapogenin); CDE: CD with 200 g ton^{-1} of enzyme complex (EC) (Alltech® Allzyme SSF e+C; guarantee levels: 700 HUT g^{-1} of protease, 300 SPU g^{-1} of phytase and 40 CMCU g^{-1} of cellulase); CDME: CD with 400 g ton^{-1} of multienzyme complex with emulsifier (Alltech® Allzyme Allsotution; guarantee levels: 640 AJDU g^{-1} pectinase, 386 HUT g^{-1} protease, 60 SPU g^{-1} phytase, 32 BGU g^{-1} of β-glucanase, 16 XU g^{-1} of xylanase, 8 CMCU g^{-1} of cellulase, 4.5 FAU g^{-1} of amylase and 52 g kg^{-1} of glyceryl polyethyleneglycol ricinoleate - PEGR); CDE+Y1: CD + EC (200 g ton^{-1}) + YSE (125 g ton^{-1}); CDE+Y2: CD + YSE (200 g ton^{-1}) + 18 YSE (250 g ton^{-1}).

The corresponding values of each component of the additives per kg of diet were calculated (Table 2).

Table 1 - Proximate and nutritional composition of control diets for piglets according to nutritional requirements

Item	Phases		
	I	II	III
Ingredient[1]			
Yellow corn (7.9%)	39.04	50.32	65.38
Soybean meal (45.4%)	22.00	22.00	22.00
Inactivated whole soy (37.3%)	14.47	10.17	6.53
Whey powder (12.3%)	17.25	10.06	-
Soy oil	2.02	2.22	0.72
Dicalcium phosphate	1.74	1.75	1.63
Limestone	1.05	0.95	0.77
L-lysine HCL	0.52	0.54	0.42
DL-methionine	0.23	0.21	0.13
L-arginine	0.22	0.21	-
L-tryptophan	0.04	0.06	0.03
L-threonine	0.26	0.26	0.16
L-valine	0.11	0.12	0.02
Mineral and vitamin supplement	0.50	0.50	0.50
Salt	0.49	0.41	0.47
BHT	0.02	0.02	0.02
Inert[2]	0.06	0.18	0.23
Total	100.00	100.00	100.00
Calculated values			
Metabolizable energy (kcal/kg)	3,400	3,375	3,250
Crude protein (%)	21.42	19.87	18.06
Calcium (%)	1.07	0.97	0.79
Crude fiber (%)	2.51	2.47	2.54
NDF (%)	10.46	11.40	12.95
ADF (%)	4.40	4.32	4.43
Available phosphorus (%)	0.53	0.48	0.39
Digestible tryptophan (%)	0.28	0.28	0.21
Digestible lysine (%)	1.45	1.35	1.12
Digestible methionine (%)	0.52	0.48	0.38
Digestible methionine+cystine (%)	0.81	0.75	0.64
Digestible threonine (%)	0.97	0.90	0.73

Phases: I - 21 to 32 (5.5 to 9 kg) of age; II - 33 to 42 (9.1 to 15 kg) of age; III - 43 to 63 (15.1 to 25 kg) of age.
[1] Nutritional values obtained from the ingredients were recommended by Rostagno et al. (2017).
[2] Sand.

Table 2 - Calculated values of enzymes, emulsifier and sapogenin of additives per kg of diet

Item	Experimental diets[1]					
	CD	CDY	CDE	CDME	CDE+Y1	CDE+Y2
Protease (HUT kg^{-1})	-	-	140.0	154.4	140.0	140.0
Phytase (SPU kg^{-1})	-	-	60.0	24.0	60.0	60.0
Cellulase (CMCU kg^{-1})	-	-	8.0	3.2	8.0	8.0
Pectinase (AJDU kg^{-1})	-	-	-	256.0	-	-
β-glucanase (BGU kg^{-1})	-	-	-	12.8	-	-
Xylanase (XU kg^{-1})	-	-	-	6.4	-	-
Amylase (FAU kg^{-1})	-	-	-	1.8	-	-
PEGR (g kg^{-1})	-	-	-	20.8	-	-
Sapogenin (mg kg^{-1})	-	8.1	-	-	8.1	16.3

[1] CD: control diet; CDY: CD with 125 g ton^{-1} of *Yucca schidigera* extract (YSE) (De-Odorase®; guarantee level: 65 mg kg^{-1} of sapogenin); CDE: CD with 200 g ton^{-1} of enzyme complex (EC) (Alltech® Allzyme SSF e+C; guarantee levels: 700 HUT g^{-1} of protease, 300 SPU g^{-1} of phytase and 40 CMCU g^{-1} of cellulase); CDME: CD with 400 g ton^{-1} of multienzyme complex with emulsifier (Alltech® Allzyme Allsotution; guarantee levels: 640 AJDU g^{-1} pectinase, 386 HUT g^{-1} protease, 60 SPU g^{-1} phytase, 32 BGU g^{-1} of β -glucanase, 16 XU g^{-1} of xylanase, 8 CMCU g^{-1} of cellulase, 4.5 FAU g^{-1} of amylase and 52 g kg^{-1} of glyceryl polyethyleneglycol ricinoleate - PEGR); CDE+Y1: CD + EC (200 g ton^{-1}) + YSE (125 g ton^{-1}); CDE+Y2: CD + YSE (200 g ton^{-1}) + YSE (250 g ton^{-1}).

2.3. Productive performance and diarrhea incidence

The animals were weighed at the beginning and end of each phase (0 to 7 days, 0 to 16 days, and 0 to 32 days), as well as the feed leftovers, obtaining the daily feed intake (DFI), daily weight gain (DWG), and feed conversion ratio (FCR).

During the performance evaluation, the incidence of diarrhea in the piglets was assessed. Fecal scores were recorded during the first 21 days of the experimental period. Fecal scores were recorded during the first 21 days of the experimental period. Fecal consistency was visually analyzed twice daily, at 08:00 and 17:00 h, according to the following scores: 1 = normal feces; 2 = pasty feces; 3 = watery feces. Scores 1 and 2 were considered non-diarrheic feces, and 3 were considered diarrhea. These identifications were always performed by the same observer.

2.4. Apparent total tract digestibility

To evaluate the digestibility of the experimental diets, the partial feces collection method was used with the inclusion of 1% Celite as a source of acid-insoluble ash (AIA) in the diet from days 43 to 60 of age as an indigestibility marker (Liu, 2022). After the start of feed consumption with the marker, the feed was provided for three days to maintain the indicator flow through the digestive tract, followed by feces collection for four days.

The partial feces collection method was used, with two feces collections per day (morning and afternoon), directly from the animals' rectum. After collection, the feces were homogenized and stored in plastic bags at −18 °C for later analysis. Feed samples were also collected and stored for analysis.

The feces were thawed at room temperature, homogenized by pen, pre-dried at 55 °C, and ground for the determination of dry matter, organic matter, ash, crude protein, gross energy, neutral detergent fiber, and acid detergent fiber (AOAC, 2005). The gross energy of the feces and feed was determined using a Parr 6100 bomb calorimeter.

Phosphorus content was analyzed by spectrophotometry using a UV-VIS spectrophotometer (UV-5100; Metash Instruments, Shanghai, China) according to the methodology proposed by Rangana (1979).

For calcium determination, samples were analyzed using an atomic absorption spectrometer with a flame atomizer (iCE 3500; Thermo Scientific, Cambridge, UK). A hollow cathode lamp containing Ca

(Photron, Victoria, Australia) was used as the primary radiation source, and background correction was performed with a deuterium lamp coupled to the equipment.

The standard curve was prepared with a calcium standard solution (Specsol, São Paulo, Brazil). Instrumental parameters were used according to the manufacturer's recommendations, and the data were processed using SOLAAR® software (Thermo Scientific, Cambridge, UK).

The apparent total tract digestibility coefficients (ATTDC) of nutrients, energy, and Ca and P availability were calculated according to Adeola (2001), as follows:

$$ATTDC = 1 - \frac{\text{Concentration of AIA in diet} \times \text{Component in feces}}{\text{Concentration of AIA in feces} \times \text{Component in diet}}$$

Retained and excreted phosphorus and nitrogen were calculated as follows:

$$\text{Retained} = \text{Component in diet} - \left(\text{Component in feces} \times \frac{\text{AIA in diet}}{\text{AIA in feces}}\right)$$

$$\text{Excreted} = \text{Component in diet} - \text{Retained component}$$

2.5. Serum biochemical parameters

At the end of each phase (35, 44, and 60 days of age), approximately 10 mL of blood was collected directly from the jugular vein from one animal per experimental unit, selected according to the average weight closest to the treatment average weight. Blood samples were centrifuged at 2500 rpm for 10 minutes to obtain serum, and aliquots were stored in plastic microtubes, identified, and kept at −20 °C until analysis.

All analyses were performed using an automated biochemical analyzer, model Labmax 240 (Labtest® Diagnóstica S.A; Brazil), using Labtest® commercial kits. The serum metabolites analyzed were urea by the enzymatic method (Ureia UV Liquiform (Ref.: 104)) and creatinine by the colorimetric method (Creatinina K (Ref.: 96)). The hepatic serum markers analyzed were: aspartate aminotransferase (AST; AST Liquiform (Ref.: 109)), alanine aminotransferase (ALT), and alkaline phosphatase (ALP; ALP (Ref.: 76)) by the kinetic method, and gamma-glutamyl transferase (GGT; GGT Liquiform (Ref.: 105)) by the modified Szasz method. Serum minerals calcium (Ca Arsenazo Liquiform (Ref.: 95)) and phosphorus (Phosphorus UV Liquiform (Ref.: 12)) were analyzed by the arsenazo and UV photometry methods, respectively.

2.6. Animal slaughter

At the end of the experimental period (60 days of age) the piglets were slaughtered after an 6-hour fast by electrical stunning followed by exsanguination in accordance with the procedures approved by CEUA/UFPB. The piglets were slaughtered following the humane slaughter protocol.

Immediately after slaughter, the animals' abdomens were opened, and the viscera removed. With the aid of forceps and a scalpel, fragments of the duodenum, jejunum, and ileum were collected as described below for subsequent analyses.

2.7. Intestinal histology and goblet cells

For the study of the small intestine structure, samples of approximately 1–10 cm from the proximal duodenum and 25–35 cm from the proximal jejunum and ileum were collected. The intestinal segments were fixed in a solution containing 60% methanol, 30% chloroform, and 10% acetic acid for twelve hours, and kept refrigerated. Shortly after, the solution was replaced with 70% alcohol for the histological analyses.

The samples remained in 70% alcohol for 24 hours, then washed in running water for five minutes, and subsequently dehydrated in a series of increasing alcohol concentrations, passed through a xylene bath, before being embedded in paraffin. Later, microtomy of the paraffin blocks was performed to prepare the histological slides.

The small intestine slides were stained using hematoxylin-eosin staining to determine the following parameters: villus height, crypt depth, and villus width. Based on this data, the villus height:crypt depth ratio (VH:CD), mucosal thickness, and absorptive area were calculated according to a modified methodology described by Moreira Filho et al. (2015).

The count of goblet cells present in the intestinal villi was performed after staining with periodic acid-Schiff (PAS) + hematoxylin. To evaluate the number of goblet cells, eight counts per sample were performed, with a line of 500 µm per villus drawn, totaling 4000 µm per sample. The result was expressed as the number of goblet cells per 2000 µm. For slide evaluation, a light microscope model Olympus BX53 and a Zeiss Axiocam camera coupled with CellSens Dimension digital image capture program was used.

2.8. Relative gene expression of TNF-α and jejunal nutrient transporters

After slaughter, jejunum fragments of approximately 1 cm were collected, washed in saline solution (0.9% NaCl), and small cuts were made in the fragments using a scalpel and scissors. Samples were stored in 2 mL microtubes, and frozen at −80 °C until mRNA isolation.

The mRNA was extracted from the samples using the Qiagen RNeasy® Mini kit (Cat. No.74106), and cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturers' recommendations.

Primers were used for the mRNA expression of the following genes: tumor necrosis factor (TNF-α), sodium-glucose cotransporter type 1 (SGLT-1), dipeptide and tripeptide transporter in enterocytes (PEPT-1), mucin type 2 (MUC-2), sodium-dependent phosphate transporter type 2 (NaPi-IIb), and the reference genes β-actin (ACTB) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Table 3).

Relative gene expression of mRNA was determined by real-time polymerase chain reaction (qPCR) using SYBR Power SYBR® Green Master Mix (Thermo Fisher Scientific) and specific primers. After verifying that the primers amplified with an efficiency of approximately 100%, qPCR tests were performed.

The final volume was calculated for 20 µL of the reaction, containing the following components: 10 µL of SYBR Green QPCR Master mix; 4 µL of each primer; 0.3 µL of diluted reference dye; 0.7 µL of distilled and deionized water, and 5 µL of cDNA. The polymerase chain reaction was performed under the following conditions: 95 °C for 3 min (1 cycle), 95 °C for 15 s, and 60°C for 20 s (40 cycles), 95 °C for 1 min, 55 °C for 30 s, and 95 °C for 30 s (1 cycle).

Table 3 - Primers used for quantitative real-time PCR (qPCR)

Gene	Primer sequence (5'-3')		PS (bp)[1]	Access no.
	Foward	Reverse		
TNF-α	tcaacctcctctctgccatc	cccaggtagatgggttcgta	91	JF831365.1
MUC-2	tccacggggactgctactac	caggtctgcttgtctgtgga	103	XM_021082584.1
NaPi-IIb	tatcccttcagctgggtgac	gggtcagagtcgacgagaac	100	JN983501.1
SGLT-1	cggttggagcttctctgttt	attccattcaaagccaccag	107	MW280290.1
PEPT-1	ccatgttctgggctttgttt	tgatccggctggattttaag	100	AY180903.1
GAPDH	acatggcctccaaggagtaaga	gatcgagttggggctgtgact	101	NM_001206359.1
β-actin	ctggcaccacaccttctaca	gggtcatcttctcacggttg	107	DQ178122.1

[1] Product size in base pairs.

After each test, a melting curve analysis was generated to verify the specificity and purity of all qPCR products, and β-actin and GAPDH were chosen as reference genes to normalize cDNA loading. The qPCR cycles were performed in a thermocycler, and the relative expression was calculated based on the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), using reference genes for expression normalization.

2.9. Statistical analysis

Normality and homogeneity of variances were tested using the Cramer-von Mises and Levene tests, respectively. Variables were subjected to analysis of variance using the GLM procedure of SAS statistical software (SAS On Demand for Academics). The data was evaluated for outliers. The pen was considered the experimental unit except for blood data, for which the animal was considered the experimental unit. The data were analyzed considering treatment and BW block as fixed and random effects, respectively.

The statistical model used for the analyses was:

$$Y = \mu + Ti + Bj + eij,$$

in which Y = observation for each parameter analyzed; μ = overall mean; Ti = treatment effect, where i = 1, 2, 3, 4, 5 and 6; Bj = block effect, where j = 1, 2, 3, 4, and 5; eij = experimental error associated with each observation.

In case of significant difference, means were compared by Tukey's test ($P<0.05$) and considered a tendency when $0.05 < P < 0.10$.

3. Results

Enzymatic additives, with or without plant extracts, influenced the productive performance of weaned piglets (Table 4). From 0 to 7 days, the average daily feed intake (ADFI) of piglets that received CDE was higher (P = 0.023), which resulted in higher average daily gain (ADG) (P = 0.028). Greater feed conversion ratio was observed for CDY (P = 0.001).

Table 4 - Daily feed intake (DFI), daily weight gain (DWG), feed conversion (FC) and final weight (FW) of weaned piglets fed diets containing enzymatic additives associated or not with plant extracts

Item	Experimental diets[1]						SEM	P-value
	CD	CDY	CDE	CDME	CDE+Y1	CDE+Y2		
				0 to 7 days				
DFI (kg)	0.570b	0.567b	0.648a	0.567b	0.580b	0.608ab	0.019	0.023*
DWG (kg)	0.437ab	0.360b	0.495a	0.429ab	0.423ab	0.454a	0.021	0.028*
FC	1.305b	1.577a	1.360b	1.324b	1.373b	1.336b	0.046	0.001*
FW (kg)	10.308	8.925	10.358	9.833	9.767	10.025	0.475	0.819
				0 to 16 days				
DFI (kg)	0.613b	0.595c	0.673a	0.588c	0.562d	0.585c	0.020	<0.001*
DWG (kg)	0.389	0.350	0.430	0.401	0.379	0.369	0.017	0.194
FC	1.577b	1.700a	1.568b	1.468b	1.485b	1.585b	0.072	0.004*
FW (kg)	13.042	12.013	13.767	13.242	12.867	12.750	0.610	0.932
				0 to 32 days				
DFI (kg)	0.783ab	0.809ab	0.831a	0.747bc	0.724c	0.758b	0.027	0.001*
DWG (kg)	0.375	0.360	0.403	0.351	0.353	0.340	0.015	0.193
FC	2.089bc	2.247a	2.064bc	2.128b	2.052c	2.230a	0.060	<0.001*
FW (kg)	19.258	17.925	19.775	18.067	18.108	17.725	0.751	0.742

SEM - standard mean error.
[1] CD: control diet; CDY: CD with 125 g ton^{-1} of *Yucca schidigera* extract (YSE) (De-Odorase®); CDE: CD with 200 g ton^{-1} of enzyme complex (EC) (Alltech® Allzyme SSF e+C); CDME: CD with 400 g ton^{-1} of multienzyme complex with emulsifier (Alltech® Allzyme Allsotution); CDE+Y1: CD + EC (200 g ton^{-1}) + YSE (125 g ton^{-1}); CDE+Y2: CD + YSE (200 g ton^{-1}) + YSE (250 g ton^{-1}).
Means in the same row followed by different letters differ (P≤0.05) by Tukey's test at a 5% probability level.

During the 0 to 16 days period, the ADFI of animals that received diets with Yucca extract (YSE) was lower (P<0.001) compared with those that consumed CD and CDE. However, the ADG did not differ (P>0.05) among the diets. A lower (P = 0.004) feed conversion ratio (FCR) was observed in piglets when exogenous enzymes were added to the diets compared with CDY.

For the entire experimental period (0 to 32 days), the ADFI was lower when the enzymatic additives were combined with YSE (P = 0.001). As in the previous phase, no significant difference in ADG (P>0.05) was observed over the total period. Piglets fed with CDE+Y1 had lower FCR (P = 0.001), while animals that received CDY or CDE+Y2 had the highest FC. The final weight did not differ (P>0.05) among dietary treatments in any of the periods analyzed.

There was no difference (P>0.05) among diets on the incidence of diarrhea (data not shown). Apparent total tract digestibility coefficients (ATTDC) of nutrients, and energy are presented in Table 5. CDME had greater (P = 0.040) dry matter (DM) digestibility compared with CDE+Y2.

The digestibility of organic matter (OM) (P = 0.022), neutral detergent fiber (NDF) (P = 0.005), acid detergent fiber (ADF) (P = 0.014), and energy (P = 0.006) were higher with CDE and CDME compared with CDE+Y2. CDME showed a trend for greater calcium (Ca) availability (P = 0.064) compared with other diets. Overall, there was an improvement with supplementation of additives compared with CD.

For phosphorus (P), CDE, CDME, and CDE+Y1 resulted in higher availability (P = 0.003) than CDE+Y2. CDE+Y2 promoted lower coefficients for DM, OM, NDF, ADF, energy, and P. Fecal nitrogen (N) retention and excretion were not altered by diets (P = 0.05). However, higher P retention (P = 0.004) and lower fecal P excretion (P = 0.007) were observed for CDE, CDME, and CDE+Y1 as compared with CDE+Y2 (Table 6).

Data on apparent total tract digestibility (ATTD) indicate improvements with enzyme supplementation; however, the effects of *Yucca schidigera* extract (YSE) remain inconsistent, highlighting the need for further research in this area.

Serum biochemical parameters characterized by metabolites (urea and creatinine), liver markers (AST, ALT, GGT, and ALP), and minerals (Ca and P) are presented in Table 7. At the end of phase I (sample day 7) GGT was higher (P = 0.014) when YSE was added, regardless of the inclusion level. Piglets fed diets without YSE addition, control diet (CD), and diet with enzymatic complex (CDE) had lower GGT levels than other groups.

At the end of phase II (sample day 16), there was a trend (P = 0.056) of greater CRC level when using CD compared with other diets and significantly lower serum phosphorus (P = 0.043) with DCE+Y2 compared with CD, CDY, and CDE+Y1. After 32 days, the end of phase III, there was greater AST (P = 0.007) in animals consuming diets supplemented with YSE, except for those receiving CDE+Y1, which

Table 5 - Total tract apparent digestibility coefficients (TTADC) of nutrients, energy and mineral availability in diets containing enzymatic additives associated or not with plant extracts for weaned piglets

Diets	DM	OM	CP	NDF	ADF	Energy	Ca	P
CD	0.937ab	0.814ab	0.773	0.652bc	0.346ab	0.80 ab	0.350	0.525ab
CDY	0.930ab	0.787ab	0.724	0.691abc	0.394ab	0.769ab	0.491	0.542ab
CDE	0.936ab	0.831a	0.790	0.797a	0.426a	0.811a	0.475	0.610a
CDME	0.942a	0.834a	0.787	0.755ab	0.459a	0.826a	0.581	0.630a
CDE+Y1	0.932ab	0.809ab	0.759	0.693abc	0.352ab	0.794ab	0.517	0.586a
CDE+Y2	0.923b	0.772b	0.730	0.591c	0.280b	0.742b	0.441	0.439b
SEM	0.002	0.007	0.012	0.018	0.019	0.008	0.022	0.017
P-value	0.040*	0.022*	0.615	0.005*	0.014*	0.006*	0.064	0.003*

DM - dry matter; OM - organic matter; CP - crude protein; NDF - neutral detergent fiber; ADF - acid detergent fiber; GE - gross energy; Ca - calcium; P - phosphorus; SEM - standard mean error.
[1] CD: control diet; CDY: CD with 125 g ton^{-1} of *Yucca schidigera* extract (YSE) (De-Odorase®); CDE: CD with 200 g ton^{-1} of enzyme complex (EC) (Alltech® Allzyme SSF e+C); CDME: CD with 400 g ton^{-1} of multienzyme complex with emulsifier (Alltech® Allzyme Allsotution); CDE+Y1: CD + EC (200 g ton^{-1}) + YSE (125 g ton^{-1}); CDE+Y2: CD + YSE (200 g ton^{-1}) + YSE (250 g ton^{-1}).
Means in the same column followed by different letters differ (P≤0.05) using the Tukey test at a 5% probability level.

Table 6 - Retention and excretion of fecal nitrogen and phosphorus in weaned piglets fed diets containing enzymatic additives associated or not with plant extracts

Diets[1]	Nitrogen (g)		Phosphorus (g)	
	Retained	Excreted	Retained	Excreted
CD	22.79	6.69	3.56ab	3.21ab
CDY	21.19	8.05	3.67ab	3.15ab
CDE	22.79	6.03	4.17a	2.67b
CDME	22.68	6.13	4.31a	2.53b
CDE+Y1	21.95	6.94	3.99a	2.79b
CDE+Y2	21.03	7.79	3.01b	3.85a
SEM	0.352	0.355	0.117	0.118
P-value	0.598	0.567	0.004*	0.007*

SEM - standard mean error.
[1] CD: control diet; CDY: CD with 125 g ton^{-1} of *Yucca schidigera* extract (YSE) (De-Odorase®); CDE: CD with 200 g ton^{-1} of enzyme complex (EC) (Alltech® Allzyme SSF e+C); CDME: CD with 400 g ton^{-1} of multienzyme complex with emulsifier (Alltech® Allzyme Allsotution); CDE+Y1: CD + EC (200 g ton^{-1}) + YSE (125 g ton^{-1}); CDE+Y2: CD + YSE (200 g ton^{-1}) + YSE (250 g ton^{-1}).
Means in the same column followed by different letters differ (P≤0.05) using the Tukey test at a 5% probability level.

Table 7 - Serum biochemical parameters of weaned piglets fed diets containing enzymatic additives associated or not with plant extracts

Item	Experimental diets[1]						SEM	P-value
	CD	CDY	CDE	CDME	CDE+Y1	CDE+Y2		
				Sample day - 7				
UR (mg dL^{-1})	6.13	6.57	1.53	4.93	4.34	2.43	0.87	0.524
CRC (mg dL^{-1})	0.93	0.58	0.71	0.47	0.85	0.43	0.07	0.192
AST (U L^{-1})	62.81	35.34	63.67	45.48	68.89	47.82	4.90	0.318
ALT (U L^{-1})	42.06	25.59	36.63	35.7	33.04	24.52	2.69	0.382
GGT (U L^{-1})	51.42b	102.25ab	58.38b	74.57ab	139.95a	76.31ab	8.12	0.014*
ALP (U L^{-1})	283.06	221.41	209.92	175.54	205.94	205.95	10.58	0.051
Ca (mg dL^{-1})	11.64	9.23	10.11	8.61	9.51	7.51	0.51	0.287
P (mg dL^{-1})	9.59	8.09	9.03	6.73	9.49	6.79	0.44	0.204
				Sample day - 16				
UR (mg dL^{-1})	9.04	11.74	10.70	12.80	14.19	6.97	1.29	0.674
CRC (mg dL^{-1})	0.91	0.71	0.79	0.55	0.61	0.42	0.05	0.056
AST (U L^{-1})	44.63	57.46	65.29	56.18	44.81	40.76	3.13	0.199
ALT (U L^{-1})	50.18	40.37	47.99	49.3	50.72	35.28	2.81	0.609
GGT (U L^{-1})	51.63	84.59	30.99	45.05	57.65	40.83	5.77	0.195
ALP (U L^{-1})	187.77	277.69	236.72	178.54	209.24	216.14	12.52	0.225
Ca (mg dL^{-1})	12.35	10.57	11.86	11.78	10.89	10.20	0.286	0.299
P (mg dL^{-1})	9.15a	9.55a	8.57ab	7.39ab	9.11a	6.43b	0.43	0.043*
				Sample day - 32				
UR (mg dL^{-1})	10.33	19.13	11.70	14.70	19.48	10.67	1.60	0.394
CRC (mg dL^{-1})	0.67	0.78	0.61	0.58	0.69	0.64	0.02	0.225
AST (U L^{-1})	39.17c	75.15ab	63.39abc	84.57a	47.93bc	62.38abc	4.27	0.007*
ALT (U L^{-1})	47.61	55.39	47.71	62.56	50.38	56.08	2.78	0.643
GGT (U L^{-1})	25.85	55.32	37.02	41.29	60.52	41.28	3.96	0.101
ALP (U L^{-1})	192.26	318.09	194.83	186.61	188.52	309.42	17.85	0.063
Ca (mg dL^{-1})	11.24	12.08	11.04	10.96	10.73	12.31	0.286	0.240
P (mg dL^{-1})	9.00	10.19	7.13	8.52	9.24	7.30	0.255	0.436

UR - urea; CRC - creatinine; AST - aspartate aminotransferase; ALT - alanine aminotransferase; ALP - alkaline phosphatase; GGT - gamma-glutamyl transferase; Ca - calcium; P - phosphorus; SEM - standard mean error.
[1] CD: control diet; CDY: CD with 125 g ton^{-1} of *Yucca schidigera* extract (YSE) (De-Odorase®); CDE: CD with 200 g ton^{-1} of enzyme complex (EC) (Alltech® Allzyme SSF e+C); CDME: CD with 400 g ton^{-1} of multienzyme complex with emulsifier (Alltech® Allzyme Allsotution); CDE+Y1: CD + EC (200 g ton^{-1}) + YSE (125 g ton^{-1}); CDE+Y2: CD + YSE (200 g ton^{-1}) + YSE (250 g ton^{-1}).
Means in the same row followed by different letters differ (P≤0.05) by Tukey's test at a 5% probability level.

maintained the same level as CD. There was also a trend (P = 0.063) for higher ALP levels in piglets fed CDY and CDE+Y2.

The addition of exogenous enzymes, with or without YSE, resulted in significantly higher (P<0.001) villus height in the duodenum, as well as crypt depth (P<0.001) compared with animals fed CD and CDY (Table 8).

The villus height:crypt depth ratio (VH:CD) in the duodenum did not differ (P>0.05) among diets. However, villus width, mucosal thickness and absorptive area increased (P<0.001) when diets with enzymatic additives combined with plant extracts were provided, especially CDME.

Jejunal villus height was greater (P<0.001) when piglets received CDME. Overall, diets with additives promoted higher villus height (P<0.001) in piglets compared with those fed CD. Deeper crypts (P<0.004) were observed in piglets fed CDME and CDE+Y2.

There was a greater (P = 0.002) VH:CD when animals received supplemented diets, especially CDE, CDME, and CDE+Y1 compared with CD. Wider jejunal villi (P<0.001) were observed with CDY, CDE, and CDME. Mucosal thickness, and jejunal absorptive area were highest (P<0.001) with CDME. Compared with other diets, supplementation of CDE with YSE regardless of level resulted in the lowest (P<0.001) in jejunal absorptive area.

Table 8 - Intestinal morphometry and goblet cells of weaned piglets fed diets containing enzymatic additives associated or not with plant extract

Item	Experimental diets[1]						SEM	P-value
	CD	CDY	CDE	CDME	CDE+Y1	CDE+Y2		
				Duodenum				
VH (µm)	396.64b	417.55b	458.56a	461.37a	464.33a	471.49a	3.41	<0.001*
CD (µm)	194.04c	208.60bc	231.37a	228.04ab	241.453a	239.321a	2.18	<0.001*
VH:CD	2.12	2.08	2.03	2.07	1.99	2.02	0.02	0.284
VW (µm)	73.77e	86.90d	104.39b	113.15a	98.23c	106.40b	0.75	<0.001*
MT (µm)	590.68b	626.15b	689.93a	689.41a	705.78a	710.81a	4.87	<0.001*
AA (µm^2)	29,18e	36,49d	47,91bc	51,86a	45,83c	50,17ab	519.04	<0.001*
GC	88	85	86	92	86	69	2.92	0.331
				Jejunum				
VH (µm)	316.00d	324.06cd	337.94bcd	395.86a	355.04b	347.54bc	3.24	<0.001*
CD (µm)	169.85b	166.26b	164.77b	188.21a	167.45b	169.67a	1.71	0.004*
VH:CD	1.93b	2.04ab	2.12a	2.15a	2.17a	2.11ab	0.02	0.002*
VW (µm)	97.53b	101.64ab	104.33a	102.20a	76.74c	79.07c	0.76	<0.001*
MT (µm)	485.85b	490.31b	502.71b	584.08a	522.49b	517.22b	4.39	<0.001*
AA (µm^2)	30,48cd	33,39bc	35,37b	40,42a	27,32d	27,73d	418.44	<0.001*
GC	61	60	66	72	83	53	2.95	0.068
				Ileum				
VH (µm)	293.71b	304.05ab	315.07ab	325.33a	295.27b	321.92a	2.69	<0.001*
CD (µm)	163.54ab	156.52abc	149.53bc	146.98c	166.38a	147.51bc	1.65	<0.001*
VH:CD	1.84c	2.02bc	2.19ab	2.28a	1.99bc	2.27a	0.02	<0.001*
VW (µm)	95.03c	89.71c	94.89c	109.11ab	115.63a	105.75b	0.84	<0.001*
MT (µm)	457.25	460.56	464.61	472.31	461.65	469.43	3.31	0.776
AA (µm^2)	28,35b	27,45b	29,68b	35,70a	33,50a	34,08a	393.74	<0.001*
GC	102ab	107a	87abc	80bc	91abc	78c	2.67	0.003*

VH - villus height; CD - crypt depth; VH:CD - villus height:crypt depth ratio; VW - villus width; MT - mucosal thickness; AA - absorptive area; GC - goblet cells (cells in 2000 µm); SEM - standard mean error.
[1] CD: control diet; CDY: CD with 125 g ton^{-1} of *Yucca schidigera* extract (YSE) (De-Odorase®); CDE: CD with 200 g ton^{-1} of enzyme complex (EC) (Alltech® Allzyme SSF e+C); CDME: CD with 400 g ton^{-1} of multienzyme complex with emulsifier (Alltech® Allzyme Allsotution); CDE+Y1: CD + EC (200 g ton^{-1}) + YSE (125 g ton^{-1}); CDE+Y2: CD + YSE (200 g ton^{-1}) + YSE (250 g ton^{-1}).
Means in the same row followed by different letters differ (P≤0.05) by Tukey's test at a 5% probability level.

Supplementations significantly increased (P<0.001) villus height in the ileum of piglets, except CDE+Y1, which also induced greater crypt depth (P<0.001). Shallower crypts (P<0.001) were detected in the ileum of animals receiving supplemented diets, especially CDME. The VH:CD was also higher (P<0.001) in piglets receiving CDME, followed by those fed CDE+Y2 and CDE.

Villus width was greatest (P<0.001) when animals received CDE+Y1, with CD, CDY, and CDE smaller than other diets. Ileal mucosal thickness did not differ (P>0.05) among diets. However, the absorptive area was greater (P<0.001) for CDME, CDE+Y1, and CDE+Y2 than the other diets.

The number of goblet cells in the duodenum was different (P = 0.331) among diets. However, there was a trend (P = 0.068) for a greater presence of goblet cells in the jejunum of animals fed CDE+Y1. In the ileum, CDY resulted in a higher (P = 0.003) number of goblet cells compared with CDME and CDE+Y2. CDE+Y2 had the lowest number of goblet cells in the jejunum and ileum.

The mRNA expressions of the evaluated genes are shown in Table 9. Tumor necrosis factor (TNF-α) was less expressed (P = 0.005) when animals consumed diets with plant extracts, with or without enzymatic supplementation. The expression of mucin type 2 (MUC-2) was lower (P<0.001) jejunum tissue of animals fed CDY, CDME, and CDE+Y2 compared with DC. The sodium-dependent phosphate transporter type 2 (NaPi-IIb) was more highly expressed (P<0.001) when animals received CDE compared with other diets. For the sodium-glucose cotransporter type 1 (SGLT-1), CDE resulted in higher expression (P = 0.001) than other diets except CDY. All supplemented diets were superior to the control diet and caused greater expression (P<0.001) of the dipeptide and tripeptide transporter in enterocytes (PEPT-1), especially CDE+Y2.

Table 9 - Effects of enzyme additives associated or not with plant extracts on the normalized relative abundance of gene mRNA in the jejunal tissue of weaned piglets

Diets[1]	Item				
	TNF-α	MUC-2	NaPi-IIb	SGLT-1	PEPT-1
CD	2.14ab	0.41a	2.78b	1.84b	0.91c
CDY	1.81ab	0.09b	2.62b	2.87ab	2.97ab
CDE	2.77a	0.24ab	5.93a	4.30a	1.54bc
CDME	1.71b	0.11b	2.71b	2.27b	2.59ab
CDE+Y1	1.60b	0.21ab	2.33b	2.07b	1.94abc
CDE+Y2	1.48b	0.12b	2.32b	1.54b	2.97a
SEM	0.173	0.025	0.318	0.245	0.172
P-value	0.005*	<0.001*	<0.001*	0.001*	<0.001*

TNF-α - tumor necrosis factor alpha; MUC-2 - mucin 2; NaPi-IIb - type II sodium-dependent phosphate transporter; SGLT-1 - sodium/glucose cotransporter 1; PEPT-1 - peptide transporter 1; SEM - standard mean error.
[1] CD: control diet; CDY: CD with 125 g ton^{-1} of *Yucca schidigera* extract (YSE) (De-Odorase®); CDE: CD with 200 g ton^{-1} of enzyme complex (EC) (Alltech® Allzyme SSF e+C); CDME: CD with 400 g ton^{-1} of multienzyme complex with emulsifier (Alltech® Allzyme Allsotution); CDE+Y1: CD + EC (200 g ton^{-1}) + YSE (125 g ton^{-1}); CDE+Y2: CD + YSE (200 g ton^{-1}) + YSE (250 g ton^{-1}).
Means in the same row followed by different letters differ (P≤0.05) by Tukey's test at a 5% probability level.

4. Discussion

4.1. Productive performance

From day 0 to 7, piglets fed the CDE+Y2 diet showed higher average daily feed intake (ADFI) and average daily gain (ADG), a beneficial outcome considering the typical decline in performance due to post-weaning stress (Shi et al., 2014). However, higher feed conversion ratios observed in piglets fed CDY and CDE+Y2 from days 0 to 16 and 0 to 32 may be linked to unknown effects of Yucca extract (YSE). Research on YSE inclusion is inconsistent—some studies report slight reductions in body weight (Gebhardt et al., 2019), whereas others show improved feed conversion (Fan et al., 2022). Further

investigation is needed to assess long-term impacts, particularly regarding potential toxicity from excessive saponins (Dos Reis et al., 2016).

The similar growth performance observed in piglets fed CDE and CDME compared with the control diet is likely due to the use of highly digestible ingredients. This may have reduced the effectiveness of enzyme supplementation, as enzyme benefits are typically more pronounced in diets with high levels of antinutritional factors or nutrient limitations (Barros et al., 2014; Ao, et al., 2020; Brandão Melo et al., 2020).

Notably, piglets fed CDE+Y1 showed improved feed conversion from days 0 to 32. Supporting this, a meta-analysis by Torres-Pitarch et al. (2017) found that 61% of studies on enzyme supplementation in weaned piglets reported improvements in growth performance and feed efficiency. These preliminary results suggest that the high digestibility of the basal diet may have masked the potential benefits of supplementation.

The use of combined exogenous enzymes in pig diets is an effective strategy to reduce antinutritional factors, enhance nutrient digestion and absorption, and improve overall performance (Duarte et al., 2019). Supplementation with *Yucca schidigera* extract (YSE) at 120–125 g ton^{-1} has also been linked to improved piglet performance, likely due to enhanced intestinal barrier function (Colina et al., 2001; Gebhardt et al., 2019; Fan et al., 2022; Yang et al., 2021). These benefits are attributed to the glyco-components and saponins present in YSE (Chen et al., 2021), which, when combined with enzyme complexes, contribute to better productive outcomes. However, no effect on diarrhea incidence was observed, differing from previous studies that reported reduced diarrheic scores with enzyme or YSE supplementation (Zhang et al., 2014; Long et al., 2021; Yang et al., 2021). This discrepancy may be due to elevated oxidative stress during weaning, which could have exceeded the mitigating capacity of YSE (Chen et al., 2021).

4.2. Apparent total tract digestibility

The improvements in dry matter (DM), organic matter (OM), NDF, ADF, and energy digestibility observed with CDE and CDME supplementation are attributed to the hydrolysis of otherwise indigestible feed substrates, which become accessible targets for exogenous enzymes (Adeola et al., 2011). The carbohydrases used - amylase, cellulase, β-glucanase, pectinase, and xylanase - are designed to break down compounds primarily found in the cell walls of plant-based ingredients (Ao et al., 2020).

Additionally, CDE, CDME, and CDE+Y1 enhanced nutrient availability and retention while reducing phosphorus (P) excretion, a result consistent with findings of Trindade Neto et al. (2021). This effect is partly due to the inclusion of exogenous protease, which helps degrade the protein matrix surrounding starch granules. When combined with phytase, which hydrolyzes phytate, this enzymatic synergy further improves nutrient and energy release.

Phytase supplementation is well documented as an effective nutritional strategy to reduce dietary and excreted phosphorus (P), addressing both environmental and economic concerns (Torres-Pitarch et al., 2017). Given that inorganic P is a limited, non-renewable resource, and that manure use as fertilizer is a major contributor to P contamination, optimizing dietary P levels in livestock is crucial (Menezes-Blackburn et al., 2015).

Combining enzymes in diets with high in non-starch polysaccharides (NSP) and fiber has shown positive effects on growth performance and feed efficiency in piglets (Kim et al., 2005; Cowieson et al., 2017; Zeng et al., 2018). These enzymes degrade β-glucans and arabinoxylans in the plant cell wall, reducing digesta viscosity in the small intestine. This reduction allows endogenous enzymes to act more effectively, as high viscosity can otherwise hinder their access to substrates (Owusu-Asiedu, 2010; Passos et al., 2015; Li et al., 2018).

The improved nutrient digestibility following enzyme complex supplementation may result from the effective degradation of non-starch polysaccharides (NSP) (Duarte et al., 2019; Li et al., 2021).

Giang et al. (2010) suggested that exogenous enzymes enhance nutrient digestibility in the first two weeks post-weaning due to the underdeveloped proteolytic and amylolytic digestive system in piglets, which matures around the six week of age.

Guo et al. (2022) reported that multi-enzyme complexes improved feed, amino acid, and energy utilization in corn- and soybean-based diets for piglets during the early post-weaning period. These findings suggest that enzyme supplementation may enable newly weaned piglets to extract nutrients and energy from simpler diets more efficiently.

In our study, combining enzyme supplementation with a high level of *Yucca schidigera* extract (YSE, 250 g ton^{-1}) resulted in lower nutrient digestibility, reduced retention, and increased phosphorus excretion. However, supplementation with 125 g ton^{-1} of YSE has been shown to improve ammonia nitrogen utilization, likely enhancing nutrient digestibility (Fan et al., 2022).

The saponins in YSE reduce ammonia levels and support nutrient digestion and absorption at doses up to 200 g ton^{-1}, though higher levels may have adverse effects (Yen and Pond, 1993). The mechanism behind YSE's action remains unclear, as it is believed not to be absorbed by the digestive tract, suggesting no direct metabolic effects (Espinosa-Muñoz et al., 2008). Kaya et al. (2003) proposed that YSE's surfactant activity, particularly from saponins, reduces cell membrane tension in microvilli, facilitating nutrient absorption. However, Shi et al. (2014) cautioned that the efficacy of saponins is dose-dependent, with low levels being beneficial but high levels potentially acting as antinutritional factors.

4.3. Serum biochemical parameters

On day seven, only the liver enzyme GGT differed significantly among treatments, with higher levels observed in diets containing YSE. However, these values remained within the normal physiological range (Kaneko, 1989). As biochemical markers reflect animal performance, and serum creatinine is closely linked to body weight and muscle mass in pigs (Wang et al., 2021), the absence of changes in creatinine levels suggests that amino acid needs were adequately met for optimal muscle growth.

At day 32, increased AST levels were observed in diets supplemented with YSE, except for CDE+Y1, which maintained the level equal to CD. However, AST levels are considered normal (32 to 84 U L^{-1}) according to Kaneko (1989). According to Kurtz and Travlos (2017), the enzymes GGT and AST are considered important markers of liver function and assessment of the enzymatic metabolic profile. Liver tissue damage causes these enzymes to leak into systemic circulation, thereby altering their serum levels.

Yang et al. (2021) found that including 120 g ton^{-1} of YSE in weaned piglet diets did not affect serum ALT levels, indicating its safety at this dosage. However, higher YSE levels led to increased ALT and potential side effects (Dos Reis et al., 2016). Although saponins are not inherently toxic, excessive intake can cause adverse effects. The elevated serum ALP levels observed in piglets fed CDY and CDE+Y2 may reflect compromised intestinal integrity and increased permeability (Bilski et al., 2017; Celi et al., 2019; Wang et al., 2022).

Serum phosphorus (P) levels are known to increase in proportion to dietary P content (Dellaert et al., 1990; Eeckhout et al., 1995). Additionally, phytase supplementation enhances serum P concentrations (Gentile et al., 2003; Beaulieu et al., 2007). Lu et al. (2016) also reported that piglets fed diets richer in calcium (Ca) and phosphorus showed higher serum levels of both minerals.

Enzyme supplementation did not affect serum Ca concentrations. However, we observed lower serum P when there was a higher inclusion of YSE (250 g ton^{-1}) at the end of the second phase (16th day). ATTD of P was 28.03% lower in CDE+Y2 compared with CDE, suggesting that supplementation with YSE at the level of 250 g ton^{-1} negatively impacted mineral utilization. This is corroborated by the 24.97% lower serum P under the same condition.

4.4. Intestinal morphometry and goblet cells

Post-weaning stress is linked to negative changes in intestinal morphology (Spreeuwenberg et al., 2001; Leonard et al., 2011) and impaired intestinal barrier function (Carey et al., 1994). Key indicators of small intestine absorptive capacity include villus height and width, crypt depth, VH:CD ratio, mucosal thickness, and absorptive area (Yang et al., 2021). Structural alterations can lead to the loss of brush border enzymes—such as aminopeptidases and carbohydrases—reducing nutrient digestibility (Kluess et al., 2010). Weaned piglets typically show reduced villus height, limiting absorption (Long et al., 2021). However, piglets receiving enzymatic supplementation, with or without plant extracts, showed improved villus morphology and mucosal thickness. Crypt depth increased proportionally, resulting in no change to the VH:CD ratio.

Jejunal villi development improved with enzymatic supplementation combined with plant extracts, particularly with the inclusion of a multi-enzyme complex plus emulsifier (CDME). Unlike in the duodenum, the VH:CD ratio increased in piglets fed CDE, CDME, and CDE+Y1 compared with the control (CD).

Treatments with CDE and CDME also resulted in wider villi, with CDME promoting greater mucosal thickness and absorptive area—likely due to its higher enzyme concentration enhanced by the emulsifier. Similar improvements were observed in the ileum, supporting the conclusion that combining exogenous enzymes with plant extracts enhances intestinal morphology, consistent with improved digestibility and performance outcomes.

Prior studies have reported positive effects of enzyme supplementation on intestinal structure in weaned piglets (Valverde Piedra et al., 2009; Yi et al., 2013; Jiang et al., 2015; Duarte et al., 2019; Long et al., 2021; Yang et al., 2021; Fan et al., 2022).

Maintaining intestinal integrity is essential for growth and health in piglets, as the villus height-to-crypt depth (VH:CD) ratio reflects nutrient absorption and digestive efficiency (Li et al., 2021). A higher VH:CD ratio is associated with better nutrient utilization (Montagne et al., 2003). These benefits may be attributed to both the improved digestibility and increased post-weaning feed intake observed in piglets fed the supplemented diets, aligning with findings by Zhu et al. (2022) on the relationship between feed intake and intestinal development.

Goblet cells produce mucins that form a protective mucus layer, which helps prevent pathogens from binding to the intestinal epithelium (Xiong et al., 2019). The thickness of this layer and the number of goblet cells are critical for intestinal defense (Song et al., 2022). Enzymatic supplementation combined with YSE at 125 g ton^{-1} (CDE+Y1) increased the number of jejunal goblet cells, likely due to improved intestinal morphology. However, a higher YSE level (250 g ton^{-1}) resulted in fewer goblet cells in both the jejunum and ileum.

In the intestine, toxins can trigger inflammation and impair the protective function of the mucus barrier (Song et al., 2022). While saponins in YSE offer benefits at low inclusion levels, higher concentrations may lead to adverse effects and act as antinutritional factors (Shi et al., 2014; Dos Reis et al., 2016).

4.5. Relative gene expression of TNF-α and jejunal nutrient transporters

The combination of exogenous enzymes and plant extracts reduced the relative mRNA expression of TNF-α, a key pro-inflammatory cytokine. While the exact mechanism behind this anti-inflammatory effect remains unclear (Moita and Kim, 2022), the PGRG emulsifier in the CDME diet may have enhanced lipid digestion and energy utilization, amplifying the benefits of the multienzyme complex. Additionally, the breakdown of NSPs and phytate by carbohydrases and phytase produces oligosaccharides and reduces digesta viscosity, both of which may help lower pro-inflammatory cytokine levels and support immune function in piglets (Agyekum et al., 2015; Lee and Bedford, 2016; Chen et al., 2020).

YSE contains resveratrol and saponins, both of which have strong anti-inflammatory properties. Resveratrol can significantly reduce TNF-α levels (Wenzig et al., 2008), while saponins—especially the protopanaxadiol saponin fraction—have been shown to suppress the expression of inflammatory cytokines (Cheeke et al., 2006; Yang et al., 2015). These properties likely explain the reduced inflammation observed in piglets receiving diets supplemented with enzymes and plant extracts during the early post-weaning phase. However, studies on the immune-modulatory effects of enzyme supplementation, with or without plant extracts, remain limited and likely depend on factors such as physiological stage, diet composition, and enzyme dosage.

The intestinal barrier consists primarily of epithelial cells and a mucosal layer (France and Turner, 2017). Fan et al. (2022) found that supplementation with 125 g/ton of YSE increased jejunal MUC-2 expression, suggesting a role for saponins, phenolics, and polysaccharides in enhancing barrier function. However, in our study, higher MUC-2 expression was observed in piglets fed the control diet, possibly indicating increased mucosal turnover due to reduced nutrient digestibility (Lu et al., 2016). Lower MUC-2 levels in enzyme-supplemented diets may reflect better jejunal integrity and reduced epithelial stress, as supported by findings from Lu et al. (2016) and Shekels et al. (2001).

Our results also showed that CDE supplementation increased jejunal NaPi-IIb mRNA expression by 53.1%, likely due to the inclusion of phytase in the enzyme blend, which enhanced phosphorus availability and retention. This supports findings by Vigors et al. (2014). Other treatments had no significant effect on NaPi-IIb expression, likely due to the predominance of passive (paracellular) phosphorus absorption when dietary P is adequate (Stein et al., 2008; Sabbagh et al., 2011).

Enzyme supplementation also influenced nutrient transporter expression. CDE increased SGLT-1 expression by 54.2%, and diets containing enzymes —especially CDY and CDE+Y2 — upregulated PEPT-1. This is likely due to the hydrolysis of antinutritional factors, which increased the availability of glucose and peptides for absorption. Similar effects were reported by Zhu et al. (2022).

Our findings are consistent with those of Fan et al. (2022), who observed increased SGLT-1 expression in piglets fed 125 g ton^{-1} of YSE. Although PEPT-1 was unaffected in that study, there were significant increases in other nutrient transporters (e.g., CAT-1, ASCT-1, rBAT, FATP-4), which support nutrient absorption and play key roles in metabolic and signaling pathways.

Given the swine industry's need for sustainable production strategies, our results highlight the potential of combining enzyme complexes with plant extracts to enhance intestinal health and nutrient metabolism in weaned piglets. Further research is needed to determine optimal inclusion rates, timing, and interactions with other feed additives to fully harness these benefits.

5. Conclusions

The inclusion of enzymatic additives, either alone or in combination with other dietary additives, positively impacted the productive performance, nutrient digestibility, and intestinal health of weaned piglets. The combination of enzymatic additives and plant extracts demonstrated a synergistic effect, enhancing the individual benefits of each additive. However, further studies are necessary to elucidate the underlying mechanisms of action and the interactions between these additives in swine diets. Future research should also focus on analyzing the intestinal microbiota and optimizing the dosage of *Yucca schidigera* extract for piglets.

Data availability

The data that support the findings of this study are available on request from the corresponding author. The data is not publicly available due to privacy or ethical restrictions.

Author contributions

Conceptualization: Silva, W. A.; Pascoal, L. A. F. and Costa e Silva, L. F. **Data curation:** Silva, W. A. and Pascoal, L. A. F. **Formal analysis:** Silva, W. A.; Almeida, J. L. S.; Medeiros, C. J.; Silva, M. B.; Soares, P. C.; and Araújo, W. J. **Funding acquisition:** Pascoal, L. A. F. and Costa e Silva, L. F. **Investigation:** Silva, W. A.; Pascoal, L. A. F.; Almeida, J. L. S.; Medeiros, C. J.; Silva, M. B. and Azevedo, M. L. **Methodology:** Silva, W. A.; Pascoal, L. A. F.; Guerra, R. R.; Soares, P. C. and Araújo, W. J. **Project administration:** Pascoal, L. A. F. **Resources:** Pascoal, L. A. F. **Supervision:** Pascoal, L. A. F.; Givisiez, P. E. N. and Watanabe, P. H. **Validation:** Silva, W. A.; Pascoal, L. A. F. and Costa e Silva, L. F. **Visualization:** Silva, W. A.; Pascoal, L. A. F.; Costa e Silva, L. F.; Guerra, R. R. and Giviziez, P. E. N. **Writing – original draft:** Silva, W. A.; Pascoal, L. A. F. and Costa e Silva, L. F. **Writing – review & editing:** Silva, W. A.; Pascoal, L. A. F.; Costa e Silva, L. F.; Guerra, R. R.; Giviziez, P. E. N. and Watanabe, P. H.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We thank Alltech do Brazil and the Coordenação de Aperfeiçoamento de Pessoal e Nível Superior (CAPES) for the financial support provided for the completion of this study.

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