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Energy-reduced diets supplemented with xylanase, *Bacillus* sp., and yeast wall maintain bone parameters, gut morphometry, economic indices, and performance of pigs

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ABSTRACT - This study aimed to investigate the effects of xylanase (Xyl) individually or in combination with *B. subtilis* and *B. licheniformis* associated or not with yeast cell wall in pig diets with a reduction of 100 kcal/kg of metabolizable energy (ME) content. Pig performance, bone parameters, intestinal morphometry, and bioeconomic indices were evaluated. A total of 75 pigs (25 females and 50 barrows; 25.02±3.21 kg) obtained from crossing Large White × Landrace were used. The experimental design consisted of randomized blocks, with five treatments and five blocks, totaling 25 experimental units subjected to treatments: basal diet (B); basal diet with reduction of 100 kcal/kg of ME (BEM); BEM with supplementation of xylanase (0.01%; BX); BX with supplementation of direct-fed microbials (composed of *B. subtilis* and *B. licheniformis*; 0.02%; BXM); and BX supplemented with 0.10% of symbiotic (which includes *B. subtilis*, *B. licheniformis*, and yeast cell wall; BXS). There were no differences in bone parameters. The BEM diet resulted in a lower villus height:crypt depth ratio in the jejunum than that seen upon using the BXS diet as feed. In the first period, pigs fed BEM diet had a 12% higher average daily feed intake than those fed the BXM diet. There were no differences in the pig performance during the second period. Over the total period, pigs fed BEM diet had greater FCR compared with pigs fed BXM diet. The inclusion of feed additives in diets with reduced ME content contributes to the maintenance of performance and characteristics of the metacarpus and jejunum of growing pigs and provides better bioeconomic indices.

Keywords: *Bacillus*, energy restriction, feed additive, nutrition, swine

1. Introduction

Antinutritional factors impact efficiency and economic return in swine production. Non-starch polysaccharides (NSP), antinutritional factors found in plant cell walls, are associated with reduced nutrient digestibility and feed efficiency (Dong et al., 2018) and impaired gut health (Adeola and Cowieson, 2011). The use of feed additives as a nutritional strategy can help overcome these issues.

Xylanase (Xyl) hydrolyses arabinoxylans, a NSP present in grains and byproducts, by breaking 1,4-β-D-xylopyranosyl bonds in xylans (Dong et al., 2018; Moita and Kim, 2022). Including it in diets

enhances digestion by reducing gut viscosity or breaking down endosperm cells, thereby facilitating enzymatic activity (Bedford and Cowieson, 2012; Cardoso et al., 2018). Xylanase also favors energy efficiency of pigs through the production of short-chain fatty acids (SCFA) in the gut, which originates from the fermentation of arabinoxylans hydrolysis products (Torres-Pitarch et al., 2019). The SCFA are fuels for intestinal cells (Montagne et al., 2003) contributing to the energy supply for maintenance.

Swine diets supplemented with direct-fed microbials (DFM), such as *Bacillus subtilis* and *Bacillus licheniformis*, have the potential to reduce energy expenditure for maintenance. This is due to improved gut integrity, decreased immune activation, and reduced endogenous nutrient losses (Jaworski et al., 2017). *B. subtilis* can modify the gut microbiota, promote intestinal and bone health (Jiang et al., 2021), and produce enzymes that improve nutrient digestibility (Leser et al., 2008). Yeasts and their products are also able to modify immunological functions and benefit animal health, metabolism, and performance (Broadway et al., 2015). The mixture of *Bacillus* spp. and yeast cell wall forms a symbiotic, a combination of live microorganisms and substrates selectively used by host microorganisms, which promotes benefit to the host's health (Swanson et al., 2020). Moreover, Xyl generates prebiotics in the pig gut (Choct, 2015).

Based on this, the feed additives could reduce the impacts of NSP, favoring intestinal health and increasing the availability of nutrients and energy for pigs fed diets with reduced metabolizable energy (ME). To our knowledge, the synergism between them has not been investigated in growing pigs. Thus, this study aimed to evaluate performance, bone parameters, intestinal morphometry, and bioeconomic indices in growing pigs fed diets with a reduction of ME content and inclusion of Xyl individually or in combination with DFM associated or not with the yeast cell wall.

2. Material and Methods

Procedures were performed under the guidelines of the Institutional Committee on the Use of Animals (No. 0031-12-2018). Experiments were performed in Seropédica, Rio de Janeiro, Brazil, (at 22°45' S and 43°41' W, at an altitude of 33 m). A total of 75 pigs (25 females and 50 barrows; 25.0±3.21 kg) obtained from crossing Large White × Landrace were used. Piglets were distributed in five groups following a randomized block design; blocks were formed according to average body weight (ABW). There were five repetitions per treatment, and experimental units consisted of two barrows and one female housed in 2.0 × 1.0 m stone pens equipped with semi-automatic feeders and nipple drinkers. Pigs were fed experimental diets *ad libitum* for 30 days. They remained in the experiment for 30 days, divided into growth phase 1 (25 to 47 kg BW; days 1 to 22) and growth phase 2 (47 to 55 kg BW; days 23 to 30). At the end of this period, one barrow from each repetition with an ABW representative of the experimental unit was maintained for seven more days until slaughter. Treatments were: basal diet (B); basal diet with reduction of 100 kcal/kg of ME (BEM); BEM with supplementation of xylanase (0.01%; BX); BX with supplementation of direct-fed microbials (composed of *B. subtilis* and *B. licheniformis*; 0.02%; BXM); and BX supplemented with 0.10% of symbiotic (which includes *B. subtilis*, *B. licheniformis*, and yeast cell wall; BXS).

The composition and nutrient content of diets provided during the grower phases 1 and 2 are presented in Tables 1 and 2; respectively. The basal diet was formulated to meet the nutritional requirements of barrows in growth phases 1 and 2, according to Rostagno et al. (2017), while the other diets did not meet the complete ME requirements of pigs. All diets contained phytase (0.01%), and its contribution to the nutritional matrix was considered with the added value for digestible aminoacids, available phosphorus, calcium, and crude protein. The Xyl contribution to the nutritional matrix of the diets (50 kcal/kg of feed) was also considered. The reduction in ME content was based on other studies that investigated the use of Xyl in pig diets (Martínez-Aispuro et al., 2015, 2017). The methods for determination of dry matter, gross energy, crude protein, and total phosphorus of the diets are described in Silva and Queiroz (2006).

Table 1 - Ingredients and composition of the experimental diets in grower phase 1 (first period from 1 to 22 days of the experiment)

Ingredient (%)	Diet[1]				
	Basal	BEM	BX	BXM	BXS
Maize	55.68	58.16	58.16	58.16	58.16
Soybean meal	18.81	18.36	18.36	18.36	18.36
Wheat bran	18.30	18.30	18.30	18.30	18.30
Soy oil	3.83	1.79	1.79	1.79	1.79
Limestone	1.08	1.08	1.08	1.08	1.08
Inert[2]	0.75	0.75	0.74	0.72	0.64
Salt	0.43	0.43	0.43	0.43	0.43
Mineral-vitamin supplement[3]	0.40	0.40	0.40	0.40	0.40
L-Lysine HCL	0.38	0.39	0.39	0.39	0.39
DL-Methionine	0.10	0.10	0.10	0.10	0.10
L-Threonine	0.14	0.14	0.14	0.14	0.14
Choline chloride	0.04	0.04	0.04	0.04	0.04
L-Tryptophan	0.03	0.03	0.03	0.03	0.03
Dicalcium phosphate	0.03	0.02	0.02	0.02	0.02
Phytase[4]	0.01	0.01	0.01	0.01	0.01
L-Valine	0.01	0.01	0.01	0.01	0.01
Xylanase[5]	-	-	0.01	0.01	0.01
Direct-fed microbials[6]	-	-	-	0.02	-
Symbiotic[7]	-	-	-	-	0.10
Total	100.00	100.00	100.00	100.00	100.00
Chemical composition					
Dry matter (%)	88.12	88.46	88.27	88.00	87.95
Gross energy (kcal/kg)	3,865	3,745	3,769	3,758	3,740
Crude protein (%)	14.47	14.67	14.20	14.50	14.27
Total phosphorus (%)	0.36	0.39	0.36	0.38	0.34
Calculated composition					
Metabolizable energy (kcal/kg)	3,250	3,150	3,150	3,150	3,150
Digestible aminoacids (%)					
Lysine	0.95	0.95	0.95	0.95	0.95
Methionine	0.31	0.31	0.31	0.31	0.31
Methionine + cystine	0.55	0.55	0.55	0.55	0.55
Threonine	0.61	0.61	0.61	0.61	0.61
Tryptophan	0.19	0.19	0.19	0.19	0.19
Valina	0.65	0.65	0.65	0.65	0.65
Leucine	1.19	1.20	1.20	1.20	1.20
Isoleucine	0.55	0.55	0.55	0.55	0.55
Phenylalanine	0.67	0.67	0.67	0.67	0.67
Phenylalanine + tyrosine	1.16	1.16	1.16	1.16	1.16
Histidine	0.38	0.38	0.38	0.38	0.38
Digestible calcium (%)	0.52	0.52	0.52	0.52	0.52
Crude fiber (%)	3.54	3.56	3.56	3.56	3.56
Sodium (%)	0.19	0.19	0.19	0.19	0.19

[1] BEM - basal diet with reduction of 100 kcal/kg of ME; BX - BEM with supplementation of xylanase (0.01%); BXM - BX with supplementation of direct-fed microbials (composed of *B. subtilis* and *B. licheniformis*; 0.02%); BXS - BX supplemented with 0.10% of symbiotic (which includes *B. subtilis*, *B. licheniformis*, and yeast cell wall).
[2] Inert: washed sand.
[3] Composition per kg of product: iron (min): 8,750 mg; copper (min): 3,750 mg; manganese (min): 6,250 mg; zinc (min): 18.75 g; iodine (min): 250 mg; selenium (min): 75 mg; vitamin A (min): 1,000,000 IU; vitamin D3 (min): 150,000 IU; vitamin E (min): 3,000 IU; vitamin K3 (min): 750 mg; vitamin B1 (min): 150 mg; vitamin B2 (min): 875 mg; vitamin B6 (min): 250 mg; vitamin B12 (min): 4,500 mcg; niacin (min): 5,000 mg; biotin (min): 7.5 mg; choline chloride (min): 40 g.
[4] Smizyme Phytase 10,000 FTU/g.
[5] Smizyme Xylanase 10,000 U/g.
[6] Smibiotics (*B. subtilis* 10^{10}, *Bacillus licheniformis* 10^{10}).
[7] Vitabiotic *B. subtilis* 10^{10}, *Bacillus licheniformis* 10^{10}, mannan-oligosaccharides 7.00%, glucans and beta-glucans 13.00%.

Table 2 - Ingredients and composition of the experimental diets in grower phase 2 (second period from 23 to 30 days of the experiment)

Ingredient (%)	Diet[1]				
	Basal	BEM	BX	BXM	BXS
Maize	71.49	73.97	73.97	73.97	73.97
Soybean meal	16.82	16.37	16.37	16.37	16.37
Wheat bran	4.19	4.19	4.19	4.19	4.19
Soy oil	2.30	0.26	0.26	0.26	0.26
Limestone	0.81	0.82	0.82	0.82	0.82
Inert[2]	3.00	3.00	2.99	2.97	2.89
Salt	0.40	0.40	0.40	0.40	0.40
Mineral-vitamin supplement[3]	0.45	0.45	0.45	0.45	0.45
L-Lysine HCL	0.32	0.33	0.33	0.33	0.33
L-Threonine	0.09	0.09	0.09	0.09	0.09
DL-Methionine	0.06	0.06	0.06	0.06	0.06
Choline chloride	0.03	0.03	0.03	0.03	0.03
L-Tryptophan	0.03	0.03	0.03	0.03	0.03
Phytase[4]	0.01	0.01	0.01	0.01	0.01
Xylanase[5]	-	-	0.01	0.01	0.01
Direct-fed microbials[6]	-	-	-	0.02	-
Symbiotic[7]	-	-	-	-	0.10
Total	100.00	100.00	100.00	100.00	100.00
Chemical composition					
Dry matter (%)	88.20	88.35	88.04	88.06	88.32
Gross energy (kcal/kg)	3,878	3,762	3,744	3,773	3,746
Crude protein (%)	12.93	12.85	12.60	12.30	12.23
Total phosphorus (%)	0.25	0.26	0.26	0.27	0.28
Calculated composition					
Metabolizable energy (kcal/kg)	3,250	3,150	3,150	3,150	3,150
Digestible aminoacids (%)					
Lysine	0.82	0.82	0.82	0.82	0.82
Methionine	0.26	0.26	0.26	0.26	0.26
Methionine + cystine	0.48	0.48	0.48	0.48	0.48
Threonine	0.53	0.53	0.53	0.53	0.53
Tryptophan	0.16	0.16	0.16	0.16	0.16
Valine	0.58	0.58	0.58	0.58	0.58
Leucine	1.16	1.17	1.17	1.17	1.17
Isoleucine	0.50	0.50	0.50	0.50	0.50
Phenylalanine	0.61	0.61	0.61	0.61	0.61
Phenylalanine + tyrosine	1.06	1.06	1.06	1.06	1.06
Histidine	0.35	0.35	0.35	0.35	0.35
Digestible calcium (%)	0.38	0.38	0.38	0.38	0.38
Crude fiber (%)	2.43	2.46	2.46	2.46	2.46
Sodium (%)	0.17	0.17	0.17	0.17	0.17

[1] BEM - basal diet with reduction of 100 kcal/kg of ME; BX - BEM with supplementation of xylanase (0.01%); BXM - BX with supplementation of direct-fed microbials (composed of *B. subtilis* and *B. licheniformis*; 0.02%); BXS - BX supplemented with 0.10% of symbiotic (which includes *B. subtilis*, *B. licheniformis*, and yeast cell wall).
[2] Washed sand.
[3] Composition per kg of product: iron (min): 8,750 mg; copper (min): 3,750 mg; manganese (min): 6,250 mg; zinc (min): 18.75 g; iodine (min): 250 mg; selenium (min): 75 mg; vitamin A (min): 1,000,000 IU; vitamin D3 (min): 150,000 IU; vitamin E (min): 3,000 IU; vitamin K3 (min): 750 mg; vitamin B1 (min): 150 mg; vitamin B2 (min): 875 mg; vitamin B6 (min): 250 mg; vitamin B12 (min): 4,500 mcg; niacin (min): 5,000 mg; biotin (min): 7.5 mg; choline chloride (min): 40 g.
[4] Smizyme Phytase 10,000 FTU/g.
[5] Smizyme Xylanase 10,000 U/g.
[6] Smibiotics (*B. subtilis* 10^{10}, *Bacillus licheniformis* 10^{10}).
[7] Vitabiotic *B. subtilis* 10^{10}, *Bacillus licheniformis* 10^{10}, mannan-oligosaccharides 7.00%, glucans and beta-glucans 13.00%.

On day 30, the barrow with ABW representative of the experimental unit was selected for the collection of biological materials. On day 37, 25 barrows were slaughtered (one barrow from each repetition, totaling five barrows per treatment). Stunning was performed using electronarcosis. Electrodes were attached to the ear of each pig, and a voltage of 220 V was applied. After 15 s of electrical discharge and by verifying the effective stunning signals, the electrical equipment was turned off and bleeding was made through the section of the vessels in the neck following the recommendations of Morés et al. (2014). All procedures were performed by a qualified team following the guidelines for euthanasia of the National Regulation Council of Animal Experimentation (CONCEA, 2013). The joint between the carpal of the proximal row and the radial and ulnar bones was ruptured to remove and store the right leg of each animal to obtain the metacarpal. Segments of the jejunum, approximately 5 cm in length, were collected and immediately stored in glass jars containing formaldehyde until the slides were prepared for intestinal histological analysis. The methodology for collection and storage of jejunum samples was based on Junqueira et al. (2009). Metacarpals were removed using a surgical scalpel, washed, and dissected. After drying at room temperature, weight *in natura* was determined using 0.0001 g precision digital scales. Bone length and vertical and horizontal diameters were measured using a digital pachymeter. The procedures from metacarpal preparation to physical analysis were adapted from Ferreira (2015) because the bones were not defatted for chemical analysis in our assay. The Seedor index was calculated by dividing the bone weight *in natura* (mg) by the bone length (mm) (Seedor et al., 1991). The methods described by Silva and Queiroz (2006) were used to determine the dry matter, mineral matter, and total phosphorus content of the metacarpals. The mineral solution was prepared using the ash, and phosphorus content was quantified via colorimetry. For bone parameters, the metacarpal collected was considered as the experimental unit, totaling five repetitions per treatment. Fragments of the jejunum were fixed in 10% neutral buffered formaldehyde and cut into 1 cm (length) × 3 mm (width) slices, which were subjected to histological procedures ending in slide staining using the hematoxylin-eosin technique. Each fragment originated five cuts which were then affixed to the histological slides. Morphometry of the intestinal epithelium was evaluated using five sections of the jejunum from each pig and observed using a microscope with a coupled camera at 10X magnification. The techniques for preparing and staining the histological slides were also based on Junqueira et al. (2009) but with some changes such as the size of the jejunum samples collected and the size of the fragments obtained from them. Images were captured using the Pro-Image J software. The height of the villi (μm) and depth of the crypts (μm) were measured using Image J. In each section, three intact villi perpendicular to the lumen and three crypts were measured, with two closest to the ends of the section and one close to the center. Mean villus height and crypt depth were calculated for each section. For the analysis of intestinal morphometry, each jejunum section was considered an experimental unit, thus totaling 25 repetitions per treatment.

The bioeconomic indices were assessed by calculating the cost of feed per kg of weight gain, according to Bellaver et al. (1985), and the economic efficiency index (EEI) and cost index (CI) were determined as proposed by Fialho et al. (1992). The price quotation of the ingredients of the diet (Table 3) was made during the trial period (beginning of 2019) using specialized portals and websites of companies selling products for animal feed to obtain the cost per kg of each diet.

Pigs were weighed without fasting on days 1, 22, and 30. Feed intake was calculated by the difference between the amount of feed provided and refusals. The feed conversion ratio was calculated by the amount of feed consumed divided by weight gain. The cost of feed per kg of weight gain was calculated using the equation: $Yi = (Qi \times Pi)/Gi$, in which Yi is the cost of feed per kg of weight gained in the treatment, Qi is the amount of feed consumed in the treatment, Pi is the price per kg of feed used in the treatment, and Gi is the weight gain of pigs in the treatment. The EEI was calculated as $EEI = (MCei/Ctei) \times 100$, in which Mcei is the lowest cost of feed per kg of weight gain observed between treatments and Ctei is the cost of feeding in the treatment (i.e., cost of kg of feed × the amount of feed consumed in the treatment). The CI was calculated as $CI = (Ctei/Mcei) \times 100$. Data were subjected to analysis of variance (ANOVA), and the mean values obtained for each treatment were subjected to Tukey's test with a 5% significance level ($P = 0.05$). Analyses were performed using SISVAR software. The following statistical model was used:

$$Yij = \mu + Bj + Ni + Eij,$$

in which *Yij* = value observed in the experimental unit of the *j*-th block that received the *i*-th treatment; μ = general average; *Bj* = effect of block j (1, 2, 3, 4, 5); *Ni* = effect of experimental treatments; *i* = B, BEM, BX, BXM, BXS; and *Eij* = random error related to each observation. A global comparison was performed for data referring to bioeconomic indices.

Table 3 - Cost of experimental diet ingredients

Ingredient	Cost/kg (R$)[1]
Maize	0.65
Soybean meal	1.36
Wheat bran	0.87
Soy oil	2.93
Limestone	6.00
Inert	1.20
Salt	0.30
Mineral-vitamin supplement	6.07
L-Lysine HCL	6.91
DL-Methionine	10.67
L-Threonine	6.18
Choline chloride	8.47
L-Tryptophan	48.77
L-Valine	20.27
Dicalcium phosphate	2.17
Phytase	11.22
Xylanase	34.44
Direct-fed microbials	25.15
Symbiotic	13.14

[1] Obtained by the quotation carried out in the beginning of 2019.

3. Results

For the diets used in the first period (1-22 days), the gross energy content was higher for the basal diet (Table 1). This difference to the diets BEM, BX, BXM, and BXS was 120, 96, 107, and 125 kcal/kg, respectively. In the second period (23-30 days), the basal diet also had the highest gross energy content—116, 134, 105, and 132 kcal/kg higher than that for diets BEM, BX, BXM, and BXS, respectively (Table 2).

In the first period, pigs fed BEM diet had an average daily feed intake (ADFI) 12% higher than those fed the BXM diet (P = 0.033) (Table 4). There were no differences (P>0.05) in the performance of pigs during the second period. During the total period (1-30 days), pigs fed the BEM diet had a greater feed conversion ratio (FCR) compared with those fed the BXM diet (P = 0.048).

There were no changes in the Seedor Index or other physical characteristics of the pig metacarpals (P>0.05) (Table 5). Furthermore, bone ash and phosphorus contents remained similar. The villus height:crypt depth ratio (VH:CD) decreased in the jejunum of pigs fed the BEM diet than in those fed the BXS diet (P = 0.030; Table 6).

The highest cost per kg of feed was observed for the basal diet, which was higher (5, 4, and 3%) than per kg costs of diets BEM and BX, BXM, and BXS, respectively (Table 7). The basal diet had the highest cost per kilogram of pigs produced—9.17, 7.20, 4.85, and 1.28% higher than that for diets BXM, BX, BXS, and BEM, respectively. The lowest cost per kilogram of pig was attributed to the BXM diet. Consequently, the best EEI and CI were achieved with the BXM diet.

Table 4 - Initial body weight (IBW), average daily feed intake (ADFI), average daily gain (ADG), feed conversion ratio (FCR), and final body weight (FBW) of growing pigs fed the experimental diets

Variable	Experimental diet[1]					CV (%)	P-value
	Basal	BEM	BX	BXM	BXS		
			First period				
IBW (kg)	25.22	25.01	24.97	25.02	24.87	1.62	0.767
ADFI (kg/d)	2.216ab	2.231b	2.044ab	1.993a	2.141ab	7.44	0.033
ADG (kg/d)	0.999	1.016	0.951	0.966	0.981	7.59	0.661
FCR	2.22	2.28	2.14	2.06	2.18	5.33	0.068
FBW (kg)	47.19	47.37	45.89	46.27	46.47	3.70	0.628
			Second period				
ADFI (kg/d)	2.725	2.899	2.871	2.589	2.710	8.07	0.214
ADG (kg/d)	1.042	1.052	1.128	0.978	1.058	7.37	0.096
FCR	2.61	2.75	2.54	2.65	2.56	4.86	0.111
FBW (kg)	55.53	55.79	54.91	54.10	54.94	3.58	0.702
			Total period				
ADFI (kg/d)	2.352	2.475	2.264	2.152	2.292	7.26	0.082
ADG (kg/d)	1.010	1.026	0.998	0.969	1.002	6.4	0.718
FCR	2.33ab	2.41b	2.26ab	2.22a	2.29ab	4.13	0.048

CV - coefficient of variation.
[1] BEM - basal diet with reduction of 100 kcal/kg of ME; BX - BEM with supplementation of xylanase (0.01%); BXM - BX with supplementation of direct-fed microbials (composed of *B. subtilis* and *B. licheniformis*; 0.02%); BXS - BX supplemented with 0.10% of symbiotic (which includes *B. subtilis*, *B. licheniformis*, and yeast cell wall).
a-b - Different letters in the same row indicate a significant difference (P<0.05) by Tukey's test.

Table 5 - Physical and chemical characteristics of the metacarpal of pigs at 107 days of age fed the experimental diets

Variable	Experimental diet[1]					CV (%)	P-value
	Basal	BEM	BX	BXM	BXS		
Fresh weight (g)	16.24	17.26	16.25	16.17	16.54	7.00	0.561
Length (mm)	63.94	65.38	63.57	62.29	63.27	4.01	0.451
Horizontal diameter (mm)	15.22	15.13	15.23	15.11	15.94	4.07	0.231
Vertical diameter (mm)	12.34	12.92	12.83	12.42	12.78	4.73	0.465
Seedor index	253.00	263.00	255.00	258.60	261.40	3.87	0.443
Ash (%)	34.37	34.03	33.95	33.92	32.15	4.13	0.146
Phosphorus (%)	12.81	12.45	12.03	11.85	11.42	6.81	0.123

CV - coefficient of variation.
[1] BEM - basal diet with reduction of 100 kcal/kg of ME; BX - BEM with supplementation of xylanase (0.01%); BXM - BX with supplementation of direct-fed microbials (composed of *B. subtilis* and *B. licheniformis*; 0.02%); BXS - BX supplemented with 0.10% of symbiotic (which includes *B. subtilis*, *B. licheniformis*, and yeast cell wall).

Table 6 - Morphometric parameters of swine jejunum at 107 days of age, fed the experimental diets

Variable	Experimental diet[1]					CV (%)	P-value
	Basal	BEM	BX	BXM	BXS		
Villus height (µm)	647.31	633.28	673.95	615.33	651.38	10.10	0.694
Crypt depth (µm)	253.77	265.41	245.20	221.69	201.68	14.06	0.052
VH:CD (µm)	2.64ab	2.41b	2.78ab	2.79ab	3.27a	13.48	0.030

VH:CD - villus height to crypt depth ratio; CV - coefficient of variation.
[1] BEM - basal diet with reduction of 100 kcal/kg of ME; BX - BEM with supplementation of xylanase (0.01%); BXM - BX with supplementation of direct-fed microbials (composed of *B. subtilis* and *B. licheniformis*; 0.02%); BXS - BX supplemented with 0.10% of symbiotic (which includes *B. subtilis*, *B. licheniformis*, and yeast cell wall).
a-b - Different letters in the same row indicate a significant difference (P<0.05) by Tukey's test.

Table 7 - Bioeconomic parameters of experimental diets

Parameter	Experimental diet[1]				
	Basal	BEM	BX	BXM	BXS
Cost kg/feed (R$)	1.03	0.98	0.98	0.99	1.00
Feed cost/kg of weight gained (R$)	2.38	2.35	2.22	2.18	2.27
Economic efficiency index	91.30	92.72	98.15	100.00	95.84
Cost index	109.52	107.85	101.89	100.00	104.34

[1] BEM - basal diet with reduction of 100 kcal/kg of ME; BX - BEM with supplementation of xylanase (0.01%); BXM - BX with supplementation of direct-fed microbials (composed of *B. subtilis* and *B. licheniformis*; 0.02%); BXS - BX supplemented with 0.10% of symbiotic (which includes *B. subtilis*, *B. licheniformis*, and yeast cell wall).

4. Discussion

One of the hypotheses of this study was that feed additives in diets with ME below the minimum recommendation could maintain the performance of pigs. When compared with the BEM diet, the best value of FCR was observed when Xyl was associated with DFM (BXM diet). The best FCR for the pigs fed the BXM diet in the total experimental period can be explained by the 12% lower ADFI during the first 22 days. The lower ADFI may be associated with a higher availability of nutrients caused by the synergistic effect between the feed additives. This is because the NSP present in vegetal ingredients increase digest viscosity and can impair nutrient digestibility, such as carbohydrates, which is a crucial factor in energy availability, in corn-and soybean meal-based diets (Suryanarayana, 2013; Dong et al., 2018). As the diets have a higher content of these ingredients, the use of Xyl with DFM can increase nutrient digestibility, which leads to a better FCR. The Xyl enzyme catalyzes the hydrolysis of glycoside bonds in xylans. This process provides nutrients for absorption, reduces digest viscosity, and releases encapsulated nutrients (Cardoso et al., 2018; Dong et al., 2018). The Xyl use enhances nutrient digestibility as well as energy and/or feed efficiency, even in pigs fed corn, soybean meal, and wheat bran-based diets (He et al., 2010; Passos et al., 2015; Lan et al., 2017; Petry et al., 2019). Xylanase can also improve the energy efficiency of pigs through the production and absorption of SCFA in the gut, which originate from the fermentation of products of the AX hydrolysis (Torres-Pitarch et al., 2019). The SCFA are used as fuel by intestinal cells (Montagne et al., 2003; Metzler and Mosenthin, 2008) contributing to the energy supply for maintenance. Strains of *B. subtilis* and *B. licheniformis* were associated with Xyl production and can be used to ameliorate the adverse effects of NSP in the gastrointestinal tract of pigs and promote nutrient availability. Upadhaya et al. (2015) reported that *Bacillus* spp. can produce enzymes that hydrolyze soybean NSP, which may also be related to the results obtained in the FCR in pigs fed the BXM diet in this study.

Diets BX and BXS did not alter pig performance. The symbiotic had yeast cell wall, which is composed of polysaccharides β-glucans and mannans (Shurson, 2018). Mannan-oligosaccharides (MOS) prevent the adhesion of pathogenic bacteria to the gut epithelium and their colonization; thus, they have been reported to benefit gut functionality with a higher supply of nutrients for the deposition of lean tissue and host immunity (Conejos et al., 2012; Spring et al., 2015). However, these beneficial effects were not observed in the present study. Similarly, Giang et al. (2011) did not detect any improvement in pig performance using a combination of *B. subtilis* and *Saccharomyces* in their diets. Oliveira (2018) did not find an association between Xyl and the yeast cell wall supplemented in piglet diets. Beneficial effects on the performance upon supplementation with yeast or its products are especially observed when an animal is raised under non-ideal hygienic conditions or exposed to a disease (Shurson, 2018). These conditions were not present in our assay, which may have generated a less favorable scenario for studying the beneficial effects of these additives on pig performance. Although the DFM and symbiotic used had the same microbiological basis, they are different products with different compositions. This important detail may justify the difference in the results obtained with diets BXM and BXS.

Other studies have also reported that supplementing Xyl in pig diets can improve utilization of energy obtained from the diet, and thus enable the use of levels below those recommended without affecting

performance. Martínez-Aispuro et al. (2015) verified that the use of Xyl compensated for a reduction of ME up to 105 kcal/kg, whereas Martínez-Aispuro et al. (2017) reported that a reduction of 75 kcal/kg was feasible. Xylanase not only helps to reduce the digest viscosity, making nutrients more available but also supports gut health by influencing the microbiota and increasing the production of SCFA, which helps to create an acidic environment that is favorable to beneficial bacteria (Dong et al., 2018; Tobias et al., 2023). Intestinal maintenance energy savings can increase tissue deposition in pigs, which also explains the maintenance of performance despite a reduction in diet energy content.

The reduction in ME levels in the BEM diet did not affect the performance of pigs when compared with the diet with no reduction. This was not expected because energy requirements were not met.

The ash and phosphorous contents of the metacarpus were not affected. No alterations were observed in the physical traits such as the Seedor Index, which is an indicator of bone density. The effects of feed additives in the gastrointestinal tract could increase the availability of nutrients that constitute the bone tissue, such as minerals and aminoacids, benefiting its chemical and physical characteristics. However, this conjecture has not yet been confirmed.

We investigated whether Xyl, individually or synergistically with *Bacillus* strains or with the yeast cell wall, could affect the integrity of the gut epithelium. The assumption is that NSP increase digest viscosity, which not only impairs nutrient digestibility but also leads to an increase in mucosal cell turnover and mucin production (Dong et al., 2018). This response can be due to mucosal damage. Although no direct effects were observed for villus height and crypt depth separately, a lower VH:CD was observed for the BEM diet when compared with the BXS diet. The BXS diet led to a desirable VH:CD, as longer villi and shallower crypts represent greater nutrient absorption capacity and lower energy expenditure with cell renewal in the intestine, respectively (Souza et al., 2021). This finding may be associated with the mechanisms of action of prebiotics, glucans, β-glucans, and MOS, and constituents of yeast cell wall since both BX and BXM diets did not affect the jejunal epithelium. β-glucan can stimulate the immune system (Eicher et al., 2006), and MOS can prevent pathogens adhesion in villus surfaces and lead to the elimination of these microorganisms (Conejos et al., 2012). Furthermore, prebiotics neutralize toxins (Narasimha et al., 2013) that can cause injuries to the gut epithelium. Liu et al. (2017) obtained similar results when they used cell walls of *Saccharomyces cerevisiae* in piglet diets, with an increase in jejunum villus height and VH:CD. Conejos et al. (2012) also reported a higher villus height in the jejunum when they supplied MOS derived from *Saccharomyces cerevisiae* to growing pigs. Conversely, Passos et al. (2015) did not observe differences in histological measures when testing Xyl levels in pigs. Some studies did not show effects on gut integrity concerning DFM (Le Bon et al., 2010; Giannenas et al., 2016). On the other hand, Cai et al. (2015) used DFM with *B. subtilis* and *B. amyloliquefaciens* on nursery piglets and observed an improvement in villus height.

We evaluated growing pigs in this study, and during all experimental periods, no clinical signs of enteric disorders were observed. More consistent and positive effects of DFM were observed in weaned piglets. Furthermore, the beneficial effects of administering these microorganisms are evident when animals are subjected to health challenges or chemical substances (Yirga, 2015; Liu et al., 2018). During our field trial, hygiene and density conditions were prioritized, imposing fewer challenges. Moreover, the NSP content of the diet may not have been sufficient to damage the gastrointestinal tract mucosa.

Because the diets contained the same ingredients, cost alterations were strictly a function of ME reduction and feed additives. The ME reduction caused a difference in diet composition, given the low quantities of soy oil and soybean meal in diets BEM, BX, BXM, and BXS, which are more expensive than corn and wheat bran. Consequently, the highest cost was incurred for the basal diet, which was higher (5, 4, and 3%) than the per kg costs of diets BEM and BX, BXM, and BXS, respectively.

Even though the feed additives used in this study are more expensive than most other ingredients, low inclusion combined with the reduction in ME resulted in a lower total cost of the diet. This is relevant in swine production systems, where the cost of feed is one of the highest. Thus, technologies used to improve pig performance must be economically viable so as not to negatively affect the producers' economic payback.

Due to the higher cost, pigs fed the basal diet required higher feed expenditure per kilogram of weight gained. Compared with other diets, this expense was higher (9.17, 7.21, 4.85, and 1.28% for diets BXM, BX, BXS, and BEM, respectively). The BXM diet was the most cost-effective for per kilogram of weight gained. This reflects the FCR of the animals and is associated with the lowest feed costs. Although the difference in FCR was significant only between animals that received diets BEM and BXM, numerical differences cannot be ignored when it comes to bioeconomic indices. If we consider a scenario of production of tons of feed and pork, cost savings of 9.17% would have a considerable economic impact.

In the calculation of the EEI and CI, the lowest feed cost per kg of weight gained was considered. The basal diet exhibited the worst EEI and CI, followed by the BEM diet. The BXM diet resulted in better EEI and CI, followed by BX and BXS diets. Similarly, Martínez-Aispuro et al. (2015) observed a higher EEI with the supply of Xyl in the diets of pigs with reduced ME. The best results were obtained for the BXM diet, which exhibited the lowest cost per kilogram of weight gained, due to the lowest cost per kilogram of this diet combined with greater feed efficiency. The observed results show that the use of Xyl combined with DFM in a diet with reduced ME maintains performance of pigs at a lower cost, resulting in greater economic viability.

5. Conclusions

Diets with reduced metabolizable energy supplemented with xylanase, xylanase plus direct-fed microbials (*B. subtilis* and *B. licheniformis*), or xylanase plus direct-fed microbials, and yeast cell wall contribute to the maintenance of performance and characteristics of the metacarpus and jejunum of growing pigs. The use of feed additives combined with the reduction of metabolizable energy in diets provides better bioeconomic indices.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Justino, L. R.; Vieira, A. A. and Lima, C. A. R. **Data curation:** Justino, L. R.; Souza, C. S.; Costa, J. A.; Quaresma, D. V.; Dilelis, F.; Vieira, A. A. and Lima, C. A. R. **Formal analysis:** Justino, L. R.; Souza, C. S.; Vasconcelos, B. S.; Costa, J. A.; Quaresma, D. V.; Dilelis, F. and Lima, C. A. R. **Funding acquisition:** Vasconcelos, B. S. and Vieira, A. A. **Investigation:** Justino, L. R.; Souza, C. S.; Vieira, A. A. and Lima, C. A. R. **Methodology:** Justino, L. R.; Souza, C. S.; Vasconcelos, B. S.; Costa, J. A.; Quaresma, D. V.; Dilelis, F.; Vieira, A. A. and Lima, C. A. R. **Project administration:** Justino, L. R.; Souza, C. S.; Vasconcelos, B. S.; Costa, J. A.; Quaresma, D. V.; Dilelis, F.; Vieira, A. A. and Lima, C. A. R. **Resources:** Vasconcelos, B. S.; Vieira, A. A. and Lima, C. A. R. **Supervision:** Souza, C. S.; Vasconcelos, B. S.; Vieira, A. A. and Lima, C. A. R. **Validation:** Souza, C. S. **Visualization:** Justino, L. R.; Souza, C. S.; Vieira, A. A. and Lima, C. A. R. **Writing – original draft:** Justino, L. R. and Lima, C. A. R. **Writing – review & editing:** Justino, L. R.; Souza, C. S.; Vieira, A. A. and Lima, C. A. R.

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