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# Energy valorization matrices for dietary carbohydrases and their effects on the behavior of grower and finisher pigs

# Matrizes de valorização energética para carboidratos dietéticos e seus efeitos no comportamento de suínos em crescimento e terminação

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## Highlights

The α-amylase (100g t$^{-1}$) with reduction of ME kg increases the visits to the feeder.
β-mannanase and xylanase with reduction of 70 or 85 kcal ME increases feed intake.
Carbohydrases in diets with reduced ME alters the behavioral traits of pigs.

## Abstract

This study aimed to assess the effect of exogenous carbohydrases (α-amylase or β-mannanase) in diets with reduced levels of metabolizable energy (ME) containing or not xylanase-phytase on the behavioral traits of grower and finisher pigs. In the first experiment, 42 crossbred male pigs (Landrace × Large White), initially weighing 25.81± 0.20 kg, were allotted to a completely randomized design with six treatments, seven replications, and one pig per pen as the experimental unit (UE). The treatments consisted of the combination of two classes of α-amylase (no enzyme and enzyme-added at 100 g α-amylase ton$^{-1}$ diet) and three levels of ME reduction (0, 50, and 100 kcal of ME kg$^{-1}$). In the second experiment, 40 crossbred male pigs (Landrace × Large White), initially weighing 26.0 ± 0.9 kg, were allotted to a randomized block design with four treatments, ten replications, and one animal per EU. The

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experimental treatments containing xylanase and valued at 40 kcal ME $kg^{-1}$ were: reference diet (RD) with no β-mannanase supplementation and RD containing 300 g β-mannanase $t^{-1}$ and reduced by 30, 45 and 60 kcal of ME $kg^{-1}$, comprising a total energy reduction (β-mannanase and xylanase) as follows: 0 + 40 (RD40), 30 + 40 (RD70), 45 + 40 (RD85), and 60 + 40 (RD100). The behavioral observations of grower and finisher pigs were recorded via closed-circuit video cameras installed in the upper part of the shed. In experiment I, an interaction between amylase enzyme (ENZ) and dietary ME reduction (ENZ*ME) was observed ($P < 0.05$) on the frequency (%) of standing, idleness, sleeping, and interactive behaviors in grower I pigs. Similarly, an interaction between ENZ and ME was observed for standing, lying down, feeding, and interactive behaviors in grower II pigs. There was an effect of ENZ*EM ($P < 0.05$) on lying down and interactive behavior in finisher I pigs and on standing, feeding, drinking, and sleeping behaviors in finisher II pigs. In the second experiment, there was an effect ($P < 0.05$) of energy valorization matrix (EVM) for β-mannanase in the idleness behavior of grower II pigs and on standing, lying down, idleness, and feeding behaviors in finisher II pigs. Including 100 g α-amylase $t^{-1}$ in diets reduced by up to 50 kcal of ME $kg^{-1}$ increases the feed intake frequency of grower II pigs. Including 300 g β-mannanase and 400 g xylanase $t^{-1}$ in diets reduced by 70 and 85 kcal ME per kg increases the percentage of feed intake of finisher II pigs.

**Key words:** Alpha-amylase. Beta-mannanase. Closed-circuit video camera. Generalized linear model. Pig behavioral frequency.

## Resumo

Este estudo teve como objetivo avaliar o efeito de carboidrases exógenas (α-amilase ou β-mananase) em dietas com níveis reduzidos de energia metabolizável (EM) contendo ou não xilanase-fitase sobre as características comportamentais de suínos em crescimento e terminação. No primeiro experimento, 42 suínos machos mestiços (Landrace × Large White), pesando inicialmente 25,81 ± 0,20 kg, foram distribuídos em um delineamento inteiramente casualizado com seis tratamentos, sete repetições e um suíno por baia como unidade experimental (UE). Os tratamentos consistiram na combinação de duas classes de α-amilase (sem enzima e com enzima adicionada a 100 g de α-amilase $ton^{-1}$ de dieta) e três níveis de redução de EM (0, 50 e 100 kcal de EM $kg^{-1}$). No segundo experimento, 40 suínos machos mestiços (Landrace × Large White), pesando inicialmente 26,0 ± 0,9 kg, foram distribuídos em um delineamento em blocos casualizados com quatro tratamentos, dez repetições e um animal por UE. Os tratamentos experimentais contendo xilanase e avaliados em 40 kcal EM $kg^{-1}$ foram: dieta de referência (RD) sem suplementação de β-mananase e RD contendo 300 g de β-mananase $t^{-1}$ e reduzida em 30, 45 e 60 kcal de EM $kg^{-1}$, compreendendo uma redução total de energia (β-mananase e xilanase) como segue: 0 + 40 (RD40), 30 + 40 (RD70), 45 + 40 (RD85) e 60 + 40 (RD100). As observações comportamentais dos suínos em crescimento e terminação foram registradas por meio de câmeras de vídeo de circuito fechado instaladas na parte superior do galpão. No experimento I, foi observada ($P < 0,05$) uma interação entre a enzima amilase (ENZ) e a redução de EM dietética (ENZ*EM) na frequência (%) de comportamentos de ficar em pé, ociosidade, sono e interação em suínos de crescimento I. Similarmente, foi observada uma interação entre ENZ e EM para comportamentos de ficar em pé, deitar, alimentar-se e interação em suínos de crescimento II. Houve um efeito de ENZ*EM ($P < 0,05$) no comportamento de ficar deitado e interação em suínos de terminação I e nos comportamentos de ficar em pé, alimentar-se, beber-se e dormir em suínos de terminação II. No segundo experimento, houve um efeito ($P < 0,05$) da matriz de valorização energética (EVM) para β-mananase no comportamento de ficar em pé, deitar-se, ociosidade e alimentação em suínos de terminação II. A inclusão de 100 g de α-amilase $t^{-1}$ em dietas reduzidas em até 50 kcal de EM $kg^{-1}$ aumenta a frequência de consumo de ração de suínos em fase de crescimento II. A inclusão de 300 g de β-mananase e 400 g de xilanase $t^{-1}$ em dietas reduzidas em 70 e 85 kcal de EM por kg aumenta a porcentagem de consumo de ração de suínos em fase de terminação II.

**Palavras-chave:** Alfa-amilase. Beta-mananase. Câmera de vídeo de circuito fechado. Modelo linear generalizado. Frequência comportamental do suíno.

## Introduction

Consumers have been concerned about how animals are raised, transported, and slaughtered. In addition to humanitarian issues, there is a perception that animal welfare provides safer and better product quality in all stages of production. This perception contributes to a high interest in animal welfare in technical, scientific, and academic fields (Dias et al., 2014).

Sensors and cameras to assess and monitor body conformation and score in pigs have been commonly used to select and rank batches in multiplier pig farms. In animal behavior-focused research, camera monitoring is more advantageous for recording information than visual methods with on-site observation due to a real-time diagnosis with no observer interference (Brewster et al., 2018).

Diet can modulate pig welfare and behavior. Corn and soybean meal are the main ingredients in Brazilian commercial diets for pigs, which have a fibrous fraction composed of non-starch polysaccharides (NSP). Pig digestive enzymes do not hydrolyze these carbohydrates; hence, NSP can impair dietary digestibility and animal performance (Sakomura et al., 2014).

Soybean meal has a high NSP concentration (170 to 270 g $kg^{-1}$) (Bach Knudsen, 2014), and corn has about 8% to 9% NSP (51 to 53.5 g $kg^{-1}$ are arabinoxylans) (Tavernari et al., 2008). Soybean meal β-mannans (13 g $kg^{-1}$) (Jackson et al., 2004) and arabinoxylans are part of the soluble fraction of hemicellulose, an NSP found in plant ingredients that can provide an energy loss of up to 90 kcal ME $kg^{-1}$ feed (Veum & Odle, 2001; Shastak et al., 2015). β-mannans, mainly from soybean meal, increase energy expenditure because the innate immune system recognizes them as strange substances in the body (Vangroenweghe et al., 2021).

Regarding the high corn starch concentration, grower and finisher pigs produce salivary and pancreatic amylases, which cleave starch into simple sugars for later intestinal absorption and energy production. However, energy is lost during this process, and digestion in the final part of the digestive tract is incomplete (Ruiz et al., 2008). Dietary exogenous enzymes can compensate for this energy loss, which, in synergy with endogenous enzymes, can improve the nutrient absorption of corn and soybean meal nutrients by increasing the catalysis efficiency of specific chemical reactions and promoting energy savings (Gomes et al., 2019).

A study was conducted based on the hypothesis that such savings would increase the frequency of active behaviors in pigs. Therefore, the present study aimed to evaluate the effect of dietary carbohydrases (α-amylase or β-mannanase) on the behavior of grower and finisher pigs fed diets with reduced levels of metabolizable energy (ME), containing or not xylanase phytase.

## Material and Methods

Experiments I (protocol no. 25/2020) and II (protocol no. 17/2021) were conducted in a pig unit (24°31′52″ S and 54°01′03″ W) located at an altitude of ≥ 387 m in Marechal Cândido Rondon, PR, Brazil. All animals were cared for under the guidelines of the institutional animal care committee.

### *Experiment I*

In the first experiment, a total of 42 entire crossbred male pigs [(Landrace × Large White, Agroceres♂, 100% AGPIC, > 80% AGPIC 337 + small part of AGPIC 359) and DanBred♀, DB 90], initially weighing 25.81 ± 0.20 kg, were allotted to one of the six treatments (two classes of the enzyme α-amylase and three levels of ME reduction) in a randomized complete design with seven replications and one animal per pen as the experimental unit.

Before starting the experiments, the animals remained for 5 days in an adaptation period to the pens and diet.

Based on the batch average body weight for changing phase, reference diets for grower I (28 days; from 25 to 50 kg) and II (18 days; from 50 to 70 kg) and finisher I (24 days; from 70 to 100 kg) and II (18 days; from 100 to 125 kg). Experimental diets (offered as mash) were corn- and soybean meal-based supplemented with industrial amino acids. Diets (Table 1) were formulated to meet the nutritional requirements (Rostagno et al., 2017) of each weight category. The treatments consisted of the arrangement of two classes of α-amylase (no enzyme and enzyme-added at 100 g α-amylase ton$^{-1}$ diet) and three ME reduction levels (0, 50, and 100 kcal ME kg$^{-1}$ diet).

**Table 1**

**Composition of diets fed to grower and finisher male pigs in experiment I (as-fed basis)**

| Item | Experimental phases and control diet (CD0)[4] | | | |
|---|---|---|---|---|
| | Grower I | Grower II | Finisher I | Finisher II |
| | CD0 | CD0 | CD0 | CD0 |
| Ingredients ( g kg$^{-1}$ diet) | | | | |
| Ground corn, 71.3 g kg$^{-1}$ CP | 690.4 | 745.0 | 803.4 | 865.0 |
| Soybean meal, 482.6 g kg$^{-1}$ CP | 244.8 | 198.2 | 146.4 | 922.0 |
| Soybean oil | 22.2 | 18.5 | 15.2 | 12.0 |
| Dicalcium phosphate | 16.7 | 14.2 | 12.4 | 09.2 |
| Calcitic limestone | 6.9 | 6.0 | 5.3 | 4.4 |
| Salt | 4.4 | 4.1 | 3.8 | 3.7 |
| Choline chloride | 2.3 | 2.3 | 2.1 | 1.9 |
| Lysine sulfate, 546 g kg$^{-1}$ | 5.5 | 5.5 | 5.5 | 5.4 |
| DL-methionine, 995 g kg$^{-1}$ | 1.6 | 1.3 | 1.0 | 0.7 |
| L-threonine, 968 g kg$^{-1}$ | 1.7 | 1.5 | 1.5 | 1.3 |
| L-tryptophan, 990 g kg$^{-1}$ | 0.4 | 0.4 | 0.4 | 0.3 |
| L-valine, 955 g kg$^{-1}$ | 0.7 | 0.6 | 0.6 | 0.3 |
| Mycotoxin adsorbent | 1.0 | 1.0 | 1.0 | 1.0 |
| Mineral premix[1] | 0.5 | 0.5 | 0.5 | 0.5 |
| Vitamin premix[1] | 0.2 | 0.2 | 0.2 | 0.2 |
| Enramycin | 0.06 | 0.06 | 0.06 | - |
| Kaolin (inert) | 0.1 | 0.1 | 0.1 | 1.5 |
| $\alpha$-amylase | - | - | - | - |
| Calculated chemical composition | | | | |
| Metabolizable energy (kcal/kg) | 3300 | 3300 | 3300 | 3300 |
| Crude protein, g kg$^{-1}$ | 175.0 | 156.0 | 135.0 | 112.4 |
| SID[2] lysine, g kg$^{-1}$ | 11.0 | 9.8 | 8.5 | 7.1 |
| SID methionine + cysteine g kg$^{-1}$ | 6.5 | 5.8 | 5.1 | 4.3 |
| SID threonine, g kg$^{-1}$ | 7.1 | 6.3 | 5.5 | 4.6 |
| SID tryptophan, g kg$^{-1}$ | 2.3 | 2.1 | 1.8 | 1.4 |
| SID valine, g kg$^{-1}$ | 8.0 | 7.1 | 6.2 | 4.9 |
| Total starch, g kg$^{-1}$ | 423.9 | 454.3 | 486.7 | 520.9 |
| Total calcium, g kg$^{-1}$ | 7.7 | 6.6 | 5.7 | 4.4 |
| STTD[3] phosphorus, g kg$^{-1}$ | 3.8 | 3.3 | 2.8 | 2.2 |
| Total sodium, g kg$^{-1}$ | 1.9 | 1.8 | 1.6 | 1.5 |

[1] Contained per kg diet: Mn sulfate, 60 mg; Zn oxide, 80 mg; Fe sulfate, 60 mg; Cu sulfate, 10 mg; I, 1 mg. Contained per kg diet: vitamin A, 3000 IU; vitamin D3, 600 IU; vitamin E, 10 IU; vitamin K3, 0.9 mg; vitamin B1, 0.4 mg; vitamin B2, 1.9 mg; vitamin B6, 0.4 mg; vitamin B12, 7 mg; niacin, 10 mg; pantothenic acid, 6.5 mg; folic acid, 0.25 mg; IF, 0.3 mg, BHT, 0.06 mg.

[2] SID: standardized ileal digestible.

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[3] STTD: standardized total tract digestible.

[4] Diferences in the composition of diets compared to CD0 (%), grower I: CD50 (soybean oil 1.62, inert 0.62, ME 3,250 kcal), CD100 (soybean oil 1.01, inert 1.21, ME 3,200 kcal), AM0 (inert 0.0, α-amylase 0.01), AM50 (soybean oil 1.62, inert 0.60, α-amylase 0.01, ME 3,250 kcal), AM100 (soybean oil 1.01, inert 1.20, α-amylase 0.01, ME 3,200 kcal); grower II: CD50 (soybean oil 1.25, inert 0.61, ME 3,250 kcal), CD100 (soybean oil 0.65, inert 1.21, ME 3,200 kcal), AM0 (inert 0.0, α-amylase 0.01), AM50 (soybean oil 1.25, inert 0.60, α-amylase 0.01, ME 3,250 kcal), AM100 (soybean oil 0.65, inert 1.20, α-amylase 0.01, ME 3,200 kcal); finisher I: CD50 (soybean oil 0.92, inert 0.61, ME 3,250 kcal), CD100 (soybean oil 0.31, inert 1.21, ME 3,200 kcal), AM0 (inert 0.0, α-amylase 0.01), AM50 (soybean oil 0.92, inert 0.60, α-amylase 0.01, ME 3,250 kcal), AM100 (soybean oil 0.31, inert 1.20, α-amylase 0.01, ME 3,200 kcal); finisher II: CD50 (soybean oil 0.60, inert 0.75, ME 3,250 kcal), CD100 (soybean oil 0.0, inert 1.35, ME 3,200 kcal), AM0 (inert 0.14, α-amylase 0.01), AM50 (soybean oil 0.60, inert 0.74, α-amylase 0.01, ME 3,250 kcal), AM100 (soybean oil 0.0, inert 1.35, α-amylase 0.01, ME 3,200 kcal).

During diet mixing, the α-amylase experimental dose (100 g ton$^{-1}$) was mixed in a single-screw vertical mixer for 15 min. The enzyme used was α-amylase (Sunamy amylase®), produced via fermentation using Bacillus subtilis, with a minimum activity of 1000 U g$^{-1}$.

### *Experiment II*

In the second experiment, 40 crossbred male pigs (Landrace × Large White), initially weighing 26.0 ± 0.9 kg, were allotted to a randomized block design (two homogeneous blocks based on initial body weight) with a 4 × 2 factorial arrangement of treatments (four diets and two periods of the day: morning and afternoon), five replications, and one pig per pen (experimental unit).

Before the beginning of the experiments, the animals were adapted to the pens and diets for five days. In both experiments, pigs were fed a commercial diet after individual housing in pens and had free access to water. Then, pigs were weighed and identified with numbered ear tags. Animals were housed in a masonry facility with a ceramic tile roof and two rows (with a central aisle) of compact concrete floor pens (1.5 × 1.5 m, 2.25 m²). All pens were equipped with a semiautomatic front feeder and a nipple waterer. Curtains, a skylight system, and trees on both sides of the shed were used to control temperature and ventilation.

Room temperature (ºC) was recorded using a data logger (Hygro-Thermometer, model RT811) in the middle of the experimental facility. Relative humidity (%) was recorded using a digital display (Vketech, model HTC-2, SP-Brazil) placed 1.5 m from the floor. Temperature (ºC) and relative humidity (%) were recorded on the two days (four times daily: twice in the morning and twice in the afternoon) of behavior assessment in grower and finisher pigs.

The reference diets of grower I (25 days; from 26.00 to 50.35 kg) and II (17 days; from 50.35 to 70.88 kg) and finisher I (22 days; from 70.88 to 98.35 kg) and II (30 days; from 98.35 to 134.97 kg) (Table 2) phases, were corn- and soybean meal-based supplemented with industrial amino acids. Diets were formulated to meet the nutritional requirements of each weight category (Rostagno et al., 2017). Water and diets were provided ad libitum throughout the 94 days of the experiment.

**Table 2**
**Composition of diets fed to grower and finisher male pigs in the experiment II (as-fed)**

| Item | Experimental phases and control diet (CD0)[4] | | | |
|---|---|---|---|---|
| | Grower I | Grower II | Finisher I | Finisher II |
| | CD | CD | CD | CD |
| Ingredients ( g kg$^{-1}$ diet) | | | | |
| Ground corn, 78.6 g kg$^{-1}$ CP | 703.6 | 727.1 | 793.5 | 906.6 |
| Soybean meal, 454 g kg$^{-1}$ CP | 247.5 | 222.0 | 162.3 | 47.6 |
| Dicalcium phosphate | 16.8 | 14.1 | 12.3 | 9.7 |
| Calcitic limestone | 6.8 | 5.9 | 5.2 | 4.5 |
| Inert (kaolin) | - | - | - | 5.9 |
| Soybean oil | 7.5 | 14.4 | 10.3 | 7.2 |
| Common salt | 4.4 | 4.1 | 3.8 | 3.7 |
| Premix[1] | 3.0 | 3.0 | 3.0 | 3.0 |
| Lysine sulfate, 546. g kg$^{-1}$ | 5.6 | 5.5 | 5.7 | 7.1 |
| DL-methionine, 995. g kg$^{-1}$ | 1.6 | 1.4 | 1.2 | 1.0 |
| L-threonine, 968 g kg$^{-1}$ | 1.7 | 1.6 | 1.5 | 1.8 |
| L-tryptophan, 990 g kg$^{-1}$ | 0.4 | 0.3 | 0.3 | 0.5 |
| L-valine, 955 g kg$^{-1}$ | 0.6 | 0.1 | 0.2 | 0.8 |
| β-mannanase | - | - | - | - |
| Enramycin | 0.06 | 0.06 | 0.06 | 0.06 |
| Calculated chemical composition | | | | |
| Metabolizable energy, kcal kg$^{-1}$ | 3260 | 3310 | 3310 | 3310 |
| Crude protein, g kg$^{-1}$ | 175.0 | 164.4 | 142.0 | 99.5 |
| SID2 lysine, g kg$^{-1}$ | 11.0 | 10.3 | 8.9 | 6.9 |
| SID methionine + cysteine, g kg$^{-1}$ | 6.5 | 6.1 | 5.3 | 4.1 |
| SID threonine, g kg$^{-1}$ | 7.1 | 6.7 | 5.8 | 4.5 |
| SID tryptophan, g kg$^{-1}$ | 2.3 | 2.1 | 1.8 | 1.3 |
| SID valine, g kg$^{-1}$ | 8.0 | 7.1 | 6.2 | 4.8 |
| Total calcium, g kg$^{-1}$ | 7.7 | 6.6 | 5.7 | 4.4 |
| STTD3 phosphorus, g kg$^{-1}$ | 3.8 | 3.3 | 2.8 | 2.1 |
| Total sodium, g kg$^{-1}$ | 1.9 | 1.8 | 1.6 | 1.5 |

[1] Content per kg of premix: Mn sulfate, 5400 mg/kg; Zn oxide, 13.50 g/kg; Fe sulfate, 10.50 g/kg; Cu sulfate, 2100 mg/kg; I, 150 mg/kg; vitamin A, 900000 IU/kg; vitamin D3, 180000 IU/kg; vitamin E, 3000 IU/kg; vitamin K3, 270 mg/kg; vitamin B1, 120 mg/kg; vitamin B2, 570 mg/kg; vitamin B6, 120 mg/kg; vitamin B12, 2100 mcg/kg; niacin, 3000 mg/kg; pantothenic acid, 1950 mg/kg; folic acid, 75 mg/kg; If, 90 mg/kg; phytase, 166.66 U/g; xylanase, 333.33 U/g.

[2] SID: standardized ileal digestible.

[3] STTD: standardized total tract digestible.

[4] Diferences in the composition of diets compared to CD (%), grower I: CD70 (soybean oil 0.39, inert 0.33, β-mannanase 0.03, ME 3,230 kcal), CD85 (soybean oil 0.21, inert 0.51, β-mannanase 0.03, ME 3,215 kcal), CD100 (soybean oil 0.03, inert 0.69, β-mannanase 0.03, ME 3,200 kcal); grower II: CD70 (soybean oil 1.07, inert 0.33, β-mannanase 0.03, ME 3,280 kcal), CD85 (soybean oil 0.89, inert 0.51, β-mannanase 0.03, ME 3,265 kcal), CD100 (soybean oil 0.71, inert 0.69, β-mannanase 0.03, ME 3,250 kcal); finisher I: CD70 (soybean oil 0.67, inert 0.33, β-mannanase 0.03, ME 3,280 kcal), CD85 (soybean oil 0.49, inert 0.51, β-mannanase 0.03, ME 3,265 kcal), CD100 (soybean oil 0.31, inert 0.69, β-mannanase 0.03, ME 3,250 kcal); finisher II: CD70 (soybean oil 0.36, inert 0.92, β-mannanase 0.03, ME 3,280 kcal), CD85 (soybean oil 0.19, inert 1.10, β-mannanase 0.03, ME 3,265 kcal), CD100 (soybean oil 0.0, inert 1.28, β-mannanase 0.03, ME 3,250 kcal).

The experimental dietary treatments added of xylanase and valued at 40 kcal ME/kg were: reference diet (RD) with no β-mannanase supplementation and RD supplemented with 300 g β-mannanase ton$^{-1}$ diet reduced by 30, 45, and 60 kcal ME kg$^{-1}$ diet. As a result of β-mannanase and xylanase supplementation, dietary energy (kcal kg$^{-1}$) was reduced by 0 + 40 (DR40), 30 + 40 (DR70), 45 + 40 (DR85), and 60 + 40 (DR100), respectively.

β-mannanase (Elanco Animal Health, Inc., São Paulo, SP, Brazil; registration SP-59122 30011, HemicellTM HT®) was obtained via fermentation (*Paenibacillus lentus*). A unit (U) of β-mannanase is the amount of enzyme that releases 0.72 mcg of reducing sugars (equivalent to D-mannose) per minute at 40°C and pH 7.5.

### *Behavioral assessments*

Behavioral records of the pigs were categorized as normal (drinking, feeding, standing, lying down, sleeping, and idleness) and social (interactive with the pig in the adjacent pen). Data were obtained with closed-circuit video cameras (Intelbras, model HD Dvr SC-Brazil) installed in the upper part of the experimental facility and connected to a DVR (Intelbras, Stand Alone model, São José, Santa Catarina, Brazil). Behavior was assessed in two periods of the day (morning and afternoon) at twelve continuous integer times (07:00h to 12:00h and 12:10h to 19:00h) and every six ten-minute intervals at each time of the day (0 to 10, 10 to 20, 20 to 30, 30 to 40, 40 to 50, and 50 to 60 min). Approximately 6,048 and 5,760 records for each phase (grower I and II; finisher I and II) were taken in the first and second experiments, respectively.

Videos were stored on an external HD and later evaluated using a ten-minute sampling interval to measure the frequency with which animals showed a certain behavior (Martin & Bateson, 1986).

The behavioral assessed variables in both experiments were the proportion or frequency observed (%) of standing (standing and moving inside the pen), lying down (at rest with the body resting on the floor), idleness (standing still with no action for a certain time), feeding (with the head next to the feeder), drinking (activating the drinker), sleeping (lying completely on the floor and motionless for a while), and interactive (playing with the pig next pen or with the drinker) (Souza da Silva et al., 2014).

### *Statistical analysis*

In the first experiment, generalized linear mixed models (GLIMMIX) were fitted to the binary data using the binomial distribution. Zero (0) and one (1) were attributed to the absence and presence of the behavior, respectively.

The effects of the fixed part of the fitted GLIMMIX were described as: $Y_{ijkl} = \mu + ENZ_i + ME_j + PER_k + ENZ*ME_{ij} + ENZ*PER_{ik} + ME*PER_{jk} + ENZ*ME*PER_{ijk} + \varepsilon_{ijk}$, where: μ, the effect associated with the overall mean; $ENZ_i$, the effect associated with the i-th class of enzyme (i = 1 and 2); MEj, the effect associated with the j-th level of reduced metabolizable energy (j = 1, 2, and 3); $PER_k$, the effect of period classes (k = 1 and 2); $ENZ*ME_{ij}$, $ENZ*PER_{ik}$, $ME*PER_{jk}$, the effects of double interactions; $ENZ*ME*PER_{ijk}$, the effect of the triple interaction, and $\varepsilon_{ijk}$, the random, normal, independent, and identically distributed errors associated with each observation. For the random part of the model, the effects of the four daily evaluations and minutes hierarchized in hours (min/hour) were analyzed as repeated measurements in the experimental plots.

In the second experiment, generalized linear models (GLM) with logit link function expressed as $g(\mu) = \ln(\mu\ 1\text{-}\mu^{-1})$ were used to model the binary data of behavioral variables. GLM parameters were estimated using the maximum likelihood method by maximizing the log-likelihood function using generalized estimating equations (GEE). This method incorporated the correlation structure among observations over time (hours), hierarchized by time (days), portraying repeated measures in the experimental unit (pig).

The binomial GLM was represented by systematic portion $\eta = g(\mu) = \mu + \beta M_i + PER_j + \beta M{*}P_{ij} + Bl_k$, where: μ, the effect of the overall mean; βMi, the effect of β-mannanase valorization matrices (i = 1, 2, 3, and 4); PERj, the effect of period classes (j = 1 and 2); $\beta M{*}PER_{ij}$, the interaction effect between β-mannanase valorization matrices and period classes; and $Bl_k$, the effect of the blocks.

The goodness-of-fit of GLM binomial to the observed data of pig behavior was based on the comparison of the deviance value (D) with the percentiles of the chi-square distribution $\chi^2_{n\text{-}p\ (\frac{\alpha}{2};\ \text{bilateral})}$, in which n - p = residual degrees of freedom. The dispersion parameter was adjusted by correcting the standard errors when under-dispersion ($D \leq \chi^2_{n\text{-}p\ (\frac{\alpha}{2}\ \text{bilateral to the left})}$) or over-dispersion ($D \geq \chi^2_{n\text{-}p\ (\frac{\alpha}{2}\ \text{bilateral to the right})}$) were detected. To test the null hypothesis, no under-dispersion or over-dispersion occurs, using quasi-binomial and quasi-likelihood models, respectively (SAS, 2022).

The effects of interaction between enzyme and metabolizable energy valorization (ENZ*ME) and the effects included in the statistical model of the first experiment were assessed via type III analysis. The effects of β-mannanase (βM), period (PER), interaction (I), and blocks on the behavioral variables of pigs in the second experiment were verified via type III GEE analysis, respectively. Whenever significant ($P \leq \alpha$), the estimated proportions associated with the effects of ENZ*ME, βM, PER, and I were compared using the test of difference among least mean squares (lsmeans), using t- and chi-square statistics ($\chi^2$), respectively. In both experiments, results were expressed as observed proportions.

Significance was set at $P \leq 0.05$ in all hypothesis tests. Statistical analyses were performed using SAS Studio software.

## Results and Discussion

In the first experiment, the average room temperature and relative humidity (RH) were 26.91 (0.23)ºC and 59.79 (0.90)%, respectively. A triple interaction among enzyme (ENZ), reduced metabolizable energy (ME), and period (PER) was observed ($P < 0.05$) for the sleeping and interactive behaviors in grower I pigs (Table 3). Although the simple effects of ENZ, ME, and triple interaction were included in the statistical model, the appropriate level of detail for the present study was expressed by the effects of double interactions ENZ*ME, ENZ*PER, and ME*PER.

**Table 3**

**Significance probability values for the effects of the model and observed means for the interaction among α-amylase and metabolizable energy and period on the behavior of grower pigs (Exp. I)**

| Item | Factorial[1] | | | | | | Period[2] | | p[3] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No enzyme | | | Enzyme-added | | | | | | | | | | | |
| | $ME_0$ | $ME_{50}$ | $ME_{100}$ | $ME_0$ | $ME_{50}$ | $ME_{100}$ | M | A | ENZ | ME | PER | ENZ*ME | ENZ*PER | ME*PER | TRI |
| Grower I (25.81 to 50.47 kg, 0 to 28 days of experiment) | | | | | | | | | | | | | | | |
| Standing | 20.21 | 19.37 | $21.28^{A}$ | 21.72 | 20.06 | $18.59^{B}$ | $16.43^{b}$ | $24.09^{a}$ | 0.817 | 0.310 | $5.48\times10^{-14}$ | 0.043 | 0.809 | 0.267 | 0.528 |
| Lying down | 77.59 | 78.86 | 76.66 | 75.64 | 77.50 | 78.86 | $81.77^{a}$ | $73.15^{b}$ | 0.644 | 0.270 | 0.000 | 0.088 | 0.959 | 0.185 | 0.388 |
| Idleness | $24.85^{b}$ | $21.87^{Bc}$ | $28.23^{a}$ | 27.30 | $27.25^{A}$ | 28.03 | $20.88^{b}$ | $31.78^{a}$ | 0.019 | 0.010 | $9.87\times10^{-6}$ | 0.029 | 0.283 | 0.641 | 0.067 |
| Feeding | 11.84 | 10.62 | 11.11 | 10.37 | 9.93 | 10.91 | 9.06b | $12.58^{a}$ | 0.227 | 0.467 | 0.000 | 0.607 | 0.811 | 0.515 | 0.739 |
| Drinking | 1.42 | 1.32 | 1.03 | 1.52 | 0.98 | 1.13 | $0.90^{b}$ | $1.57^{a}$ | 0.738 | 0.329 | 0.033 | 0.588 | 0.697 | 0.198 | 0.552 |
| Sleeping | $59.25^{b}$ | $63.94^{aA}$ | $56.85^{b}$ | 58.61 | $58.81^{B}$ | 58.17 | $66.68^{a}$ | $51.65^{b}$ | 0.128 | 0.009 | $8.76\times10^{-5}$ | 0.018 | 0.312 | 0.107 | 0.011 |
| Interactive | 3.18 | 2.50B | $3.08^{A}$ | $3.18^{a}$ | $3.72^{Aa}$ | $2.25^{Bb}$ | 2.70 | 3.27 | 0.792 | 0.125 | 0.054 | 0.000 | 0.696 | 0.299 | 0.001 |
| Grower II (50.47 to 69.93 kg, 28 to 46 days of experiment) | | | | | | | | | | | | | | | |
| Standing | $19.47^{a}$ | $18.00^{bB}$ | $18.98^{ab}$ | $18.69^{a}$ | $20.74^{aA}$ | $16.73^{b}$ | $15.67^{b}$ | $21.96^{a}$ | 0.577 | 0.024 | 0.000 | 0.028 | 0.788 | 0.074 | 0.163 |
| Lying down | $77.50^{b}$ | $79.26^{aA}$ | $74.66^{bB}$ | $78.28^{ab}$ | $75.93^{bB}$ | $79.26^{aA}$ | $81.82^{a}$ | $73.02^{b}$ | 0.209 | 0.512 | 0.000 | 0.005 | 0.766 | 0.068 | 0.252 |
| Idleness | 23.87 | 22.11 | 27.40 | 24.27 | 20.06 | 22.99 | $20.11^{b}$ | $26.88^{a}$ | 0.018 | $5.93\times10^{-5}$ | $1.60\times10^{-29}$ | 0.820 | 0.075 | 0.444 | 0.227 |
| Feeding | $13.31^{aA}$ | $11.06^{bB}$ | $10.86^{b}$ | $9.30^{bB}$ | $12.62^{aA}$ | $10.57^{b}$ | $9.88^{b}$ | $12.73^{a}$ | 0.5199 | 0.137 | $1.37\times10^{-5}$ | $4.30\times10^{-6}$ | 0.444 | 0.078 | 0.997 |
| Drinking | 1.86 | 2.45 | 2.05 | 2.84 | 1.96 | 1.86 | $1.25^{b}$ | $3.11^{a}$ | 0.4856 | 0.239 | $1.05\times10^{-9}$ | 0.097 | 0.495 | 0.477 | 0.055 |
| Sleeping | 60.08 | 63.60 | 58.41 | 62.62 | 63.99 | 63.70 | $68.21^{a}$ | $55.75^{b}$ | 0.0136 | 0.002 | $4.03\times10^{-16}$ | 0.097 | 0.025 | 0.032 | 0.206 |
| Interactive | 1.08 | 1.22 | 1.76 | $1.37^{ab}$ | $1.71^{a}$ | $1.03^{b}$ | $0.97^{b}$ | $1.77^{a}$ | 0.9676 | 0.515 | 0.000 | 0.049 | 0.876 | 0.160 | 0.865 |

[1] $ME_0$, $ME_{50}$, and $ME_{100}$: reference diet (RD) reduced by 0, 50, and 100 kcal ME $kg^{-1}$ diet, respectively; Enzyme-added: RD + 100 g α-amylase $t^{-1}$ diet;

[2] M: morning period (07:00 to 12:00h); A: afternoon (12:10 to 19:00h.).

[3] Significance probability of chi-square test of the type III analysis; ENZ: enzyme; ME: metabolizable energy; PER: period; ENZ*ME: interaction between ENZ and ME; ENZ*PER: interaction between ENZ and PER; ME*PER: interaction between ME and PER; TRI: triple interaction among ENZ*ME*PER; Means followed by different lowercase letters for the effect of ME/ENZ and different uppercase letters for the effect of ENZ/ME differ by difference among *lsmeans* test ($P < 0.05$).

An interaction between ENZ and reduced dietary ME was observed for the standing (P = 0.043), idleness (P = 0.029), sleeping (p = 0.018), and interactive (p = 0.000) behaviors in grower I pigs. The same interaction effect was observed for the standing (p = 0.028), lying down (P = 0.005), feeding ($P = 4.30\times10^{-6}$), and interactive (P = 0.0491) behaviors in grower pigs II. No interaction (P > 0.05) between ENZ and reduced dietary ME was observed for the other variables in grower I and II pigs (Table 3).

In experiment I, neither grower nor finisher pigs exhibited any significant health issues that implied the removal from pens for treatment, suggesting that the overall health status of the herd did not influence the behavior of pigs throughout the experiment.

Grower I pigs showed a higher frequency of idleness and interactive behaviors, and grower pigs II showed a higher frequency of standing and feeding behaviors when fed diets supplemented with enzyme (ENZ) than those fed diets not supplemented with enzyme (NENZ) reduced by 50 kcal ME $kg^{-1}$ diet (ME50). Regarding sleeping behavior, the opposite was observed; grower pigs I fed NENZ showed higher frequency than those in the ENZ/ME50 group. These results showed that α-amylase supplementation increases the frequency of active behaviors if the diet is reduced by 50 kcal ME/kg.

Rupolo et al. (2023) conducted total tract apparent digestibility and nitrogen uptake studies using the same treatments as the present study. They reported that α-amylase increased the nutritional value of diets with reduced ME and supported the performance of pigs during the grower and finisher phases. Feeding behavior and intake tend to increase with energy-reduced diets when enzymes are supplemented, which agrees with the results from the present study.

Grower I pigs fed the NENZ diet showed a higher proportion of standing and interactive behavior than those fed the ENZ diet reduced by 100 kcal ME $kg^{-1}$ diet (ME100). Proportions of the lying-down behavior were changed. For instance, pigs fed the ENZ diet had a greater proportion than those fed the NENZ/ME100 diet.

When no ME reduction (ME0) was applied, grower II pigs fed the NENZ diet showed greater feeding behavior than those fed the ENZ diet. This result suggests that including enzymes in diets without energy reduction did not favor feed search. Energy is the main factor driving feed intake in pigs; nutrients and their metabolites regulate short- and long-term feed intake through direct or indirect endocrine secretions interacting with processes. The digestion of carbohydrates, fats, and proteins directly affects the release of hormones from the gastrointestinal tract and pancreas, regulating feed intake (Li & Patience, 2017).

When studying the hierarchical effect of the ME matrix for α-amylase supplementation in grower II pigs, we observed that pigs fed the ME50 diet had a greater proportion of visits to the feeder than pigs fed the ME0 and ME100 diets. In grower I and grower II pigs, a similar trend for proportions was observed for interactive and standing behaviors, respectively, in which greater values were observed in pigs fed ME0 and ME50 diets compared to those fed ME100 diets.

In general, diets reduced by 50 kcal ME/kg promoted better results, corroborating the interpretation of the hierarchical effect of the enzyme with reduced dietary ME levels. Supplementing grower pigs with α-amylase compensated for the reduced dietary ME. This result agrees with those reported by Rupolo et al. (2023), where the effects of α-amylase and the tests suggested a total tract apparent digestibility improvement and growth performance maintenance in pigs fed diets with reduced ME without impairing variables such as average weight gain and feed to gain ratio.

For the hierarchical effect of the period in each class of enzyme, a greater percentage ($P < 0.05$) of grower II pigs sleeping in the morning than in the afternoon was observed in those fed diets with no enzyme (NENZ) and with enzyme (ENZ). Average values were 68.34% (morning) and 53.67% (afternoon) for PER/NENZ, and 68.56% (morning) and 57.80% (afternoon) for PER/ENZ.

Interaction between dietary enzyme (ENZ) and metabolizable energy reduction (ME) was observed for lying down ($P = 0.033$) and interactive ($P = 0.014$) behaviors in finisher I pigs, and for standing ($P = 0.0094$), feeding ($P = 0.042$), drinking ($P = 0.010$) and sleeping ($P = 0.038$) in finisher II pigs. No effect of ENZ*ME ($P > 0.05$) on the other variables was observed in finisher I and II pigs (Table 4).

**Table 4**

**Significance probability values for model effects and observed means for the interaction among α-amylase and metabolizable energy and period on the behavior of finisher pigs (Exp. I)**

| Item | Factorial[1] | | | | | | Period[2] | | p[3] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No enzyme | | | Enzyme-added | | | | | | | | | | | |
| | ME$_{0}$ | ME$_{50}$ | ME$_{100}$ | ME$_{0}$ | ME$_{50}$ | ME$_{100}$ | M | A | ENZ | ME | PER | ENZ*ME | ENZ*PER | ME*PER | TRI |
| Finisher I (69.93 to 100.21 kg, 46 to 70 days of experiment) | | | | | | | | | | | | | | | |
| Standing | 18.49 | 16.44 | 16.14 | 17.17 | 16.98 | 15.41 | 13.84$^{b}$ | 19.79$^{a}$ | 0.562 | 0.026 | $2.91\times10^{-19}$ | 0.478 | 0.241 | 0.237 | 0.760 |
| Lying down | 78.72$^{b}$ | 81.51$^{a}$ | 80.14$^{ab}$ | 80.68$^{ab}$ | 79.75$^{b}$ | 82.05$^{a}$ | 84.36$^{a}$ | 76.47$^{b}$ | 0.395 | 0.131 | $2.81\times10^{-14}$ | 0.033 | 0.352 | 0.098 | 0.358 |
| Idleness | 21.62 | 20.45 | 24.07 | 24.07 | 25.00 | 25.73 | 17.70$^{b}$ | 29.45$^{a}$ | 0.000 | 0.051 | $1.12\times10^{-51}$ | 0.205 | 0.962 | 0.319 | 0.151 |
| Feeding | 12.18 | 9.44 | 10.08 | 9.88 | 9.34 | 9.83 | 9.04$^{b}$ | 11.24$^{a}$ | 0.138 | 0.061 | 0.009 | 0.163 | 0.685 | 0.705 | 0.342 |
| Drinking | 1.96 | 1.61 | 2.20 | 2.25 | 1.86 | 1.76 | 1.09$^{b}$ | 2.81$^{a}$ | 0.386 | 0.454 | $3.93\times10^{-11}$ | 0.927 | 0.178 | 0.064 | 0.463 |
| Sleeping | 65.41 | 68.88 | 64.38 | 64.48 | 64.33 | 63.26 | 72.33$^{a}$ | 57.72$^{b}$ | 0.034 | 0.054 | $7.95\times10^{-5}$ | 0.125 | 0.635 | 0.142 | 0.111 |
| Interactive | 0.294$^{b}$ | 0.489$^{b}$ | 1.027$^{aA}$ | 0.538 | 0.538 | 0.391$^{B}$ | 0.515 | 0.579 | 0.674 | 0.168 | 0.306 | 0.014 | 0.794 | 0.158 | - |
| Finisher II (100.21 to 123.71 kg, 70 to 88 days of experiment) | | | | | | | | | | | | | | | |
| Standing | 18.54$^{aA}$ | 15.80$^{b}$ | 14.48$^{b}$ | 14.68$^{B}$ | 16.39 | 14.63 | 13.61$^{b}$ | 17.96$^{a}$ | 0.133 | 0.029 | $1.12\times10^{-11}$ | 0.009 | 0.744 | 0.358 | 0.581 |
| Lying down | 79.89 | 81.51 | 82.53 | 83.07 | 81.26 | 83.41 | 84.99$^{a}$ | 78.82$^{b}$ | 0.125 | 0.091 | 0.000 | 0.092 | 0.292 | 0.417 | 0.264 |
| Idleness | 17.76 | 16.14 | 17.86 | 18.98 | 19.67 | 17.51 | 12.23$^{b}$ | 23.91$^{a}$ | 0.038 | 0.389 | 0.000 | 0.170 | 0.064 | 0.004 | 0.923 |
| Feeding | 13.16$^{aA}$ | 10.71$^{b}$ | 10.32$^{b}$ | 10.27$^{B}$ | 11.11 | 10.13 | 10.60 | 11.31 | 0.134 | 0.109 | 0.079 | 0.042 | 0.851 | 0.942 | 0.255 |
| Drinking | 0.783$^{bB}$ | 1.86$^{a}$ | 1.61$^{a}$ | 1.81$^{A}$ | 1.13 | 1.22 | 1.03 | 1.79 | 0.637 | 0.667 | 0.052 | 0.010 | 0.180 | 0.247 | - |
| Sleeping | 68.79$^{b}$ | 72.50$^{aA}$ | 71.33$^{ab}$ | 69.91$^{ab}$ | 68.59$^{bB}$ | 71.92$^{a}$ | 76.71$^{a}$ | 64.14$^{b}$ | 0.300 | 0.064 | $9.02\times10^{-5}$ | 0.038 | 0.182 | 0.009 | 0.528 |
| Interactive | 0.147 | 0.245 | 0.147 | 0.342 | 0.245 | 0.098 | 0.097$^{b}$ | 0.314$^{a}$ | 0.898 | 0.434 | 0.015 | 0.511 | 0.740 | - | - |

[1] ME$_{0}$, ME$_{50}$, and ME$_{100}$: reference diet (RD) reduced by 0, 50, and 100 kcal MEkg$^{-1}$ diet, respectively; Enzyme-added: RD + 100 g α-amylase t$^{-1}$ diet;

[2] M: morning period (07:00 to 12:00h); A: afternoon (12:10 to 19:00h.).

[3] Significance probability of chi-square test of the type III analysis; ENZ: enzyme; ME: metabolizable energy; PER: period; ENZ*ME: interaction between ENZ and ME; ENZ*PER: interaction between ENZ and PER; ME*PER: interaction between ME and PER; TRI: triple interaction among ENZ*ME*PER; Means followed by different lowercase letters for the effect of ME/ENZ and different uppercase letters for the effect of ENZ/ME differ by difference among *lsmeans* test ($P < 0.05$).

Finisher II pigs fed a diet with no enzyme (NENZ) showed a higher proportion ($P < 0.05$) of standing and feeding behaviors than those fed a diet supplemented with enzyme (ENZ) with no reduced metabolizable energy (ME0). Under the same conditions, a higher frequency of drinking behavior was observed in pigs fed the ENZ diet than in those fed the NENZ diet (Table 4).

Higher frequencies were recorded for interactive behavior in finisher I pigs and for sleeping behavior in finisher II pigs when fed NENZ diets reduced by 100 and 50 kcal of ME kg$^{-1}$, respectively (Table 4).

Finisher I pigs fed a diet reduced by 50 kcal ME kg$^{-1}$, with no α-amylase (NENZ), tended to show greater lying down behavior than those fed a diet without energy reduction (Table 4).

On the other hand, greater standing and feeding behaviors were observed in finisher II pigs in the reference group (ME0) compared to pigs fed diets reduced by 50 and 100 kcal ME kg$^{-1}$ (Table 4). These findings showed that the best behavioral results were observed in pigs fed diets without energy reduction, as long as the α-amylase enzyme was not supplemented. This result suggests that the diets from the present study formulated to contain 3,300 kcal ME kg$^{-1}$ (Rostagno et al., 2017) met the ME requirement of finisher pigs.

When α-amylase was supplemented, lower lying and sleeping behaviors were observed, respectively, in finisher I and finisher II pigs fed a diet reduced by 50 kcal ME kg$^{-1}$ compared to those fed the diet with the greatest energy reduction (ME100). For instance, reduced passive behaviors were observed in pigs fed the ME50 diet, as shown in Table 4. These results suggest that diets reduced by up to 50 kcal ME kg$^{-1}$ can decrease the frequency of these behaviors when supplemented with α-amylase.

Partially aligning with findings on the hierarchical effect of ME/ENZ in grower pigs and based on criteria of greater proportions of active (feeding, interactive, drinking, and standing) and lower proportions of passive behaviors (lying down, idleness, and sleeping), better results were observed in pigs fed a diet reduced by 50 kcal ME kg$^{-1}$ compared to those fed the EM100 diet.

No interaction between ENZ and PER was observed on the behavioral variables in finisher pigs. For the hierarchical effect of the period at each level of reduction of metabolizable energy (0, 50, and 100 kcal kg$^{-1}$ diet), a greater proportion of morning sleeping behavior ($P < 0.05$) was observed in grower II and finisher II pigs compared to afternoon (Tables 3 and 4). Indeed, pigs have been reported (Paiano et al., 2007) to be more active (feeding, drinking, standing, exploring, and interactive) in the afternoon and less active (sleeping) in the morning, corroborating the results of the present study. The greater lying behavior observed in finisher pigs than in grower pigs may be associated with their greater body mass and weight, which could have interfered with their locomotion.

For the above-mentioned hierarchical effect, a greater idleness proportion ($P < 0.05$) was observed in finisher II pigs during the afternoon compared to the morning (Table 4).

There was a period effect ($P < 0.05$) on all behavioral variables, except for interactive behavior in grower I and finisher I pigs and feeding and drinking behaviors in finisher II pigs (Tables 3 and 4). Higher proportions of sleeping and lying down behaviors were observed in the morning in all phases. However, higher frequencies of standing and idleness were observed in the afternoon in grower and finisher pigs. These results may be mainly related to a normal individual variation affected by environmental conditions, animal interactions with the environment, and animal exploratory behavior (Massari et al., 2015; Cross et al., 2020).

The same comparative pattern for feeding and drinking behaviors was observed in the afternoon compared to the morning in the grower I and finisher I pigs (Tables 3 and 4). Finisher II pigs tended ($P = 0.079$ and $P = 0.052$) to show a greater proportion of these two behaviors in the afternoon compared to the morning (Table 4).

In the second experiment, the energy valorization matrix of the enzyme (EVM) and the time of day tended to affect, respectively, sleeping ($P = 0.067$) and drinking behaviors ($P = 0.011$) in grower I pigs. There was no effect ($P > 0.05$) of EVM, period, and their interaction on the other variables (Table 5).

**Table 5**

**Comparison among observed proportions (%) of behavioral variables in grower I and II pigs according to the simple effect of energy valorization matrix (EVM) for β-mannanase in the reference diet (RD) and period (Exp. II)**

| Item | EVM[1] | | | | Period (PER)[2] | | P[3] | | |
|---|---|---|---|---|---|---|---|---|---|
| | RD | $RD_{70}$ | $RD_{85}$ | $RD_{100}$ | M | A | EVM | PER | EVM*PER |
| | | | | | Grower I | | | | |
| Standing | 21.58 | 22.19 | 21.44 | 22.95 | 21.01 | 23.09 | 0.460 | 0.580 | 0.761 |
| Lying down | 77.12 | 76.71 | 77.67 | 76.16 | 77.87 | 75.94 | 0.531 | 0.617 | 0.573 |
| Idleness | 34.52 | 31.30 | 31.71 | 31.23 | 30.98 | 33.44 | 0.171 | 0.621 | 0.168 |
| Feeding | 10.96 | 11.10 | 11.16 | 12.12 | 11.01 | 11.67 | 0.581 | 0.766 | 0.344 |
| Drinking | 1.85 | 1.58 | 1.10 | 1.10 | 0.95$^{b}$ | 1.88$^{a}$ | 0.131 | 0.011 | 0.383 |
| Sleeping | 44.45$^{b}$ | 47.26$^{ab}$ | 47.67$^{a}$ | 46.10$^{ab}$ | 48.61 | 44.06 | 0.067 | 0.574 | 0.249 |
| Interactive | 7.95 | 8.49 | 8.15 | 9.18 | 8.04 | 8.85 | 0.263 | 0.684 | 0.753 |
| | | | | | Grower II | | | | |
| Standing | 18.84 | 22.33 | 19.52 | 20.55 | 17.53 | 23.16 | 0.294 | 0.099 | 0.315 |
| Lying down | 80.62 | 77.33 | 79.93 | 78.70 | 82.16 | 76.04 | 0.293 | 0.087 | 0.280 |
| Idleness | 28.56$^{a}$ | 21.85$^{b}$ | 23.84$^{b}$ | 24.59$^{b}$ | 20.14$^{b}$ | 29.41$^{a}$ | 0.010 | 0.046 | 0.364 |
| Feeding | 10.62 | 12.33 | 11.30 | 12.47 | 10.44 | 12.95 | 0.457 | 0.290 | 0.587 |
| Drinking | 1.78 | 1.71 | 1.44 | 1.64 | 1.28 | 2.01 | 0.851 | 0.076 | 0.681 |
| Sleeping | 52.74 | 55.75 | 56.58 | 54.18 | 62.40$^{a}$ | 47.01$^{b}$ | 0.180 | 0.031 | 0.351 |
| Interactive | 5.89 | 7.95 | 6.44 | 6.64 | 5.64 | 7.85 | 0.169 | 0.149 | 0.305 |

[1] EVM: energy valorization matrix for the enzyme(s) (kcal ME $kg^{-1}$ diet); RD: reference diet with 400 g xylanase $t^{-1}$ reduced by 40 kcal ME/kg; $RD_{70}$, $RD_{85}$, and $RD_{100}$: RD added 300 g β-mannanase $t^{-1}$ with EVM for β-mannanase of 30, 45 and 60 kcal of ME $kg^{-1}$, respectively (all diets with an EVM for xylanase of 40 kcal of ME$kg^{-1}$);

[2] M: morning period (07:00 to 12:00h); A: afternoon (12:10 to 19:00h.).

3Significance probability of chi-square test of the type III GEE analysis; Means followed by different letters on the row differ by the test of the difference among means of least squares, for the simple effect of EVM, and by the chi-square test of the type III GEE analysis, for the simple effect of period (P < 0.05).

The greater frequency of visits to the drinker in the afternoon (Table 5) may have been due to greater water intake. The behavior was assessed for two days (12 hours $day^{-1}$). According to the average temperature data between periods (considering the two days), the temperature in the afternoon was 22.8 ºC. In contrast, the average temperature in the morning was 15.8 ºC, a difference of 7ºC. Therefore, room temperature had an important influence on the effect of the period. Thus, the search for the drinker was unrelated to the thermal discomfort in grower I pigs. The average relative humidity values were very close.

Grower I pigs fed the RD85 diet showed a greater frequency of sleeping behavior than those fed the diet (RD). The result was not significant for the contrast between RD70 × RD, although the significance probability was slightly greater than the set alpha error (P= 0.072). We can not state why pigs fed a diet with a greater EVM did not show a greater frequency of sleeping behavior than pigs fed the RD diet. Based on our findings, grower I pigs fed diets reduced by 45 kcal ME kg$^{-1}$ (RD85) containing β-mannanase showed the best results (Table 5).

In grower II pigs, an effect (P< 0.05) of the assessment of EVM for β-mannanase was observed on the idleness behavior. An effect of period (P < 0.05) was observed on idleness and sleeping behaviors. No effect (P> 0.05) of EVM, period, and their interaction was observed on the other variables (Table 5).

In general, grower II pigs fed diets containing the β-mannanase and xylanase reduced by 70, 85, and 100 kcal ME kg$^{-1}$ showed lower idleness proportions than those fed the reference diet with no β-mannanase, regardless of the period of day (Table 5). These results suggest that corn and soybean meal energy was driven to other behavioral activities attributed to the different EVM for β-mannanase.

The average temperature in the morning and afternoon during the grower II phase was 12.1°C and 22.8ºC, respectively. These temperatures may explain the tendency (P = 0.087) for a greater frequency of lying down (82.16%) behavior in the morning than in the afternoon (76.04%) we observed in pigs (Table 5). With low temperatures, animals lie down longer and reduce exploratory activity to retain energy and maintain body temperature (Massari et al., 2015; Cross et al., 2020).

As a result of the low temperature, the effect of the period of the day on the idleness behavior (P = 0.048) suggests a need for the animals of all groups (RD, RD70, RD85, RD100) to increase feed intake to maintain body temperature. However, no significant effect of EVM was observed on the feeding behavior among the groups (Table 5). Li and Patience (2017) reported that the additional feed to support extra heat production is calculated using a model that estimates the efficiency of energy usage, nutrients absorbed for all body functions, and dietary digestible energy.

The estimated additional feed that should be consumed for each unit (°C) below thermal comfort is 25 g day$^{-1}$ and 39 g day$^{-1}$ for grower and finisher pigs, respectively. The estimated average daily gain is reduced by 10 g day$^{-1}$ to 22 g day$^{-1}$ in grower and finisher pigs, respectively. However, the extent to which these parameters are affected may depend on other factors, such as group size and individual genotypic traits (Nyachoti et al., 2004).

There was no effect (P > 0.05) of EVM, period, and their interaction on the behavior of finisher I pigs (Table 6). However, the significance probabilities of the EVM effect on standing and lying down were close (P = 0.09) to the one set in the present study (α = 0.05), which may suggest a possible trend. For instance, there is a probability of occurrence of type β error (false negative) that should not be ignored. Numerically, pigs fed the reference diet (RD) with no EVM for β-mannanase showed lower standing (15%) and greater lying down (82.81%) behavior frequencies compared to those fed diets with EVM for β-mannanase (Table 6).

**Table 6**

**Comparison among observed proportions (%) of behavioral variables of finisher I and II pigs according to the simple effect of energy valorization matrix (EVM) for β-mannanase in the reference diet (RD) and period (Exp. II)**

| Item | EVM[1] | | | | Period (PER)[2] | | P[3] | | |
|---|---|---|---|---|---|---|---|---|---|
| | RD | RD$_{70}$ | RD$_{85}$ | RD$_{100}$ | M | A | EVM | PER | EVM*PER |
| | | | | | Finisher I | | | | |
| Standing | 15.00 | 18.22 | 17.40 | 17.67 | 16.05 | 18.13 | 0.096 | 0.584 | 0.870 |
| Lying down | 82.81 | 79.38 | 79.38 | 79.59 | 81.76 | 78.78 | 0.092 | 0.483 | 0.682 |
| Idleness | 26.64 | 23.97 | 22.67 | 23.42 | 20.27 | 28.19 | 0.149 | 0.126 | 0.392 |
| Feeding | 9.32 | 10.96 | 10.89 | 11.58 | 10.71 | 10.66 | 0.139 | 0.982 | 0.914 |
| Drinking | 1.51 | 1.51 | 1.23 | 1.71 | 1.52 | 1.46 | 0.672 | 0.943 | 0.558 |
| Sleeping | 56.64 | 56.10 | 58.36 | 57.12 | 62.40 | 51.56 | 0.534 | 0.177 | 0.168 |
| Interactive | 5.62 | 7.19 | 6.51 | 5.75 | 5.10 | 7.47 | 0.457 | 0.133 | 0.804 |
| | | | | | Finisher II | | | | |
| Standing | 10.55$^{b}$ | 13.01$^{a}$ | 14.86$^{a}$ | 13.29$^{a}$ | 10.68$^{b}$ | 15.24$^{a}$ | 0.007 | 0.025 | 0.529 |
| Lying down | 83.90$^{a}$ | 80.96$^{ab}$ | 79.32$^{b}$ | 82.05$^{a}$ | 85.00$^{a}$ | 78.02$^{b}$ | 0.004 | 0.029 | 0.438 |
| Idleness | 28.84$^{a}$ | 24.45$^{b}$ | 24.52$^{b}$ | 28.84$^{a}$ | 19.09$^{b}$ | 34.44$^{a}$ | 0.014 | 0.001 | 0.832 |
| Feeding | 7.53$^{b}$ | 9.59$^{a}$ | 9.73$^{a}$ | 9.32$^{ab}$ | 8.61 | 9.48 | 0.065 | 0.675 | 0.591 |
| Drinking | 2.26 | 2.26 | 2.26 | 1.44 | 1.25$^{b}$ | 2.88a | 0.227 | 0.009 | 0.892 |
| Sleeping | 51.99 | 51.78 | 52.60 | 50.07 | 62.43$^{a}$ | 40.49$^{b}$ | 0.577 | 0.002 | 0.608 |
| Interactive | 4.66 | 6.85 | 6.44 | 5.27 | 3.65$^{b}$ | 8.02$^{a}$ | 0.057 | 0.010 | 0.952 |

[1] EVM: energy valorization matrix for the enzyme(s) (kcal ME/kg diet); RD: reference diet with 400 g xylanase t$^{-1}$ reduced by 40 kcal ME kg$^{-1}$; RD$_{70}$, RD$_{85}$, and RD$_{100}$: RD added 300 g β-mannanase t$^{-1}$ with EVM for β-mannanase of 30, 45 and 60 kcal of ME/kg, respectively (all diets with an EVM for xylanase of 40 kcal of ME kg$^{-1}$);

[2] M: morning period (07:00 to 12:00h); A: afternoon (12:10 to 19:00h.).

[3] Significance probability of chi-square test of the type III GEE analysis; Means followed by different letters on the row differ by the test of the difference among means of least squares, for the simple effect of EVM, and by the chi-square test of the type III GEE analysis, for the simple effect of period (P < 0.05).

Although there were no differences (P > 0.05), finisher I pigs fed diets with EVM for β-mannanase and xylanase (RD70, RD85, RD100) tended to show greater physical activity than those fed the RD diet, with positive effects on standing and feeding behaviors and lower lying down behavior (Table 6).

These results may be related to the energy savings that β-mannanase provides, which occurs due to an unnecessary immune deactivation caused by the plant β-mannans, providing the animal with an extra energy supply. Feed β-mannans can create a false signal by activating the immune system that recognizes the presence of pathogens in the gut, which causes unnecessary immune activation, wasting energy and nutrients (Vangroenweghe et al., 2021), also known as a feeding-induced immune response (Arsenault et al., 2017).

An effect ($P < 0.05$) of the EVM on standing, lying down, and idleness behaviors was observed in finisher II pigs. The period of the day also affected the standing, lying down, idleness, drinking, sleeping, and interactive behavior. There was no effect ($P > 0.05$) of EVM, period, and their interaction on the other variables (Table 6).

The significance level for the test of the difference between lsmeans (RD × RD70 contrast) of lying behavior was 0.066. This finding indicates a potential risk of a Type II error (false negative), implying a potential for significance between these means (notably, the mean of 80.96 for the RD70 diet is assigned the designation “b”).

The proportions of idleness and lying down behaviors in pigs fed RD and RD100 were greater ($P < 0.05$) than in pigs fed RD70 and RD85, which suggests a weak association between greater metabolizable energy availability and little physical activity behaviors of the pigs, except for those fed the RD100 diet.

EVM tended ($0.05 < P \leq 0.10$) to affect feeding ($p = 0.065$) and interactive ($P = 0.057$) behaviors of finisher II pigs. Accepting this trend as a significant effect ($p < 0.05$), the means 9.59 (RD70) and 9.73 (RD85) differ ($P < 0.05$) from 7.53 (RD). The average of 9.32 (RD100) is similar to the others. Thus, considering the lower frequencies of lying down and idleness, together with the greater frequency of active feeding behavior, the best results related to the greater energy availability of nutrients in diets containing β-mannanase + xylanase were provided by RD70 and RD85 diets (Table 6).

Genova et al. (2023) assessed protein and digestible energy levels in finisher I and II pigs. The authors found that feed to gain ratio and energy and protein usage were optimized in pigs fed the RD85 diet (reduced by 85 kcal ME $kg^{-1}$) without impairing the metabolism and intestinal ecosystem. In the present study, pigs fed the same diet (RD85) showed better behavioral indexes than pigs fed RD and RD100 diets, suggesting an effective contribution of the enzymatic valorization from β-mannanase and xylanase in increasing the frequency of feeding and interactive behaviors, as well as in reducing the frequency of lying down and idleness in finisher II pigs.

In addition to the beneficial effects on the behavior of finisher II pigs we observed in the present study, dietary β-mannanase, xylanase, and phytase made energy more available to the animals and allowed the reduction of oil in the nutritional matrix without impairing the dietary metabolizable energy, which is corroborated the study of Sakomura et al. (2014).

The effect of the period on the drinking behavior was associated with the assessment on the second day when the average temperature reached 32 °C in the afternoon (morning and afternoon average temperatures of 21 °C and 26°C, respectively). This result is due to the need for hydration to reduce body temperature. Alves et al. (2020) reported that the ideal temperature for grower pigs ranges from 18 to 26 °C and that pigs change their behavior according to room temperature, possibly reducing the number of visits to the feeder and the time spent on feeding at high temperatures.

## Conclusions

A dose of 100 g exogenous α-amylase/t diet compensates for a reduction of up to 50 kcal metabolizable energy kg$^{-1}$ diet in grower pigs. This dose increases the proportion of visits to the feeder and standing behavior in grower II pigs, and in grower I and II pigs, it increases the proportion of interactive behavior. Including 100 g exogenous α-amylase t$^{-1}$ in diets without energy reduction does not favor the search for feed and the standing behavior in finisher II pigs.

The energy valorization matrices (70, 85, and 100 kcal metabolizable energy kg$^{-1}$) for 300 g of β-mannanase and 400 g of xylanase t$^{-1}$ diet change the sleeping behavior of grower I pigs, reduce the proportion of idleness in the grower II pigs, and reduce the lying down behavior in finisher I pigs. Finisher II pigs fed a diet reduced by 70 and 85 kcal metabolizable energy/kg and supplemented with 300 g of β-mannanase and 400 g of xylanase show the best results for standing and feeding behaviors, with lower proportions for lying down and idleness passive behaviors. A diet reduced by 85 kcal metabolizable energy/kg supplemented with 300 g of β-mannanase and 400 g of xylanase is recommended for finisher II pigs.

## References

Alves, C. P., Eugênio, D. S., Souza, L. F. de, Santos, J. P. A. de S., Silva, J. O. N. da, Silva, A. R., Santos, T. S. dos, & Silva, T. G. F. da. (2020). Influence of temperature and relative humidity in food consumption of swine in the Brazilian semiarid region. *Brazilian Journal of Animal and Environmental Research, 3*(4), 4263-4269. doi: 10.34188/bjaerv3N4-122

Arsenault, R. J., Lee, J. T., Latham, R., & Carter, B. (2017). Changes in immune and metabolic gut response in broilers fed β-mannanase in β-mannan containing diets. *Poultry Science, 96*(12), 4307-4316. doi: 10.3382/PS/PEX246

Bach Knudsen, K. E. (2014). Fiber and non-starch polysaccharide content and variation in common crops used in broiler diets. *Poultry Science, 93*(9), 2380-2393. doi: 10.3382/PS.2014-03902

Brewster, L. R., Dale, J. J., Guttridge, T. L., Gruber, S. H., Hansell, A. C., Elliott, M., Cowx, I. G., Whitney, N. M., & Gleiss, A. C. (2018). Development and application of machine learning algorithm for classification of elasmobranch behavior from accelerometry data. *Marine Biology, 165*(4), 62-81. doi: 10.1007/s00227-018-3318-y.

Cross, A. J., Brown-Brandl, T. M., Keel, B. N., Kassady, J. P., & Rohrer, G. A. (2020). Feeding behavior of grow-finish swine and the impacts of heat stress. *Translational Animal Science, 4*(2), 986-992. doi: 10.1093/tas/txaa023

Dias, C. P., Silva, C. A., & Manteca, X. (2014). *Pig welfare.* UEL.

Genova, J. L., Rupolo, P. E., Azevedo, L. B., Henz, D., Carvalho, S. T., Kipper, M., Gonçalves, G. A. C., Vilela, H. L. O., Pasquetti, T. J., Oliveira, N. T. E., Dietrich, A. R. M., & Carvalho, P. L. O. (2023). β-mannanase supplementation in diets reduced in 85 kcal metabolizable energy/kg containing xylanase-phytase improves gain to feed ratio, nutrient usage, and backfat thickness in finisher pigs. *Frontiers in Veterinary Science, 10,* 1144692. doi: 10.3389/fvets.2023.1144692

Gomes, B. K., Cony, B. S. L., & Stella, L. (2019). Exogenous enzymes in pig feed. *Nutritime Electronic Journal, 16*(3), 8477-8487.

Jackson, M. E., Geronian, K., Knox, A., Mcnab, J., & Mccartney, E. (2004). A dose response study with the feed enzyme β-mannanase in broilers provided with corn-soybean meal based diets in the absence of antibiotic growth promoters. *Poultry Science, 83*(12), 1992-1996. doi: 10.1093/ps/83.12.1992

Li, Q., & Patience, J. F. (2017). Factors involved in the regulation of feed and energy intake of pigs. *Animal Feed Science and Technology, 233,* 22-33. doi: 10.1016/j.anifeedsci.2016.01.001

Martin, P., & Bateson, P. (1986). *Measuring behaviour: an introductory guide.* Cambridge University Press.

Massari, J. M., Curi, T. M. R. C., Moura, D. J., Medeiros, B. B. L., & Salgado, D. D. (2015). Behavioral characteristics of grower and finisher pigs in a “wean to finish” system. *Agricultural Engineering, 35*(4), 646-656. doi: 10.1590/1809-4430-Eng.Agric.v35n4p646-656/2015

Nyachoti, C. M., Zijlstra, R. T., Lange, C. F. M., & Patience, J. F. (2004). Voluntary feed intake in grower-finisher pigs: a review of the main determining factors and potential approaches for accurate predictions. *Canadian Journal of Animal Science, 84*(4), 549-566. doi: 10.4141/A04-001

Paiano, D., Barbosa, O. R., Moreira, I., Quadros, A. R. B., Silva, M. A. A. da, & Oliveira, C. A. L. de. (2007). Behavior of pigs housed in partially slatted floor stalls or with water depth. *Acta Scientiarum. Animal Sciences, 29*(3), 345-351. doi: 10.4025/actascianimsci.v29i3.575

Rostagno, H. S., Albino, L. F. T., Donzele, J. L., Oliveira, R. F., Barreto, S. L. T., Hannas, M. I., Donzele, J. L., Sakomura, N. K., Perazzo, F. G., Saraiva, A., Abreu, M. L.T., Rodrigues, P. B., Oliveira, R. F., Barreto, S. L. T., Brito, C. O. (2017). *Brazilian tables for poultry and pork: feed composition and nutritional requirements.* UFV.

Ruiz, U. D. S., Thomaz, M. C., Hannas, M. I., Fraga, A. L., Watanabe, P. H., & Silva, S. Z. da. (2008). Enzyme complex for pigs: digestion, metabolism, performance and environmental impact. *Brazilian Journal of Animal Science, 37*(3), 458-468. doi: 10.1590/S1516-35982008000300011

Rupolo, P. E., Monteiro, D. P., Ribeiro, T. P., Azevedo, L. B., Gregory, C. R., Careli, P. S., Carvalho, S. T., Paiano, D., Hannas, M. I., Nunes, R. V., Silva, M. A. A., Genova, J. L., & Carvalho, P. L. O. (2023). Effects of supplementation of α-amylase alone in a model of grower and finisher pigs fed metabolizable energy-reduced diets. *Livestock Science, 278,* 105361. doi: 10.1016/j.livsci.2023.105361

Sakomura, N. K., Silva, H. V., & Costa, G. P. C. (2014). *Nutrition of non-ruminants.* Funep.

Shastak, Y., Ader, P., Feuerstein, D., Rühle, R., & Matuschek, M. (2015). ß-Manan and mannanase in poultry nutrition. World´s *Poultry Science Journal., 71*(1), 161-174. doi: 10.1017/s0043933915000136

Souza da Silva, C., Haenen, D., Koopmans, S. J., Hooiveld, G. J. E. J., Bosch, G., Bolhuis, J. E., Kemp, B., Müller, M., & Gerrits, W. J. J. (2014). Effects of resistant starch on behaviour, satiety-related hormones and metabolites in grower pigs. *Animal, 8*(9), 1402-1411. doi: 10.1017/S1751731114001116

Tavernari, F. C., Carvalho, T. A., Assis, A. P., & Lima, H. J. D. (2008). Soluble non-starch polysaccharide in the diet of pigs and poultry. *Nutritime Electronic Journal, 5*(5), 673-689.

Vangroenweghe, F. A., Poulsen, K., & Thas, O. (2021). Supplementation of a β-mannanase enzyme reduces post-weaning diarrhea and antibiotic use in piglets on an alternative diet with additional soybean meal. *Porcine Health Management, 7*(1), 1-12. doi: 10.1186/S40813-021-00191-5

Veum, T. L., & Odle, J. (2001). Feeding neonatal pigs. In A. J. Lewis, & L. L. Southern (Eds.), *Swine nutrition* (pp. 671-691). Boca Raton.