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Nutrient and energy balance, and amino acid digestibility in weaned piglets fed wheat bran and an exogenous enzyme combination

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*The digestive system of the weaned piglets can be affected by the type of ingredients present in the diet, and a high fibre content in diets can limit the use of other nutrients and energy. The study was conducted to determine the effects of multicarbohydrase (**MC**) and phytase (**Phy**) supplementation on the nutritive value of wheat bran (**WB**) in weaned piglets. Multicarbohydrase preparation had 700 U α-galactosidase, 2200 U galactomannanase, 3000 U xylanase and 22 000 U β-glucanase per kilogram of diet, and Phy had 500 phytase units – FTU/kg of diet. Twenty-five weaned piglets (6.1 ± 0.63 kg) at 21 days old were fed five diets in a completely randomised experimental design with a 2 × 2 + 1 (0 and 200 mg/kg MC; 0 and 50 mg/kg Phy; and basal diet – **BD**) factorial arrangement used to determine treatment effects. An additional group of piglets was fed a corn-basal diet during apparent digestibility of nutrients, and fed a 5% casein-corn starch basal diet during apparent and standardised ileal digestibility (**SID**) of amino acid evaluations. Piglets were individually caged until 38 days old, when Ileal digesta was collected at slaughter. Test diets were made by mixing the basal diets and WB 7 : 3 (w/w), with or without MC, Phy or the combination. There was an interaction trend (P = 0.07) between MC and Phy in the balance of ash, digestible energy (**DE**) and metabolisable energy (**ME**). Effects of MC (P < 0.01) on DM, N retention, DE and ME, as well as an effect of Phy (P < 0.05) on ash, DE and ME and a trend in protein digestibility (P = 0.07) also was observed. The enzyme combination showed effect (P < 0.05) on SID of Lys, Pro and Ser; as a trend (P < 0.07) on His, Thr and Val. Isolated, MC also suggested improving (P < 0.07) on SID of His, Lys, Ala (P < 0.05), while Phy improved (P < 0.06) SID of Leu, Lys, Met (P < 0.01), Thr, Val, Ala (P < 0.01), Pro and Ser (P < 0.05). The MC carbohydrate complex was characterised as a viable alternative to increase the apparent nutrients digestibility and SID of amino acids when WB was used in the diet of young pigs and, when combined with Phy, suggested an additive effect on the apparent use of energy.*

Keywords: improvement, multicarbohydrase, nutritional, phytase, digestion

Implications

Piglets have a limited capacity to digest fibre compounds, while dietary plant ingredients contain fibre, which can reduce the use of energy and nutrients. However, the dietary inclusion of exogenous enzymes becomes an alternative to increase the efficiency of digestion by weaned piglets. As the present study shows, the use of caboidrase alone or in combination with phytase allows changes in diet formulation when wheat bran is included.

Introduction

Protein sources from vegetable by-products are used in pig diets, usually in order to reduce feeding costs. However, plant ingredients are rich in fibrous carbohydrates whose compounds are resistant to digestion in the small intestine. Among these,the most prominent of the resistant carbohydrates are non-starch polysaccharides in the plant cell walls (Englyst *et al.*, 2007), whose digestibility is very limited in pigs. Inaddition, at weaning the piglet presents digestive physiological limitations, mainly for the digestion of vegetal ingredients. Other difficulties involved in the study of digestibility in early weaned swine are those related to the implantation of T-cannula in the distal ileum. Thus, these conditions limit the accuracy of the nutritional information published in pig food tables. However, the physiological development of the digestive tract in the early stages of growth should be considered. In contrast, most of ingredients used in commercial piglet diets are based on nutritional specifications obtained from older pigs. For example, the values of

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amino acid digestibility should be obtained with younger pigs to support diet formulations for weaned piglets.

The ability of pigs to digest more fibrous diets, without affecting production efficiency, needs to be focused to maintain a normal nutritional efficiency. Wheat bran (**WB**) is a by-product of the dry milling of wheat to obtain flour. It contains around 20% starch, 15% CP, 10% ADF and 30% NDF (National Research Council, 2012). It can be partially used as a corn replacement to minimise feed costs. The high levels of crude fibre and phytic phosphorus, combined with the limited production of digestive enzymes in piglets, restrict the inclusion of WB in diets. High fibre intake or non-starch polysaccharide increases intestinal viscosity, reducing intestinal transit time and nutrient utilisation, and may induce physiological changes in the gastrointestinal tract, as well as increase the energy demand for the intestine (Adeola and Cowieson, 2011). On the other hand, dietary supplementation with exogenous enzymes, alone or in combination, could favour the use of certain plant ingredients for swine and poultry. Synergies when these enzymes are combined and marketed as a mixture of additives should also be understood (Kiarie *et al.*, 2010).

The importance of phosphorus (P) in animal nutrition is widely known, but in plant ingredients, it is in phytate form, which can only be degraded to a limited extent in non-ruminant gastrointestinal tracts (Slominski, 2011). Wheat phytate molecules encapsulate proteins within the cells of the aleurone layer and are surrounded by carbohydrates that limit the availability of nutrients (Heard *et al.*, 2001). However, carbohydrases increase the digestibility of nutrients in broilers (Gallardo *et al.*, 2018) and weaned piglets (Dadalt *et al.*, 2017) by reducing digesta viscosity (Amerah, 2015). Carbohydrases also improve the endogenous enzymes' access to nutrients and the transformation of phytate by phytase (**Phy**; Woyengo *et al.*, 2016).

The hypothesis of this study was that the digestibility of WB, in young piglets between 8 and 11 kg, is lower than the values described in the literature. Thus, the expected differences would suggest the need for readjustments of the tables for the chemical composition of WB, with or without enzymes. This study evaluated the effects of multicarbohydrase (**MC**) and Phy on the apparent digestibility of nutrients and energy use, and the standardised ileal digestibility (**SID**) of amino acid content of WB in weaned piglets.

Materials and methods

All research methods and procedures have been approved by ethics and animal 97 experimentation committee at the University of São Paulo (project 2843/2012), Brazil, 98 and were followed according to animal welfare.

Wheat bran and enzymes

Wheat bran included on basal diets (Table 1) was a hard red spring wheat variety that was obtained from the feed mill of Sao Paulo University. The chemical composition (as fed basis) of WB is shown in Tables 2 and 3. The nutritional profile of WB was similar to that described in the literature, with the exception of GE, which was 14.41 MJ/kg compared to 16.84 MJ/kg from Rostagno *et al.* (2011) and 16.15 MJ/kg from the NRC (2012).

The enzymes used were a MC blend described as Endopower Beta and Phy described as Genophos, as reported in Gallardo *et al.* (2017). Exogenous enzyme activities were

Table 1 *Ingredient composition and nutrient content of basal and experimental diets for piglets*[1]

	Total collection diet		Ileal collection diet	
Ingredientes, %	BD	BDI	BD_1	BDI_1
Corn	61.35	42.95	–	–
Corn starch	5.00	3.50	41.56	29.09
Test ingredient	–	30.00	–	30.00
Soybean oil	1.98	1.38	3.13	2.19
Dry whey	15.00	10.50	–	–
Skim milk powder	10.00	7.00	–	–
Dextrose	–	–	21.00	14.70
Lactose	–	–	20.00	14.00
Casein	–	–	5.00	3.50
Choline	0.01	0.01	0.01	0.01
Potassium chloride	–	–	1.04	0.73
Sodium bicarbonate	0.43	0.30	1.04	0.73
Dicalcium phosphate	1.71	1.19	3.01	2.11
Limestone	0.54	0.38	0.22	0.15
Cromium oxide	0.30	0.21	0.30	0.21
Zinc oxide	0.35	0.25	0.35	0.25
Vitamin premix[2]	0.10	0.07	0.10	0.07
Mineral premix[3]	0.10	0.07	0.10	0.07
Cellulose	3.00	2.10	3.00	2.10
Antioxidant	0.01	0.01	0.12	0.08
Flavouring	0.12	0.08	0.02	0.01
Total	100	100	100	100
Calculated composition[4], %				
DM	90.41	–	93.84	–
ME (MJ/kg)	14.19	–	13.94	–
CP	9.01	–	4.26	–
Ca	0.85	–	0.85	–
P	0.45	–	0.45	–
Sodium	0.28	–	0.28	–
Chlorine	0.31	–	0.49	–
Potassium	0.61	–	0.52	–
Linoleic acid	2.21	–	1.65	–

BD and BD_1 = basal diet; BDI and BDI_1 = basal diet + test ingredient (WB); ME = metabolisable energy.

[1]Test ingredient replaced 30% of basal diet, with or without multicarbohydrase (200 g/ton); phytase (50 g/ton) and multicarbohydrase (200 g/ton) + phytase (50 g/ton), respectively.

[2]Vitamin px. provided per ton of diet: Vitamin A, 10 000 000UI; Vitamin D_3, 1 650 000UI; Vitamin E, 60 000 IU; Vitamin K, 2000 mg; Vitamin B_1, 1200 mg; Vitamin B_2, 4000 mg; Vitamin B_6, 2200 mg; Vitamin B_{12}, 22 000 mcg; pantotenic acid, 18 000 mg; folic acid, 400 mg; niacin, 30 000 mg; biotine, 150 mg.

[3]Mineral px. provided per ton of diet: Se, 361 mg; I, 1100 mg; Iron, 90 000 mg; Cu, 10 500 mg; Zn, 117 800 mg; Mn, 40 200 mg.

[4]Values were calculated using NRC (2012) database.

Table 2 *Analysed composition of WB and experimental diets used for the apparent digestibility of nutrients and energy determination in postweaned piglets*[1]

	Ingredient	Experimental diets				
Item, %	WB	BD	WB	WB+MC	WB+Phy	WB+MC+Phy
DM	87.64	88.95	89.09	88.91	89.10	89.17
GE (MJ/kg)	16.44	15.98	16.09	16.29	16.32	16.32
CP	15.36	10.56	11.51	11.63	12.06	12.52
Ash	5.15	5.20	5.11	4.96	5.11	5.25
Crude fibre	6.62	–	–	–	–	–
NDF	40.72	–	–	–	–	–
ADF	11.98	–	–	–	–	–
Fat	3.15	–	–	–	–	–

WB = wheat bran; BD = basal diet; MC = multicarbohydrase; Phy = Phytase; GE = gross energy.
[1]Data on as-fed basis.

Table 3 *Analysed amino acids content of WB and experimental diets used for the AID and SID determination in postweaned piglets*

	Ingredient	Experimental diets				
Item, %	WB	BD	WB	WB+MC	WB+Phy	WB+MC+Phy
DM	87.64	88.95	89.09	88.91	89.10	89.17
Indispensable amino acids						
Arg	1.18	0.16	0.44	0.43	0.43	0.44
His	0.46	0.14	0.23	0.22	0.22	0.22
Iso	0.52	0.26	0.33	0.31	0.32	0.32
Leu	0.97	0.44	0.59	0.59	0.60	0.59
Lys	0.71	0.36	0.47	0.45	0.46	0.46
Met	0.19	0.12	0.14	0.14	0.14	0.13
Phe	0.57	0.36	0.47	0.45	0.45	0.46
Thr	0.64	0.23	0.33	0.33	0.33	0.33
Val	0.89	0.36	0.48	0.47	0.47	0.48
Dispensable amino acids						
Ala	0.79	0.17	0.34	0.33	0.33	0.34
Asp	1.37	0.43	0.69	0.67	0.67	0.68
Cys	0.16	0.020	0.05	0.05	0.05	0.04
Glut	2.61	0.98	1.44	1.43	1.43	1.43
Gly	0.86	0.10	0.31	0.30	0.30	0.29
Pro	0.92	0.45	0.56	0.55	0.57	0.56
Ser	0.76	0.28	0.40	0.40	0.40	0.40
Tyr	0.45	0.25	0.33	0.32	0.33	0.31
Total amino acids	14.03	5.11	7.58	7.46	7.49	7.47

WB = wheat bran; AID = apparent ileal digestibility; SID = standardised ileal digestibility; BD = basal diet; MC = multicarbohydrase; Phy = phytase.

suggested by GNC Bioferm Inc. (Saskatoon, Saskatchewan, Canada), and technical information was provided by Uniquimica, São Paulo, Brazil.

Diets and experimental design

Twenty-five weaned piglets at 21 days of age were fed five diets, in a completely randomised experimental design with a $2 \times 2 + 1$ (0 and 200 mg/kg MC; 0 and 50 mg/kg Phy, and basal diet – BD). The factorial arrangement was used to determine the effects of treatment of enzyme supplementation alone or in combination. An additional group of piglets was fed a corn-basal diet (**BD**) during apparent nutrients digestibility and fed a 5% casein-corn starch diet (BDI) during the apparent ileal digestibility (**AID**) and SID of WB. Piglets were individually grated in metabolism crates. Test diets were made by mixing the BD or BDI and WB 7 : 3 wt/wt basis. Thus, test diets were with or without MC, Phy or their combination. Therefore, five corn/dry whey/milk powder–based diets were used to calculate the apparent nutrients digestibility and energy of the WB. At the end of apparent nutrients digestibility evaluation, piglets were fed five corn starch-based diets containing 5% casein

to quantify the AID and SID of amino acids. The nutritional composition and energy of WB and the experimental diets are presented in Tables 2 and 3. Five piglets were used per treatment in each evaluation period. For the apparent digestibility of nutrients and energy, the BD was used in the test ingredient. For the determination of the SID, the additional group of piglets fed BDI (5% casein-corn starch diet) was used to estimate the loss of basal endogenous amino acids. A 0.25% inclusion of ferric oxide (Fe_2O_3) in the experimental diets (apparent digestibility of nutrients determination) was added to determine the time of collection by the appearance of marked faeces. As an indigestible marker (0.3% of the diet) for the calculation of AID and SID of amino acids, chromium oxide (Cr_2O_3) was used. The MC was added at 200 g/ton and Phy added at 50 g/ton in the diet. All diets were supplemented with minerals and vitamins to meet or exceed recommended nutrient specification for growing pigs (Rostagno *et al.*, 2011).

Feeding and sample collection

Weaned piglets were fed basal corn dry-whey milk-powder diet for 5 days and subsequently introduced to experimental diets for 10 days of which 7-day adaptation period to the diets and 3-day collection of faeces and urine, according to Trindade Neto *et al.* (2010). Ferric oxide (0.25%) was added to the morning meal on days 8 and 10 to determine the beginning and the end of collections. The pigs' BW during the initial adaptation period was 8.0 ± 0.5 kg. The daily amount of feed provided to the pigs was calculated as three times the estimated requirement for maintenance energy (i.e. 106 kcal of ME/$kg^{0.75}$; NRC, 2012) and provided in two equal meals at 0700 and 1700 h. Pigs were allowed *ad libitum* access to water throughout the experiment. Fecal samples were collected twice daily at 0730 and 1730 h and stored at −20°C immediately after collection.

At the end of the total collection, an endogenous loss diet (**ELD**) or BDI for the determination of amino acids was used. Another four ELDs (BDI with 30% WB replacement) with or without MC, Phy or MC+Phy, were also used. All 25 piglets were fed their respective treatment diets in replicates of 5, as was done for the apparent nutrients digestibility. After 3 days of adaptation to the treatments, the pigs were slaughtered and digesta samples were collected at the end of the ileum (an approximately 30 cm-long segment). Before slaughter, pigs were weighed and then given an intramuscular injection of acepromazine maleate (0.3 mg/kg of BW) mixed in a single syringe with 15 mg of ketamine hydrochloride/kg BW as a pre-anesthetic tranquiliser. Once tranquilised sufficiently, approximately 20 mg of thiopental/kg BW was administered by intracardiac injection.

Chemical analysis

Faeces were stored (300 g/kg) in a freezer at −10°C, and at the end of the experiment were homogenised, sampled and dried in an oven at 60°C for 72 h. Excreted urine was drained to plastic buckets with 5 ml H_2SO_4. The volume was weighed daily, and an aliquot of 300 g/kg was withdrawn and kept under refrigeration at −10°C. The urine samples were oven dried and analysed for GE. Ileal samples were freeze-dried for 72 h at −40°C (LH 0401, Terroni, Sao Carlos, Brazil), as described by Gallardo *et al.* (2017) for all preparations of diets, WB, faeces and ileal digesta samples that were finely milled and analysed, according to Association of Official Analytical Chemists (AOAC, 2004) procedures for DM, ash, gross energy (**GE**), nitrogen (N) and NDF determinations. Test diets and ileal digesta samples were further analysed for amino acids, GE content, N, ash and chromium concentration in the ileal digesta, as reported by Gallardo *et al.* (2017). Cysteine was expressed as cystine.

Digestibility calculation and statistical analyses

As reported by Dadalt *et al.* (2017), all formulas related to apparent nutrients digestibility and amino acid digestibility (AID and SID) were:

$$\text{Apparent nutrients digestibility } (\%) = 100 \times [(\text{NI} - \text{NO}_{\text{faeces}})/\text{NI}],$$

where NI is the nutrient or energy intake (g or MJ/kg), and NO_{faeces} is the nutrient or energy output in faeces (g or MJ/kg).

The digestible energy (**DE**) and metabolisable energy (**ME**) values of the WB were calculated as following equations:

$$\text{DEWB}_{\text{Diet}} = (\text{IWB}_{\text{Diet}} \times \text{GEWB}_{\text{Diet}} - \text{DM}_{\text{Excreted}} \times \text{GE}_{\text{Faeces}}),$$

where $DEWB_{Diet}$ is DE (MJ/kg) of the WB diet (test diet); IWB_{Diet} is intake of test diet on DM base; $GEWB_{Diet}$ is gross energy (MJ/kg) of the test diet; $DM_{Excreted}$ is excreted DM; and GE_{Faeces} is gross energy (MJ/kg) of the faeces.

$$\text{MEWB}_{\text{Diet}} = (\text{IWB}_{\text{Diet}} \times \text{GEWB}_{\text{Diet}} - \text{DM}_{\text{Excreted}} \times \text{GE}_{\text{Faeces}} - \text{U}_{\text{Excreted}} \times \text{GE}_{\text{Urine}})$$

where $MEWB_{Diet}$ is ME (MJ/kg) of the WB diet (test diet); IWB_{Diet} is intake of test diet (g/kg) on DM base; $GEWB_{Diet}$ is gross energy (MJ/kg) of the test diet; $DM_{Excreted}$ is excreted (g/kg) DM as faeces; GE_{Faeces} is gross energy (MJ/kg) of the faeces; $Urine_{Excreted}$ is DM excreted (g/kg) as urine; and GE_{Urine} is gross energy (MJ/kg) of the urine.

$$\text{DEWB} = [\text{DEWB}_{\text{Diet}} - (\text{DE}_{\text{BD}} \times \text{BD}_{\text{inclusion}})]/\text{WB}_{\text{inclusion}}$$

where DEWB is the DE (MJ/kg) of the WB; $DEWB_{Diet}$ is the DE (MJ/kg) of the WB diet (test diet); DE_{BD} is the DE (MJ/kg) of the BD; $BD_{inclusion}$ is the BD content (g/kg); and $WB_{inclusion}$ is WB inclusion (g/kg) on BD.

Apparent ileal amino acids digestibility were calculated using formula cited by Opapeju *et al.* (2006):

$$\text{AID } (\%) = 100 - [100 \times (\text{AA}_{\text{digesta}} \times \text{Cr}_2\text{O}_{3\text{diet}})/(\text{AA}_{\text{diet}} \times \text{Cr}_2\text{O}_{3\text{digesta}})]$$

where AA_{diet} and $AA_{digesta}$ are the concentrations (mg/kg DM) of amino acids in the diet and digesta, respectively, and Cr_2O_{3diet} and $Cr_2O_{3digesta}$ are the concentrations (mg/kg DM)

of the indigestible marker in the diet and digesta, respectively. Apparent ileal amino acids digestibility were standardised using average values for basal endogenous amino acids losses calculated using the following formula:

$$AA_{EL}(g/kg) = AA_{digesta} \times (Cr_2O_{3diet}/Cr_2O_{3digesta})$$

where AA_{EL} = average endogenous amino acids loss in (g/kg of DM).

Determined endogenous ileal amino acids losses (g/kg of DMI) were as follows: Arg, 0.50; His, 0.31; Ile, 0.50; Leu, 0.80; Lys, 0.41; Met, 0.21; Phe, 0.52; Thr, 0.93; Val, 0.67; Ala, 0.76; Asp, 0.60; Glu, 1.13; Pro, 0.95; Ser, 0.99, and Tyr, 0.35 g/kg.

Standardised ileal digestibility was calculated according to the following equation:

$$SID\ (\%) = [AID + (AA_{EL}/AA_{diet})] \times 100$$

Data were subjected to ANOVA using the GLM procedure of SAS (2008) (SAS Software Release 9.2, SAS Institute, Inc., Cary, NC, USA). Significant main effects were declared when $P < 0.05$. The homogeneity of variances was evaluated by Hartley test and the normality of residuals by the Shapiro-Wilk test (UNIVARIATE procedure). The statistical model used was:

$$Y_{ij} = \mu + a_i + b_j + (a_i \times b_j) + e_{ij}$$

where Y_{ij} = variable response of piglets fed MC (i) and Phy (j); μ = overall effect of average; a_i = fixed effect for MC; b_j = fixed effect for Phy; $(a_i \times b_j)$ = interaction between MC and Phy; e_{ij} = error term (residue).

Results

This study was conducted to evaluate the nutritive value of WB for weaned piglets, with or without MC and Phy supplementation. On as-feed-basis, DE (10.32 MJ/kg) and ME (10.09 MJ/kg) of WB, without the use of enzymes, were close to those described by Rostagno *et al.* (2011) (10.35 and 9.89 MJ/kg) and slightly above those described by the NRC (2012) (10.13 and 9.70 MJ/kg). However, the apparent digestibility of WB protein, without the addition of enzymes, showed in Table 4 was 10.91% lower than that described by the Brazilian Tables for Poultry and Swine (Rostagno *et al.*, 2011).

Although the effect of the MC × Phy interaction (Table 4) was not significant ($P > 0.05$), the current results suggested a trend for the additive effect of Phy on the DE ($P = 0.074$) and ME ($P = 0.073$) in weanling piglets at about 35 days of age. The DE and ME increased by 9.5% and 9.1%, respectively, when MC and Phy were combined. The same additive effect is observed in Table 5, when comparing the mean AID of all essential amino acids in WB withoutenzymes (77.60 ± 3.89%), in WB-MC (84.77 ± 5.39%), in WB-Phy (85.34 ± 4.90%) and in WB-MC+Phy (85.85 ± 3.89%), whose standard deviations gradually decreased. For amino acids that are considered dispensable, an increase in averages was also observed: 72.51 ± 4.98% in WB without enzymes; 80.75 ± 6.39% with WB-MC; 82.92 ± 5.65% with WB-Phy; and 84.56 ± 6.18% with WB-MC+Phy. These trends were shown (Table 5) in the AID of His ($P = 0.071$), Leu ($P = 0.068$), Lys ($P = 0.064$) and Thr ($P = 0.083$), and on SID of His ($P = 0.071$), Leu ($P = 0.098$), Lys ($P = 0.054$), Thr ($P = 0.072$) and Val ($P = 0.069$).

In its isolated form, there was a beneficial effect ($P < 0.01$) of MC on the apparent digestibility of DM, protein and energy of WB, but there was no significant effect ($P > 0.05$) on the AID and SID. Only one beneficial trend of the effect of MC was observed on the SID of His ($P = 0.066$), Lys ($P = 0.073$) and on the dispensable Ala ($P = 0.051$) and Tyr ($P = 0.083$), as shown in Table 6. In addition, Phy had an effect ($P < 0.001$) on the digestibility of the mineral fraction and energy ($P < 0.01$), with a tendency to improve protein digestibility ($P = 0.07$). The inclusion of Phy in the WB diet led to a 29% improvement in ash utilisation, while the inclusion of MC corresponded to a 20% improvement,

Table 4 *Apparent digestibility of nutrients and energy of WB, with or without enzymes, for postweaned piglets*[1]

	WB diets treatments					*P*-value[2]		
Item, %	WB Control	WB+MC	WB+Phy	WB+MC+Phy	SEM	MC	Phy	MC+Phy
DM	65.50	68.33	66.61	69.59	0.548	0.005	0.207	0.934
CP	71.23	74.97	72.84	81.27	1.277	0.009	0.069	0.265
N retention	51.76	55.24	50.87	55.89	1.294	0.012	0.072	0.772
Ash	38.72	48.52	54.77	54.67	1.948	0.092	0.001	0.066
DE, MJ/kg	10.32	11.35	11.19	11.60	0.109	<0.001	0.001	0.074
ME, MJ/kg	10.09	11.21	10.99	11.30	0.119	0.002	0.028	0.073
ME/GE%	62.53	68.16	66.84	68.74	0.722	0.002	0.028	0.073

WB = wheat bran; MC = multicarbohydrase; Phy = phytase; DE = digestible energy; ME = metabolisable energy; GE = gross energy.
[1]Data on as-fed basis.
[2]Effect of MC, Phy and MC+Phy, respectively.

Table 5 *AID of amino acids for WB, with or without enzymes, for postweaned piglets*

	Treatments					*P*-value[1]		
Item, %	WB	WB+MC	WB+Phy	WB+MC+Phy	SEM	MC	Phy	MC+Phy
Indispensable amino acids								
Arg	65.38	71.50	70.86	70.93	1.281	0.233	0.340	0.244
His	66.10	75.43	74.70	74.49	1.465	0.074	0.154	0.071
Iso	62.07	69.86	70.86	69.44	1.788	0.374	0.247	0.205
Leu	66.00	74.96	76.28	74.62	1.558	0.198	0.066	0.068
Lys	62.02	69.90	70.28	71.26	1.468	0.079	0.074	0.064
Met	66.39	77.25	76.75	76.58	1.532	0.073	0.009	0.219
Phe	74.20	80.69	80.81	80.68	1.299	0.213	0.197	0.197
Thr	48.88	46.66	49.44	50.89	1.718	0.118	0.077	0.083
Val	63.11	69.46	70.30	71.17	1.590	0.258	0.168	0.387
Indispensable amino acids								
Ala	46.50	56.11	59.56	61.56	1.947	0.080	0.008	0.238
Asp	55.74	59.95	62.99	62.94	1.795	0.574	0.177	0.564
Cys	69.61	77.66	78.91	77.16	1.710	0.346	0.194	0.151
Glut	56.28	65.44	68.45	70.05	2.109	0.170	0.040	0.328
Gly	51.83	57.39	59.09	63.13	1.889	0.200	0.088	0.835
Pro	63.79	75.49	76.29	75.15	1.708	0.184	0.122	0.117
Ser	65.38	71.50	70.86	70.93	1.281	0.233	0.340	0.244
Tyr	66.10	75.43	74.70	74.49	1.465	0.094	0.154	0.081

AID = apparent ileal digestibility; WB = wheat bran; MC = multicarbohydrase; Phy = phytase.
[1]Effect of MC, Phy and MC+Phy, respectively.

Table 6 *SID of amino acids for WB, with or without enzymes, for postweaned piglets*

	Treatments					*P*-value[1]		
Item, %	WB	WB+MC	WB+Phy	WB+MC+Phy	SEM	MC	Phy	MC+Phy
Indispensable amino acids								
Arg	76.81	83.09	82.58	83.13	1.299	0.191	0.262	0.269
His	80.00	89.64	88.85	89.30	1.494	0.066	0.116	0.071
Iso	77.32	85.79	86.55	85.83	1.819	0.182	0.102	0.205
Leu	79.20	88.43	89.67	88.68	1.581	0.149	0.056	0.098
Lys	70.63	78.83	79.13	80.58	1.496	0.073	0.067	0.054
Met	75.68	86.34	86.98	86.79	1.541	0.167	0.004	0.109
Phe	85.25	92.22	92.35	91.92	1.320	0.202	0.185	0.151
Thr	76.67	74.92	77.57	80.56	1.747	0.168	0.057	0.072
Val	76.80	83.67	84.38	85.89	1.624	0.113	0.061	0.069
Dispensable amino acids								
Ala	68.84	78.86	82.45	85.56	2.028	0.051	0.005	0.282
Asp	64.50	68.90	71.93	72.34	1.810	0.516	0.153	0.589
Cys[2]	–	–	–	–	–	–	–	–
Glut	77.49	85.60	86.83	85.53	1.717	0.310	0.173	0.167
Gly[2]	–	–	–	–	–	–	–	–
Pro	73.14	82.52	85.17	87.81	2.136	0.128	0.034	0.042
Ser	76.68	82.28	84.08	89.63	1.956	0.139	0.052	0.036
Tyr	74.40	86.31	87.01	86.46	1.728	0.083	0.097	0.102

SID = standardised ileal digestibility; WB = wheat bran; MC = multicarbohydrase; Phy = phytase.
[1]Effect of MC, Phy and MC+Phy, respectively.
[2]Not identified.

both in comparison to the WB diet without enzymes. The trend for the positive effect of Phy ($P = 0.07$) on protein was reflected in the AID and SID of Leu, Lys, Thr and Val, and an effective action on Met ($P = 0.004$), as well as on the non-essential amino acids Ala, Pro and Ser ($P < 0.05$). Compared to the values described by Rostagno *et al.* (2011) and the NRC (2012), the WB diet without enzymes presented the lowest AID and SID for most analysed amino acids.

Discussion

Wheat bran and physiological considerations

Most of the vegetable ingredients used in pig and poultry diets contain significant amounts of antinutritional factors. The non-starch polysaccharide constitutes a major resistant carbohydrate fraction in most foods (Englyst *et al*., 2007), limiting its nutritional value and its viability for inclusion in diets. Thus, exogenous enzymes are currently used to improve digestibility, allowing for a more viable use of these raw materials. As reviewed by Bedford and Cowieson (2012), the animal response to xylanase, β-glucanase and even Phy, as reported in the literature, tends to vary; the authors point out that many factors have a significant effect on the enzyme response, such as the enzyme source, ingredient variety, the environment under which the ingredient was grown, stored and processed in animal feed, the animal's age, interaction with other dietary ingredients and the animal's health status. Thus, in the present evaluation, differences in the nutrient and energy composition of WB, compared to those described in the literature, may be related to the intrinsic characteristics of the ingredients used in the different evaluations. A combination of characteristics of the WB nutrient profile and physiological characteristics of the piglets may have contributed to the higher energy use (DE and ME), compared to data from the literature for WB without enzymes. Compared to data in the literature, the lower digestibility coefficient of WB protein, without enzymes, may be related to the crude fibre (7.77%) of the ingredient (NRC, 2012). It may also be related to the gastrointestinal limitations of piglets in using the nitrogen fraction of WB, under normal conditions. When fibres are dissolved in the small intestine, they become viscous (gel) materials that affect the use of nutrients (Choct and Annison, 1992). A higher dietary fibre concentration results in increased substrate in the large intestine and the production of volatile fatty acids due to increased fermentation at this site (Yen *et al*., 1991), but this condition can be minimised by exogenous MC, Phy or by a combination of both enzymes.

Apparent digestibility of nutrients and energy

The results of this study, which point to an increase of DM, CP, nitrogen (N) retention, DE and ME with MC supplementation, could be associated with total or partial arabinoxylan cleavage within the crude fibre, as reported Woyengo *et al*. (2016). However, the combination of MC and Phy tended to increase ($P = 0.07$) the DE and ME of WB by 9.5% and 9.1%, respectively, relative to WB without enzymes. The present observations are in agreement with Gallardo *et al*. (2018), who reported that birds fed WB plus a Phy–MC combination showed higher energy retention compared to a control diet and diets with isolated enzymes. Thus, the probable additive effect from the combination of MC and Phy on non-starch polysaccharide degradation, especially arabinoxylans, could facilitate the access of endogenous enzymes, increasing the nutrient and energy use by the piglets. The current observations confirm the literature reports that the addition of carbohydrases to fibre-rich diets can improve nutrient utilisation in monogastric animals (Jacela *et al*., 2010). However, the current results disagree with those of Woyengo *et al*. (2016), who reported that the dietary addition of MC or Phy for growing pigs did not improve energy, the digestibility of nutrients or the nitrogen balance of wheat dried distillers' grain.

Our results also showed that Phy alone increased ($P < 0.001$) the apparent digestibility of the WB mineral fraction, and this increase in ash absorption suggests the clear and large hydrolysis of mineral-phytate complexes. The inclusion of Phy in the WB diet led to a 29% improvement in ash utilisation, while the inclusion of MC in the WB diet corresponded to a 20% improvement, both in comparison to the WB diet without enzymes. Related to the mineral fraction, Woyengo *et al*. (2016) observed that Phy supplementation not only increased P digestibility but also increased P retention, reducing the faecal P in growing pigs. In broilers, Ravindran *et al*. (2000) also reported beneficial effects of Phy in calcium and P retention when testing some feedstuffs.

Besides the effect of Phy ($P < 0.001$) on DE, CP and N, data suggest a similar trend of this enzyme on N retention. The current results also showed a mean improvement in energy utilisation, as DE and ME, of about 6.3% with Phy, 7.9% with MC and 9.3% with MC + Phy inclusions. As reported by Wu *et al*. (2015), Phy may be favourable to animal performance not only due to the release of phosphate, but also by increasing energy use. Therefore, the present effects of Phy suggest an increase in MC efficacy by the reduction of the phytate chelating effects on the improving energy utilisation (Slominski, 2011; Gallardo *et al*., 2018). In this study, these increases in DE and ME when piglets were provided the dietary inclusion of exogenous enzymes are consistent with reports by Nortey *et al*. (2007a and 2008), who found a greater energy utilisation of WB supplemented with xylanase for pigs. Thus, the compatibility of the supplementary enzyme with the substrate reflected in the ingredient digestibility, as reported by Bedford (1995) and Nortey *et al*. (2007b), suggesting higher nutrient and energy utilisation when adding carbohydrase to pig and poultry diets. Thus, the benefits of exogenous enzymes on the nutrient absorption in the intestinal lumen can be obtained by reducing the digesta viscosity from fibre in the gastrointestinal tract (Slominski, 2011; Amerah, 2015).

Amino acid digestibility

With respect to the digestibility of amino acids, the present results indicated that amino acids digestibility was improved in the piglets fed WB diets supplemented with exogenous enzymes, characterising the beneficial effects on N retention leading to the increased absorption of amino acids (Dadalt *et al*., 2017). The MC and Phy combination tended to increase amino acids digestibility, as the SID of His, Lys, Thr, Val, Pro and Ser. There was also a positive effect ($P < 0.01$) of Phy on the SID of methionine. In a previous study using broilers in the initial phase, Gallardo *et al*. (2018) also observed positive responses in the amino acids digestibility in broilers fed a

wheat-based diet with isolated or combined MC and Phy. Our study is in agreement with Diebold *et al.* (2004), Barrera *et al.* (2004) and Vahjen *et al.* (2007), who also observed positive responses of Phy supplementation on the digestibility of amino acids in pigs. Our results also agree with Cowieson and Ravindran (2007), who proposed that a great part of the effects of Phy on N retention and the reduction of endogenous amino acids losses were due to the cleavage of the phytate-protein site, and not due to the increased digestibility of dietary amino acids in broiler chickens. According to Cowieson *et al.* (2017), phytate binds with proteins under acidic conditions, forming binary phytate-protein complexes, making it impossible for pepsin to act in these complexes.

Observations from this study also confirm those of Eklund *et al.* (2014), who reported lower digestibility for most WB amino acids in growing pigs, compared to the NRC (2012). According to King *et al.* (2000), as the animal grows, the appearance and development of intestinal digestive hydrolases and the nutrient absorption capacity are observed when animals are able to assimilate ingested macromolecules. Studies on the development of digestive functions, including hydrolases and transport systems, suggested differences in hydrolase activity and transport rates in the small intestine with age (Biviano *et al.*, 1993). However, the current results showed that the isolated dietary supplementation of MC or Phy provided a better response against the antinutritional factors of WB, increasing the use of some amino acids. Other studies also suggested that the improvement on N retention by the action of carbohydrases was due to lower losses of endogenous and exogenous N and an increase of dietary protein hydrolysis (Adeola and Cowieson, 2011; Gallardo *et al.*, 2018). Thus, the literature reports that the increase in the digestibility of N and amino acids with the use of carbohydrases seems to occur indirectly, through the release of complexed proteins to the fibre, facilitating the action of digestive proteases. The positive effects of the use of carbohydrase on amino acids digestibility were also described by Adeola and Cowieson (2011) and Gallardo *et al.* (2018) in WB. However, the main action of carbohydrase seems to occur on fibre and fractions of non-starch polysaccharides and not necessarily on the amino acids. As reviewed by Simon (1998), carbohydrase enzymes that act on non-starch polysaccharides can have several modes of action, partially hydrolysing the antinutritional factors, decreasing the viscosity of the digesta and de-structuring the non-starch polysaccharides of the cell walls. The same effect seems to occur with Phy in the hydrolysis of phytic acid and the nutrient complexes. In the present experiment, a positive effect of exogenous enzymes on the SID of some amino acids was observed; however, Adeola and Cowieson (2011) reported that, although many studies prove the efficiency of the Phy enzyme in the use of phosphate by the phytate decomplexing, effects on other nutrients are not always observed. Some studies have demonstrated an increase in amino acids digestibility with the use of Phy in wheat-based diets (Barrera *et al.*, 2004; Diebold *et al.*, 2004; Vahjen *et al.*, 2007), and with wheat dried distillers' grain (Emiola *et al.*, 2009) for pigs. As reported by Cowieson *et al.* (2017) and Cowieson and Ravindran (2007), phytate can be decomplexed by the dietary inclusion of Phy, reducing endogenous N flow. In addition to its effects on digestibility, Gallardo *et al.* (2018) observed lower liver and pancreas weights in birds fed diets with MC, compared to control birds, and attributed this to positive effects of the MC complex in the nutrient absorption by fibre degradation, and a likely decrease in the secretory activity of these organs, with consequent decreased weights.

The apparent total tract digestibility of nutrients, DE and ME of WB, as well as the SID of amino acids in piglets at 35 days of age, from five replications, were lower than those reported in the literature, probably because most of the data from the tables originate from pigs at more advanced stages of growth. This suggestion is made by comparing the present results with data from the food tables for pigs. Thus, our hypothesis was confirmed: between 5 and 6 weeks of age, piglets have a lower capacity to use WB nutrients and energy, compared to pigs in the subsequent performance stages. With respect to WB, it is suggested that adjustments should be considered to the nutritional values for young piglet diets after weaning.

The MC complex was characterised as a viable alternative to increase the apparent digestibility of nutrients and energy and the SID of amino acids when WB was used in the diet of young pigs, and combined with Phy, suggested an additive effect on the apparent use of energy.

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Declaration of interest

Authors declare that there is no conflict of interest.

Ethics statement

All research methods and procedures were approved by the ethics and animal experimentation committee at the University of São Paulo (project 2843/2012), Brazil, and were followed according to animal welfare.

Software and data repository resources

This study was a doctoral thesis; the data were deposited in online archives of University of Sao Paulo: http://www.teses.usp.br/teses/disponiveis/10/10135/tde-17122015-141929/pt-br.php.

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