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Original article

Inclusion of wheat gluten as a protein source in diets for weaned pigs

María BLASCO, Manuel FONDEVILA*, José Antonio GUADA

Departamento de Producción Animal y Ciencia de los Alimentos, Universidad de Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain

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Abstract – The effect of substitution of fish meal (FM) by wheat gluten (WG) or hydrolysed wheat gluten (HG) in weaned pig diets was studied in two experimental periods. The tested protein sources were included at proportionally 0.10 and 0.06 in two diets formulated to meet pig requirements for weeks 0 to 2 and 3 to 5 after weaning, respectively. During each period, 5 lots of 5 weaned piglets (21 d old) per treatment were used to estimate growth, feed intake and nutrient digestibility. In addition, in the first period, one pig per replicate was slaughtered at the end of each phase in order to estimate ileal digestibility and pH, ammonia concentration and intestinal histology in different sections of the tract. There were no significant differences in daily growth, but the pigs consumed more of the FM diet than of the WG or HG diets on phase 3–5 (875, 807 and 827 $g \cdot d^{-1}$; $P < 0.10$). Total apparent dry matter, organic matter and crude protein digestibility were higher with wheat gluten diets ($P < 0.001$), but no differences were observed in ileal digestibility. However, ammonia concentration was higher in FM than WG in phase 0–2 ($P < 0.01$). Overall villous height tended ($P = 0.10$) to be higher with HG than FM. In conclusion, substitution of fish meal by wheat gluten in weaning pig diets does not affect growth up to 5 weeks after weaning. In addition, wheat gluten diets had higher apparent digestibility, maybe as a result of a reduced negative impact of weaning on enteric mucosa. There were no differences that might be attributed to the enhanced protein solubility of the hydrolysed wheat gluten.

wheat gluten / hydrolysed protein / intestinal histology / weaned pigs

Résumé – Inclusion de gluten de blé comme source de protéines dans les rations des porcelets sevrés. L'effet de la substitution de la farine de poisson (FM) par le gluten de blé naturel (WG) ou hydrolysé (HG) dans les rations de porcelets sevrés a été étudié lors de 2 périodes expérimentales. Les sources de protéines ont été incorporées dans une proportion de 0,10 et de 0,06 dans deux aliments formulés pour couvrir les besoins de croissance des animaux pendant deux phases, respectivement, des semaines 0 à 2 et des semaines 3 à 5 après le sevrage. Au total 5 groupes de 5 porcelets ont été utilisés pour chaque traitement afin de déterminer les quantités ingérées, la digestibilité des aliments et la croissance des animaux. A la fin de chaque phase, lors de la première période, un porcelet de chaque répétition a été abattu pour mesurer la digestibilité iléale, le pH, la concentration en ammoniaque et les caractéristiques histologiques de l'intestin. Aucune différence n'a été observée

* Corresponding author: mfonde@unizar.es

pour le gain moyen quotidien, même si la quantité de matière sèche ingérée avec la ration FM a été supérieure à celle des rations WG et HG (875, 807 and 827 $g \cdot d^{-1}$; $P < 0,10$) durant la seconde phase. Les digestibilités apparentes de la matière sèche, de la matière organique et des protéines ont été les plus élevées avec WG et HG ($P < 0,001$), tandis que les digestibilités iléales n'ont pas présenté de différences significatives. En revanche, la concentration en ammoniaque a été supérieure avec FM comparativement à WG ($P < 0,01$) durant les deux premières semaines après le sevrage. Dans l'ensemble, la hauteur des villosités a eu tendance ($P = 0,10$) à être plus élevée avec HG qu'avec FM. En conclusion, la substitution de la farine de poisson par le gluten de blé n'a pas eu d'effet sur la croissance des porcelets durant les cinq premières semaines après le sevrage. La substitution de FM par le gluten de blé a amélioré la digestibilité apparente de la matière organique, ceci peut être dû à la réduction de l'impact négatif du sevrage sur la muqueuse entérique.

gluten de blé / protéine hydrolysée / histologie intestinale / porcelets sevrés

1. INTRODUCTION

The restriction of using animal products in livestock feeding has led in Europe to a renewed interest in vegetable protein sources for substituting animal protein sources. This need becomes more apparent when the diets are focused on weaned pigs. Weaning stress, with its consequent drop in feed intake causes atrophy of the villous structure in the small intestine, which drives to a lower absorption capacity and a higher risk of digestive disorders. In this situation, feed quality is critical in order to achieve a good adaptation to solid feeding and consequently to ensure high productive performances [11].

Wheat gluten has a high crude protein content (about 800 $g \cdot kg^{-1}$), highly digestible, although as a cereal product its lysine and threonine content is slightly low (14.4 and 21.9 $g \cdot kg^{-1}$, respectively; [6]). However, its high glutamine proportion could have a positive effect against post-weaning villous atrophy since this amino acid is a preferred energy source for intestinal tissue [17], playing an important role in gut physiology and immunity [16, 22]. Inclusion of wheat gluten in weaned pig diets results in similar, or even higher, productive performances and digestibility than milk or soy derived products [4, 15, 19]. One of the outstanding features of wheat gluten among other protein sources is its viscoelasticity, which has been traditionally utilised by the baking industry [7] and is currently applied for the development of biodegradable bioplastics [8]. This feature may affect both the transit and absorption of digestive contents. The hydrolysis of wheat gluten with protease enzymes in order to increase its solubility would result in a faster absorption of amino acids and peptides [15, 21], and can avoid the restriction of utilisation of wheat protein isolates in animal feeding, and in fact it has been successfully applied in calf milk replacers.

The aim of this work was to study the effect of including wheat protein isolate, either hydrolysed or not, as a protein source in weaned pig diets on productive performances, diet digestibility and gut morphology, and to what extent wheat gluten may substitute for a widely used, high quality protein source such as LT fishmeal, chosen as a positive control to contrast this effect.

2. MATERIALS AND METHODS

2.1. Diets and animals

Two growth phases were considered from piglet weaning: the first 2 weeks and from the 3rd to 5th weeks. Three experimental diets for each time after weaning were formulated based on fishmeal (Danish Fishmeal LT-999; FM), wheat gluten (Amytex 100, Amylum Europe; WG) or hydrolysed wheat gluten (Solpro 300, Amylum Europe; HG), to include proportionally 0.33 (weeks 0–2) or 0.24 (weeks 3–5) of their total protein content as these sources. The ingredient

Table I. Ingredient ($g \cdot kg^{-1}$) composition of the pig diets for weeks 0–2 and 3–5 after weaning.

	weeks 0–2			weeks 3–5		
	FM	WG	HG	FM	WG	HG
Extruded cereal mix[1]	490	490	490	----	----	----
Maize grain	----	----	----	390	399	399
Barley grain	----	----	----	250	250	250
Whey powder	123.5	123.5	123.5	52.5	52.5	52.5
Soybean meal	110	107.5	107.5	135	130	130
Expanded soy full fat	130	120	120	65	65	65
Sunflower oil	9	9	9	20	10	10
Protein source tested[2]	100	95	95	60	57.5	57.5
L-Lysine 50	0.5	10	10	---	5	5
DL-methionine	0.5	0.5	0.5	1	---	---
L-threonine	---	0.5	0.5	---	---	---
Dicalcium phosphate	11.5	20	20	7.5	12	12
Calcium carbonate	16	15	15	10	10	10
Sodium chloride	4	4	4	4	4	4
Mineral-vitamin mix[3]	4	4	4	4	4	4
Chromic oxide	1	1	1	1	1	1

FM: fish meal; WG: wheat gluten; HG: hydrolysed wheat gluten.
[1] 0.85 maize grain and 0.15 barley grain.
[2] Fishmeal LT (diet FM), amytex 100 (diet WG) and solpro 300 (diet HG).
[3] To give per kg feed: 13000 IU vit. A; 2500 IU vit. D3; 13 mg vit. E; 1.5 mg vit. K; 5 mg riboflavin; 1 mg tiamin; 2.2 mg vit. B6; 0.02 mg vit. B12; 25 mg niacin; 10 mg calcium pantothenate; 200 mg choline chloride; 110 mg Zn; 50 mg Mn; 100 mg Fe; 165 mg Cu; 0.5 mg Co; 0.22 mg Se; 0.5 mg I.

composition of the diets is shown in Table I. The diets for weeks 0–2 and 3–5 were formulated to contain similar energy and crude protein levels, and when necessary were supplemented with synthetic amino acids. In addition, proportionally 0.01 of chromic oxide was included as digestibility marker. The diets were prepared in different batches for each period according to the same ingredient composition. The calculated composition of the diets and the analysed chemical composition for periods 1 and 2 are presented in Table II.

The experiment consisted of two periods (May–June 2001 and November–December 2001), each lasting 5 weeks. In the first period, 75 (York × Pietrain) × Dalland pigs (gilts and barrows) 19–23 day old weighing initially 6.5 ± 0.16 kg, were chosen. The animals were obtained from a commercial farm, and during lactation they received a commercial creep feed from day 14 to weaning. The piglets were weaned, allocated by weight in 5 blocks of increasing weight (n =15), and distributed within each block to the experimental diets in a randomised block design, resulting in 5 replicates of 5 pigs (three gilts and two barrows) per treatment. In the second period, weaning age and distribution were the same, but the animals were Duroc × Dalland and weighed 5.4 ± 0.14 kg. In both periods, the animals were placed in 1.45 × 1.20 m pens provided with a plastic slatted floor and an automatic drinking device in a temperature-controlled barn (23–25 °C).

Table II. Calculated and analysed chemical composition ($g \cdot kg^{-1}$) of pig diets for weeks 0–2 and 3–5 after weaning (period 1/period 2).

	weeks 0–2			weeks 3–5		
	FM	WG	HG	FM	WG	HG
DE[1]	3.40	3.41	3.41	3.47	3.43	3.43
Crude protein	217	220	220	183	186	186
Lysine	14.6	14.3	14.3	10.9	10.4	10.4
Methionine + cystine	8.5	8.4	8.4	6.9	6.4	6.4
Threonine	9.3	8.4	8.4	7.2	6.5	6.5
Tryptophan	2.6	2.3	2.3	2.1	2.0	2.0
Analysed composition:						
Organic matter	818 / 821	816 / 828	819 / 835	858 / 855	835 / 837	835 / 836
Crude protein	202 / 220	224 /222	227 / 230	185 / 190	184 / 194	185 / 196
Crude fibre	25 / 29	24 / 28	24 / 28	30 / 33	30 / 31	29 / 33
Ether extract	42 / 52	36 / 50	38 / 52	41 / 49	28 / 46	32 / 44

FM: fish meal; WG: wheat gluten; HG: hydrolysed wheat gluten.
[1] Mcal per kg dry matter.

2.2. Experimental procedures

Daily feed for each pen was offered ad libitum according to the previous day refusals and pig weight and feed refusals were weekly controlled per replicate. At the end of weeks 0–2 and 3–5 (14th and 35th day of each experimental period) samples of fresh faeces were taken at 9:00 and 16:00 from one representative animal from each pen for the determination of diet digestibility. The chosen piglets were isolated in individual pens for about 30 minutes and the faeces were taken from the floor immediately after excretion. Faecal samples were dried (60 °C, 48 h), pooled and stored until chromium determination. In addition, only in the first period, one pig from each pen (the same as for faecal collection) was slaughtered at the end of weeks 0–2 (14th and 15th day) and weeks 3–5 (35th and 36th), resulting in 30 slaughtered piglets, with 15 animals on each growing phase. The pigs were deprived from feed and water at 9:00 and slaughtered by exsanguination 3 h later after CO_2 stunning. Both animal management and slaughter conditions through this experiment were approved by the Comisión Etica Asesora para la Experimentación Animal (Universidad de Zaragoza). The entire gastrointestinal tract was immediately removed and separated into the stomach, first, second and third parts of the small intestine (at proportional distances from the gastric pylorus to the ileo-caecal valve; SI1, SI2 and SI3, respectively), caecum and mid-colon. The pH of the contents of each digestive site was immediately recorded (CRISON pH meter 507-3). Samples (5 mL) of the contents of the stomach and SI1 were diluted 1:1 with 0.2 N HCl and stored frozen at –20 °C for the determination of ammonia concentration. In addition, SI3 was longitudinally excised and content samples were collected and stored frozen for determination of ileal digestibility. One segment (approx. 10 cm) from each part of the small intestine was cut, rinsed in ice-cold 10% phosphate buffered formalin (pH 7.4) and stored in this solution for histological examination.

2.3. Analyses

The feeds were sampled daily and pooled weekly for the analysis of dry matter (DM),

organic matter (OM), crude protein (CP), crude fibre and ether extract according to the Association of Official Analytical Chemists methods [2]. The apparent digestibility of dry matter (DMD), organic matter (OMD) and crude protein (CPD) and ileal digestibility of DM (iDMD) and CP (iCPD) were determined using chromic oxide as a marker. Chromium concentration from feeds and daily pooled faecal samples were analysed by atomic absorption spectrometry, using a Perkin-Elmer 2100 equipment, after hydrolysis with nitric:perchloric acids (5:1), as in Vega and Poppi [20]. Ammonia concentration in digesta samples was determined colorimetrically following the procedure proposed by Chaney and Marbach [5]. For histological examination, samples of intestinal mucosa were stained with haematoxylin and eosin, and seven well-orientated villi and their adjacent crypts were measured for each intestinal portion under the microscope (50× magnification) using an eyepiece micrometer, in order to calculate mean villous height and crypt depth.

The results were analysed statistically by ANOVA, using the Statistix 7 software package [1]. Each experimental period was considered as a replicate in time and the initial weight of each replicate was nested within each experimental period as a block. The time after weaning (0–2 and 3–5 weeks) and diet were considered as main factors, and these and their interaction were compared against the residual. For pH, ammonia concentration and intestinal histology (only from the first period), the site of the gastrointestinal tract was also considered as the main factor. In cases of significant differences, treatment means were compared by the least significant difference procedure at $P < 0.05$.

3. RESULTS

There were no severe cases of diarrhoea (apparent liquid faeces) observed during the first period and when it appeared, the pigs recovered in one or two days. A higher incidence of diarrhoea (around 12%) was observed in the second period on days 6 to 9 after weaning, irrespective of the experimental treatment, and as a result 3 pigs died. However, the rest of the animals affected totally recovered in one or two days. In order to avoid any bias on treatment comparison, no antibiotic treatment was provided in any case.

The initial average weight of pigs for each experimental treatment was 6.4, 6.5 and 6.6 kg for treatments FM, WG and HG in the first period, and 5.4 kg for all treatments in the second period. Final average weight (after 5 weeks of the experiment) for FM, WG and HG diets was 19.9, 19.9 and 19.2 kg in the first period and 19.0, 18.5 and 17.9 kg in the second period. When average daily gain (ADG) and daily feed intake were studied throughout the whole experimental period (i.e., not considering the effect of time after weaning), no significant differences were observed among FM, WG and HG on growth (387, 380 and 387 $g \cdot d^{-1}$; s.e.m.= 9.72) or feed to gain ratio (2.01, 1.80 and 1.66 $kg \cdot kg^{-1}$; s.e.m.= 0.192), but intake was significantly higher ($P = 0.03$) with FM than WG (626, 586 and 596 g $DM \cdot d^{-1}$ respectively; s.e.m.= 11.2). When the effect of time after weaning on performance parameters was considered, no differences among the overall diet means were observed, except for a trend ($P = 0.09$) for a higher intake observed with the FM diet. The effect of the interaction time after weaning × diet on performance parameters was also not significant (Tab. III).

The total tract digestibility of dry matter (DMD), organic matter (OMD) and crude protein (CPD) of the diets after 2 or 5 weeks of the experiment are also presented in Table III. Overall values from diet FM were lower ($P < 0.05$) than WG and HG for DMD (in 0.036 and 0.024), OMD (in 0.033 and 0.025) and CPD (in 0.072 and 0.056), and there were no differences in the apparent digestibilities between WG and HG diets ($P > 0.05$). The lack of a significant effect for the interaction time after weaning × diet

Table III. Productive performances (average daily gain, $g \cdot d^{-1}$; dry matter intake, $g \cdot d^{-1}$; feed to gain ratio, F:G, $kg \cdot kg^{-1}$) and apparent dry matter, organic matter and crude protein digestibility (DMD, OMD and CPD) coefficients of pigs receiving the experimental diets from 0 to 2 weeks and from 3 to 5 weeks in two experimental periods (n = 10), together with ileal digestibility dry matter (iDMD) and crude protein (iCPD) coefficients in the first period (n = 5).

	weeks 0–2			weeks 3–5			
	FM	WG	HG	FM	WG	HG	s.e.m.
Daily gain	152	153	164	543	531	527	13.9
DM intake	253	254	250	875	807	827	16.9
F:G ratio	1.83	1.73	1.59	1.62	1.52	1.57	0.098
DMD	0.766c	0.805b	0.799b	0.809b	0.842a	0.825ab	0.0084
OMD	0.785c	0.824b	0.821b	0.826b	0.853a	0.840ab	0.0081
CPD	0.712c	0.798a	0.780ab	0.750bc	0.808a	0.795a	0.0110
iDMD	0.716	0.649	0.704	0.642	0.706	0.662	0.0357
iCPD	0.740	0.637	0.633	0.647	0.751	0.701	0.0333

FM: fish meal; WG: wheat gluten; HG: hydrolysed wheat gluten.
s.e.m.: standard error of means.
Within a row, different letters show significant differences ($P < 0.05$).

indicates that diet comparison was not different at 2 than at 5 weeks after weaning. Dry matter and crude protein ileal digestibilities (iDMD and iCPD) from the slaughter trial only corresponding to the first experimental period are also shown in Table III. There were no differences among diets on iDMD or iCPD, but the interaction time × diet in the latter ($P = 0.02$) indicates that the ileal crude protein digestibility coefficient for the FM diet was higher than for WG at 2 weeks after weaning, whereas the opposite occurred after 5 weeks.

The pH values of digestive contents recorded in the slaughter trial are shown in Table IV. Despite no significant differences being observed for the interactions site × diet or site × time × diet, the means for the triple interaction are presented to facilitate the interpretation of the results. Although there were significant differences between pH on the gastrointestinal sites, no significant differences among times after weaning or diets were detected. However, the interaction time × site ($P = 0.03$) showed a lower pH at the fundus as the pig matured (3.79 vs. 2.95 on weeks 0–2 and 3–5, respectively). The ammonia concentration in the stomach and SI1 contents is also presented in Table IV. Ammonia concentration was higher in the stomach than SI1 contents 5 weeks after weaning ($P = 0.04$). No significant differences among diets were observed on the overall ammonia concentration in weeks 3–5, but in weeks 0–2 it was lower with WG than with FM (47.5, 31.5 and 38.2 $mg \cdot L^{-1}$ with FM, WG and HW in weeks 0–2; $P = 0.01$). No differences were detected for interactions site × treatment or site × time × diet ($P > 0.10$).

The results from the histological study of the intestinal mucosa, either on average or at three different sites are summarised in Table V. No significant differences were detected for the interactions between intestinal site and the other main factors ($P > 0.10$). Villous height was lower at two weeks ($P < 0.001$), whereas no differences between times after weaning were observed in crypt depth, which consequently affected the height to depth ratio ($P < 0.05$). Among the diets, HG tended ($P = 0.10$) to promote higher villi than FM (overall villous height 364, 402 and 415 µm for FM, WG and HW; s.e.m. = 17.5), whereas crypt depth tended to be higher ($P = 0.07$) with FM than WG

Table IV. Mean pH values at different sites of the gastrointestinal tract, together with ammonia concentration ($mg \cdot L^{-1}$) in the stomach and SI1 contents of pigs receiving the experimental diets from 0 to 2 weeks and from 3 to 5 weeks. Standard error of means (s.e.m.) are referred to the means of the interaction time after weaning × diet × digestive site (n = 5).

	weeks 0–2			weeks 3–5			
	FM	WG	HG	FM	WG	HG	s.e.m.
pH:							0.313
stomach / cardiac	4.30	4.51	3.86	4.92	4.30	4.56	
stomach / fundus	4.00	4.00	3.38	3.98	2.43	2.45	
SI1	5.65	6.09	5.72	6.17	5.73	5.92	
SI2	6.14	6.25	6.32	6.27	6.42	6.41	
SI3	6.94	6.99	6.52	6.62	6.75	6.74	
caecum	5.84	5.97	6.15	5.80	5.73	5.66	
colon	6.12	6.12	6.21	6.06	5.61	5.80	
Ammonia:							5.39
stomach	49.8	29.2	44.4	52.8	58.6	57.8	
SI1	45.2	33.8	32.0	34.0	44.1	37.7	

FM: fish meal; WG: wheat gluten; HG: hydrolysed wheat gluten.
SI1, SI2 and SI3: the first, second and third part of the small intestine, respectively.

Table V. Average villous height (µm), crypt depth (µm) and height to depth ratio in SI1, SI2 and SI3 of pigs receiving the experimental diets from 0 to 2 weeks and from 3 to 5 weeks. Standard error of means (s.e.m.) are referred to the means of the interaction time after weaning × diet × digestive site (n = 5).

	weeks 0–2			weeks 3–5			
	FM	WG	HG	FM	WG	HG	s.e.m.
Villous height:							42.8
SI1	339	369	407	445	444	472	
SI2	349	342	342	407	488	465	
SI3	288	369	346	380	396	457	
Crypt depth:							19.5
SI1	308	253	284	268	230	292	
SI2	273	273	257	250	234	276	
SI3	215	242	230	207	219	273	
Height to depth:							0.229
SI1	1.13	1.53	1.46	1.63	2.02	1.80	
SI2	1.28	1.26	1.35	1.68	2.15	1.74	
SI3	1.37	1.56	1.56	1.85	1.83	1.78	

FM: fish meal; WG: wheat gluten; HG: hydrolysed wheat gluten.
SI1, SI2 and SI3: the first, second and third part of the small intestine, respectively.

(overall means of 252, 243 and 269 µm for FM, WG and HW; s.e.m. = 8.0). However, the magnitude of the error term (variation coefficient = 0.31) prevented to detect significant differences among diets on the height to depth ratio.

4. DISCUSSION

4.1. Wheat gluten vs. LT fish meal

Daily weight gain (Tab. III) remained unaffected by the experimental diets although DM intake was higher with FM, especially between the 3rd and the 5th weeks. However, this effect was not reflected in a significantly lower feed to gain ratio for wheat gluten diets possibly due to the common variability of this parameter (coefficient of variation 0.19) which prevented the observation of a significant dietary effect. The lack of differences in growth rate with wheat gluten diets compared with LT fish meal must be considered as a promising result, since the latter is considered to be a good protein source for weaned pigs. In this way, Leibholz [10] found that weight gain for a lysine supplemented wheat gluten diet tended to be higher than with meat meal. Similar growth rates either in the first 3 weeks after weaning or from the 4th to 5th weeks were also observed by Chae et al. [4] when wheat gluten was compared with dried skim milk, isolated soybean protein or spray-dried porcine plasma. Vente-Spreeuwenberg et al. [21] did not find differences on growth rate during the first two weeks after weaning among soybean based diets with 0.10 wheat gluten (hydrolysed or not) or potato protein.

Both wheat gluten diets showed higher total tract apparent DMD, OMD and CPD than fish meal along the experiment, but especially in weeks 0–2 (Tab. III). However, the results from ileal apparent DMD and CPD did not differ among the diets. The latter results must be considered with caution, because of the difficulties in obtaining a representative ileal sample in small pigs and the potential bias of contamination with enteric mucosa in slaughter trials, as shown by the high variation in ileal digestibility (coefficients of variation of 0.12 vs. 0.03 for iDMD and DMD and 0.11 vs. 0.05 for iCPD and CPD). Different responses in total tract and ileal digestibility coefficients may be due to variations in the hind gut fermentation. However, the lack of differences in hind gut pH as an index of microbial fermentation reduces the feasibility of this hypothesis.

Richert et al. [15] did not observe differences in total tract DM or N digestibility among wheat gluten diets and dried skim milk or soy protein isolate, nor Vachon et al. [19] between apparent N digestibility of wheat gluten and meat meal and lactalbumin. This agrees with the results observed by Butts et al. [3] when ileal N digestibility of wheat gluten was compared with fish meal, lactalbumin and soy protein isolate. Ammonia-N concentration in digestive contents might be considered as an index of microbial deamination and therefore reduced the amino acid availability for absorption in the small intestine. However, in order to have a full sense, this parameter would have to be related to the dry matter contents of these organs (data not measured). In any case, the animals were all deprived from feed and water three hours before being slaughtered, thus minimising the possible bias of water. Ammonia-N concentration on weeks 0–2 was significantly higher with FM than with WG, and it was 0.25 higher with FM than HG ($P > 0.05$).

Some amino acids, notably glutamine [16, 22] but also glutamate and aspartate [14] are involved in gut metabolism, as the major energy source for the mucosa and also involved in a variety of biosynthetic functions in the intestine. A beneficial influence of wheat gluten on digestion and absorption, alleviating the negative impact of weaning over the intestinal villous [9, 12] may be expected due to its high glutamine content. It has been shown that 10 $g \cdot kg^{-1}$ glutamine supply preserves villous height and crypt depth 7 days after weaning [22], and similar results were reported by Touchette et al. [18]. However, this effect is equivocal, since it occurred only in one site of the small intestine and not further along, and Vente-Spreeuwenberg et al. [21] failed to find a positive effect of adding 0.02 glutamine on villous architecture.

According to the manufacturer's analysis, glutamate plus glutamine and aspartate contents of wheat gluten sources used are 295 and 24 $g \cdot kg^{-1}$, respectively, which implies 30 and 18 g of these amino acids of wheat origin per kg feed in weeks 0–2 and 3–5. Their declared content in LT fishmeal is 94 and 66 $g \cdot kg^{-1}$, giving one half proportions per kg feed on each time after weaning (16 and 10 g per kg feed in diets for weeks 0–2 and 3–5, respectively). We observed that HG promoted on average 14% higher villi than FM ($P = 0.10$), and although non-significantly, villi with WG were 0.10 higher than with FM. Wu et al. [22] and Pluske et al. [13] recorded differences in villous height during the first 5 to 7 days after weaning, but these differences became smaller as the piglets matured. Therefore an elapse of more than 2 weeks together with the high individual variation of this parameter (coefficient of variation 0.24) might have reduced the possibility of detecting significant differences. According to the literature [13, 21], the level of intake rather than the diet composition is the main factor affecting histological changes after weaning. Since there were no diet differences on intake in weeks 0–2, it can be concluded that in our case, the response to wheat gluten on villous height, if any, must be caused by the positive effect of glutamine. The villous height to crypt depth ratio 2 weeks after weaning shows higher values for untreated and hydrolysed gluten diets than for fish meal, for 0.31 to 0.37 and for 0.13 in the first and third parts of the small intestine, respectively.

4.2. Enzymatic hydrolysis of wheat gluten

As in our case, the results in the bibliography do not show a positive response of protein hydrolysis of wheat gluten on growth of weaned piglets, nor on total tract digestibility. Richert et al. [15] contrasted wheat gluten with the same product modified enzymatically in order to increase the solubility of its protein, and found that unmodified wheat gluten tended to promote higher weight gains and lower feed to gain ratio during the first two weeks after weaning. In this sense, no differences were observed by Vente-Spreeuwenberg et al. [21] between unmodified and hydrolysed wheat gluten. Richert et al. [15] also failed to observe differences in apparent N digestibility between hydrolysed and untreated wheat gluten sources, and even N retention and weight gain tended to be higher with the latter. Probably an increased availability of amino acids after the enzymatic solubilisation treatment is balanced by the higher time of exposure to intestinal absorption of the WG because of the visco-elastic properties of vital wheat gluten [7].

Differences between gluten diets on intestinal histological parameters are minimal, which suggests that the availability of amino acids involved in a positive response is enough in the non-hydrolysed gluten. On phase 3–5, villous crypts with HG were deeper than with WG, in agreement with Vente-Spreeuwenberg et al. [21], although they only found this effect of protein hydrolysing at the proximal small intestine. Deeper crypts might indicate an increased rate of cell production [13] which might lead to a faster mucosa recovery.

In conclusion, the substitution of fish meal by wheat gluten in weaning pig diets does not affect pig growth up to 5 weeks after weaning, but reduces daily intake. In addition, wheat gluten diets had higher apparent digestibilities, maybe as a result of a reduced negative physiological impact of weaning on the enteric mucosa. There were no differences in productive performances, nutrient utilisation or intestinal villous height that might be attributed to the enhanced protein solubility of the hydrolysed wheat gluten assayed in this experiment.

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