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Addition of oat hulls to an extruded rice-based diet for weaner pigs ameliorates the incidence of diarrhoea and reduces indices of protein fermentation in the gastrointestinal tract

Jae Cheol Kim[1,2]*, Bruce P. Mullan[2], David J. Hampson[1] and John R. Pluske[1]

[1]School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, WA 6150, Australia
[2]Department of Agriculture and Food, Animal Research and Development, Department of Agriculture and food, Locked Bag No. 4, Bentley Delivery Centre, WA 6983, Australia

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An experiment was conducted to determine whether adding oat hulls to weaner pig diets based on extruded rice or unprocessed wheat influenced post-weaning diarrhoea (PWD) and protein fermentation in the large intestine. Ninety-six male piglets (5·16 (SEM 0·08) kg) were allocated to (i) extruded rice plus animal proteins (RAP); (ii) RAP with added oat hulls (20 g/kg); (iii) wheat plus animal proteins (WAP); (iv) WAP with added oat hulls (20 g/kg). Blood and faecal samples were collected on days 7 and 14 after weaning at about age 21 d. Pigs fed RAP had more PWD than pigs fed WAP ($P<0·05$). Oat hull supplementation to diet RAP decreased the incidence of PWD ($P<0·05$). The total-tract digestibility of DM, starch and energy was higher in rice-based diets than in wheat-based diets ($P<0·001$); however, oat hulls decreased digestibility of DM and gross energy ($P<0·001$). Pigs fed RAP had higher plasma creatinine concentrations ($P<0·01$), which were positively correlated to cumulative β-haemolytic *Escherichia coli* scores after weaning (R^2 0·928; $P=0·015$). Addition of oat hulls decreased plasma urea concentrations only in pigs fed RAP (interaction; $P<0·05$). Pigs fed RAP had lower faecal total biogenic amine concentrations than pigs fed WAP ($P<0·001$). Oat hull supplementation tended to decrease total biogenic amine concentrations ($P=0·103$). These data indirectly suggest that a mostly insoluble dietary fibre source such as oat hulls can decrease PWD in dietary situations where there may be a misbalance of carbohydrate to protein entering the hindgut.

Oat hulls: Post-weaning diarrhoea: Digestibility: Plasma urea: Plasma creatinine: Biogenic amines

Piglets often experience a growth check and may develop diarrhoea in the period after weaning. Post-weaning diarrhoea (PWD) is a multifactorial condition associated with proliferation of β-haemolytic strains of *Escherichia coli* in the small and large intestine[1]. Consequently, many studies have examined how components of a weaner diet may influence piglet health and production after weaning. A number of studies now show that cooked or raw white rice can be used in weaner diets as a replacement for other cereals such as maize and sorghum[2–5], and that these diets may reduce the incidence of PWD in pigs experimentally infected with β-haemolytic *E. coli*[6–8]. A recent study conducted in our laboratory, however, found that weaner pigs not experimentally infected with β-haemolytic *E. coli* and fed diets containing different types of cooked rice supplemented with animal proteins showed inconsistent shedding of β-haemolytic *E. coli* in their faeces, with pigs fed particular types of rice developing more diarrhoea than those fed other types or the wheat-based diet[9]. We reasoned that an imbalance between the amounts and/or types of carbohydrates and N entering the large intestine in pigs fed cooked rice-based diets might have caused this result that, in turn, could have influenced the occurrence of PWD. Differences in methods of cooking the rice[10] leading to different quantities of carbohydrate entering the large bowel, and/or differences in protein quality (for example, in meat and bone meal[11]), could also have influenced the outcomes.

The means by which the type and/or quantity of carbohydrate and N entering the large intestine can influence the development of PWD are uncertain, but they may differentially change the ratio of endogenous saccharolytic and proteolytic microbiota at this site. For example, a diet containing a limited amount of fermentable carbohydrates (NSP and resistant starch) and/or a high N content cause the proteolytic microbiota to predominate over saccharolytic bacteria[12,13]. The proliferation of proteolytic bacteria in the large intestine can produce metabolic by-products such as branched-chain volatile fatty acids, ammonia (NH_3), volatile phenols, indoles and biogenic amines that could cause digestive disturbances

Abbreviations: CP, crude protein; DE, digestible energy; OH, oat hulls; PWD, post-weaning diarrhoea; RAP, extruded medium-grain rice plus animal proteins; TTAD, total-tract apparent digestibility; WAP, wheat plus animal proteins.

* **Corresponding author:** Jae Cheol Kim, fax +61 8 9474 1295, email jkim@agric.wa.gov.au

after weaning[14,15]. Previous studies have demonstrated that adding sources of mostly insoluble and/or slowly fermentable NSP to diets for weaner pigs can ameliorate protein fermentation and reduce PWD[16–18].

In light of these observations, the present study tested whether oat hulls (OH), a source of mostly insoluble NSP, added to an extruded-rice based diet would reduce proteolysis in the large intestine, and help to prevent PWD. A more conventional diet based on unprocessed wheat, with and without added OH, was used as a control. The specific objectives of the study were (1) to determine whether the source of cereal (extruded rice *v.* wheat) and the addition of OH influence the excretion of β-haemolytic *E. coli* and incidence of PWD, and (2) to examine the comparative performance and total-tract digestibility of selected dietary components, indices of protein metabolism and faecal excretion of biogenic amines with the four diets.

Experimental methods

Experimental design

A 2 × 2 factorially designed experiment was conducted, with the respective factors being cereal source (extruded medium-grain rice *v.* wheat) and OH addition (with or without 20 g OH/kg), to test the effects of cereal source and insoluble NSP supplementation on faecal moisture content, the incidence of PWD, faecal scores of β-haemolytic *E. coli*, blood urea and creatinine, the faecal excretion of biogenic amines, the total-tract apparent digestibility (TTAD) of dietary components and performance after weaning. The study was approved by the Murdoch University Animal Ethics Committee.

Animals, diets, feeding and sample collection

Ninety-six male pigs (Large White × Landrace) weaned at an average age of 21 d were obtained from a commercial supplier (Wandalup Farms, Mandurah, WA, Australia). The pigs were transported to an isolated animal house at Murdoch University where they were housed in metal wire-meshed pens with a floor space of 2·5 m^2. The experiment was conducted in two replicates with forty-eight pigs (i.e. three pens of four pigs per dietary treatment) constituting a replicate. The average live weights of pigs at arrival on the day of weaning were 5·5 (SEM 0·05) kg for replicate 1 and 4·9 (SEM 0·08) kg for replicate 2. Pigs were randomly accommodated to a pen (four pigs per pen and six pens per treatment; twenty-four pigs per treatment combination) based on their live weight at arrival. The ambient temperature was maintained at 29 ± 1°C. Water and feed were freely accessible during the whole experiment through a nipple drinker and feed trough, respectively, set in each pen.

The pigs were offered their respective experimental diet *ad libitum* for 3 weeks. The diets used were (i) extruded medium-grain rice (variety *Amaroo*) plus animal proteins (RAP); (ii) RAP with added OH (20 g/kg; RAPOH); (iii) wheat plus animal proteins (WAP); (iv) WAP with added OH (20 g/kg; WAPOH). All diets were formulated to contain similar digestible energy (DE) and approximately 12·8 g standardised ileal digestible lysine/kg. Tabulated nutrient compositions were used for diet formulation[19]. The OH (Glen Forrest Stock Feeds, Glen Forrest, WA, Australia) were passed twice through a hammer-mill fitted with a 4·5 mm screen before incorporation into diets. Extrusion of rice was performed using a single-screw Wenger extruder at the Pig and Poultry Research Institute (University of Adelaide, Roseworthy, SA, Australia). Rice was first ground through a 1 mm screen in a hammer-mill to a particle size of approximately 250 μm, and was pre-conditioned in steam to increase the moisture content of grain to approximately 160 g/kg before extruding through a 7 mm die. Rapeseed oil was sprayed in the process of extrusion at the rate of 21 g/kg rice to improve the extrusion process. The extruded rice was passed through a hammer-mill without the screen to achieve a more uniform particle size. The wheat was passed through the same hammer-mill but with a 4·5 mm screen to reduce particle size. Titanium dioxide (TiO_2) was added as an inert marker for estimation of the coefficient of the TTAD. No antimicrobials were included in the diets. The composition and analysed chemical composition of experimental diets are presented in Table 1.

Pigs were weighed every week and feed intake was recorded on a weekly basis as feed disappearance from the feeder. Pigs were monitored for the presence of diarrhoea daily. Faeces were scored daily depending on their consistency using the following criteria: 1 = well-formed faeces, firm to cut; 2 = formed faeces, soft to cut; 3 = faeces falling out of shape upon contact with surfaces and sloppy; 4 = pasty and liquid diarrhoea. Piglets were counted as having diarrhoea when the faecal consistency score was 4. For ethical reasons, pigs with diarrhoea were treated immediately with an intramuscular injection of Trisprim-480 (trimethropin 80 mg/ml, sulfadiazine, 400 mg/ml; Troy Laboratories, Smithfield, NSW, Australia), and this was repeated daily until the diarrhoea ceased. Faecal swabs for culture were taken from the rectum from all pigs on days 0, 2, 5 and 6 after weaning. Blood samples (10 ml) from two randomly selected pigs per pen were taken from the anterior vena cava into heparinised EDTA-vacutainer tubes on days 7 and 14. The samples were placed on ice and analysed for plasma urea and creatinine contents on the same day. Faecal 'grab' samples (all faeces voided without contamination) were collected from each pen at 08.00, 10.00, 12.00, 14.00 and 16.00 hours on days 7 and 14 of the experiment. The samples were then kept at −20°C and later thawed, mixed, freeze-dried and ground through a laboratory hammer-mill (1 mm screen, Cyclotec 1093; Tecator AB, Höganäx, Sweden) before chemical analysis.

Chemical and microbial analyses

All analyses were made in duplicate. DM content of the diets and the faeces collected on days 7 and 14 were measured using Association of Official Analytical Chemists official method 930.15[20]. Faecal moisture content was then calculated from the DM content in faeces. Total starch was determined using a Megazyme total starch kit (Megazyme International Ireland Ltd, Bray, Co. Wicklow, Republic of Ireland). The N content was determined with a LECO FP-428 Nitrogen Analyser using a combustion method (Association of Official Analytical Chemists official method 990.03[20]). The gross energy content was determined using a ballistic bomb calorimeter (SANYO Gallenkamp, Loughborough, Leics, UK). NSP and their constituent sugar contents were determined as alditol acetates by GLC using

Table 1. Diet composition (g/kg, as-fed basis) and nutrient content (g/kg dry matter)

Diet...	RAP	RAPOH	WAP	WAPOH
Ingredients				
Rice	704·8	684·9	–	–
Wheat	–	–	782·6	762·6
Meat and bone meal	51·6	51·6	50·0	50·0
Whey powder	100·0	100·0	50·0	50·0
Blood meal	30·0	30·0	25·0	25·0
Fish meal	100·4	100·4	50·0	50·0
Oat hulls	–	20·0	–	20·0
Rapeseed oil	5·0	5·0	28·3	28·3
L-lysine-HCl	2·8	2·8	6·0	6·0
DL-Methionine	0·4	0·4	1·2	1·2
L-Threonine	1·5	1·5	2·7	2·7
Tryptophan	0·3	0·3	0·4	0·4
Choline chloride	0·4	0·4	0·4	0·4
Dicalcium phosphate	–	–	0·7	0·7
Salt	1·0	1·0	1·0	1·0
Vitamin and mineral mix*	0·7	0·7	0·7	0·7
Titanium dioxide	1·0	1·0	1·0	1·0
Calculated composition (g/kg)				
DE (MJ/kg)	14·8	14·5	14·9	14·6
Crude protein	204	203	196	194
Neutral-detergent fibre	39·7	52·7	102·3	113·8
Acid-detergent fibre	11·6	18·9	26·7	33·7
SID lysine	12·88	12·92	12·76	12·79
SID methionine	4·19	4·20	3·91	3·92
SID threonine	8·55	8·60	8·54	8·57
SID tryptophan	2·11	2·13	2·14	2·15
SID isoleucine	6·52	6·57	5·78	5·79
SID leucine	14·59	14·67	12·86	12·88
SID valine	9·83	9·99	8·41	8·45
Analysed nutrient content (g/kg DM)				
Crude protein	195	188	204	190
Starch	569	568	543	563
GE (MJ/kg DM)	19·03	19·03	19·32	19·35
Total NSP	11·94	23·02	76·99	94·07
Insoluble NSP	8·95	20·19	65·74	83·36
Soluble NSP	3·00	2·82	11·24	10·71
Free sugars	31·66	31·20	32·37	28·34
Biogenic amines (mg/kg DM)				
β-Phenylethylamine	15	19	22	22
Putrescine	24	24	17	17
Cadaverine	41	43	26	24
Histamine	4	4	3	3
Water-holding capacity (g/g dried diet)	3·67	3·71	1·69	1·65

RAP, rice animal protein; RAPOH, rice animal protein and oat hulls; WAP, wheat animal protein; WAPOH, wheat animal protein and oat hulls; DE, digestible energy; SID, standardised ileal digestible; GE, gross energy.

* Provided the following nutrients (per kg air-dry diet): vitamin A, 1470 μg (4900 IU); vitamin D_3, 24·5 μg (980 IU); vitamin E, 14 mg; vitamin K, 0·7 mg; vitamin B_1, 0·7 mg; vitamin B_2, 2·1 mg; vitamin B_6, 1·05 mg; vitamin B_{12}, 10·5 μg; calcium pantothenate, 7·5 mg; folic acid, 0·13 mg; niacin, 8·4 mg; biotin, 21 μg; Co, 0·14 mg (as cobalt sulfate); Cu, 7 mg (as copper sulfate); I, 0·35 mg (as potassium iodide); Fe, 42 mg (as ferrous sulfate); Mn, 28 mg (as manganese oxide); Se, 0·21 mg (as sodium selenite); Zn, 70 mg (as zinc oxide); antioxidant as Endox (Kemin Industries, Inc.), 14 mg (BJ Grower 1, BioJohn Pty Ltd, WA, Australia).

the method of Theander & Westerlund[21]. The water-holding capacity of diets was determined using the method of Kyriazakis & Emmans[22]. Titanium dioxide (TiO_2) was determined as described by Short *et al.*[23].

The *E. coli* score for each pig was determined by inoculating a 5 % sheep blood agar plate with a faecal swab, streaking the inoculum out and incubating overnight at 37°C in air[9]. The presence of β-haemolytic *E. coli* was then scored on a six-point scale from no growth (0) to heavy growth extending to the last streak on the plate (5). Plasma urea content was determined using a commercial enzymic (urease) kinetic method (Randox, Crumlin, Co. Antrim, UK). The plasma creatinine assay was performed on an automated analyser (Daytone RX; Randox) using alkaline picrate without deproteinisation. The concentration of biogenic amines in the feed and faeces of pigs was determined using the method of Moret *et al.*[24]. Briefly, biogenic amines were extracted with 0·1 M-HCl in an Ultra Turrax homogeniser (IKA® Works Inc., IL, USA). Biogenic amines were then derivatised with dansyl chloride and determined by HPLC using a Sphereclone ODS(2) column with a UV/VIS detector at 254 nm (Phenomenex, Torrance, CA, USA).

Statistics

There was a significant replicate effect in average starting weights between the two replicates (mean live weight of 5·5 (SEM 0·05) kg for replicate 1 and 4·9 (SEM 0·08) kg for replicate 2; $P<0·001$). Accordingly, the interaction between replicate and dietary treatment was examined for all dependent variables analysed. The interaction was significant for diarrhoea and therefore they were separately analysed and presented. For all other variables with non-significant replicate and treatment interactions, data were pooled and analysed for treatment effects. The pen was considered as an experimental unit for all statistical analyses except faecal consistency score, incidence of PWD, *E. coli* score and blood metabolites in which the pig was considered as the experimental unit. For pig performance, the treatment effects were assessed by ANOVA for a factorial arrangement with the main effects being cereal source and OH supplementation and interactions between the variables. For TTAD, blood metabolites, faecal excretion of biogenic amines and faecal moisture content, the treatment effects were assessed by repeated-measures ANOVA. Data for faecal consistency were expressed as the mean faecal consistency value of pigs within a diet having score 1 (a value of 0 %), score 2 (a value of 33 %), score 3 (a value of 66 %) or score 4 (a value of 100 %)[9]. Data for incidence of PWD were expressed as the mean proportion of days with diarrhoea with respect to 14 d after weaning[5]. All statistical analyses were conducted using the statistical package StatView 5.0 for Windows (SAS Institute Inc., Cary, NC, USA), and statistical significance was accepted at $P<0·05$.

Results

Experimental diet specifications

Based on standardised ileal digestible amino acid contents of ingredients[19], the nutrient composition of diets was estimated. The results showed that the experimental diets contained ideal amino acid patterns except for isoleucine. Ratios for isoleucine:lysine were lower (51 and 45 % for rice-based and wheat-based diets, respectively) than the proposed ideal pattern of essential amino acids[25] (60 %) for weaner pigs.

Incidence of post-weaning diarrhoea and Escherichia coli *score*

No PWD occurred in the pigs from replicate 1. The incidence of PWD in pigs in replicate 2, expressed as the mean

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proportion of days with diarrhoea with respect to 14 d after weaning, is shown in Table 2. PWD was only observed after day 5. Overall, only eight out of forty-eight (16·7 %) piglets in this replicate developed PWD, and more of those fed a rice-based diet had diarrhoea than piglets fed a wheat-based diet ($P=0·017$). There was a tendency for an interaction between cereal source and OH (interaction; $P=0·069$). Fisher's protected LSD showed that OH significantly decreased PWD in pigs fed a rice-based diet ($P=0·033$), whilst no effect was detected in pigs fed a wheat-based diet. The β-haemolytic *E. coli* score for both replicates during the first week after weaning is shown in Table 3, and was not different ($P>0·05$) between treatments. However, the *E. coli* score on day 5 was significantly higher in replicate 2 compared with replicate 1 (0·31 *v.* 0·04; $P<0·01$; data not shown).

Faecal consistency

Faecal consistency on days 2, 5 and 6 after weaning and faecal moisture content on days 7 and 14 are presented in Table 3. Pigs fed the rice-based diets had significantly firmer faeces than pigs fed the wheat-based diets on day 2 (cereal main effect; $P<0·01$), but the difference was not significant on days 5 and 6. Faecal consistency on day 2, however, was affected by a cereal × OH interaction ($P<0·05$), such that in the absence of OH, piglets fed rice-based diets had significantly lower faecal consistency than piglets fed wheat-based diets (7 *v.* 21 %), whilst faecal consistency was not different between cereal sources in the presence of OH (33 *v.* 23 %). Measurement of faecal moisture content at 1 and 2 weeks after weaning showed a main effect only for cereal, with a higher moisture content in pigs fed wheat-based diets compared with pigs fed rice-based diets ($P<0·05$). Significant replicate effects were found for faecal consistency on days 5 and 6, such that piglets in replicate 1 had firmer faeces compared with piglets in replicate 2 (17 *v.* 30 %; $P<0·05$; data not shown).

Blood metabolites

Plasma urea content tended to be higher in pigs fed the rice-based diets than in pigs fed the wheat-based diets ($P=0·064$). There was no significant main effect ($P=0·160$) of OH in lowering plasma urea levels; however, a significant cereal source × OH interaction was observed, such that supplementation of OH significantly reduced plasma urea concentration only in the pigs fed RAP ($P=0·015$). Plasma creatinine content was significantly higher in pigs fed the rice-based diets than in pigs fed the wheat-based diets ($P<0·01$), and there was no significant effect of OH supplementation (Table 4). Plasma creatinine concentration was positively correlated to cumulative β-haemolytic *E. coli* scores (Fig. 1; $P=0·015$).

Faecal excretion of biogenic amines

There were significantly greater faecal contents of putrescine, cadaverine and histamine ($P<0·001$) in pigs fed the wheat-based diets compared with pigs fed the rice-based diets. Also, the content of β-phenylethylamine tended to be higher in pigs fed the wheat-based diets ($P=0·066$). Adding OH tended to decrease the contents of β-phenylethylamine ($P=0·066$) and cadaverine ($P=0·104$) but not the contents of putrescine and histamine. There was a tendency for an interaction between cereal source and OH supplementation for the content of faecal β-phenylethylamine ($P=0·077$), such that OH decreased β-phenylethylamine content in pigs fed the wheat-based diets but did not alter β-phenylethylamine content in pigs fed the rice-based diets. Overall, total amine excretion was significantly higher in pigs fed wheat-based diets ($P<0·001$), and OH supplementation tended to decrease total amine excretion in the large intestine ($P=0·103$) (Table 4).

Total-tract apparent digestibility of dietary components

Cereal source significantly influenced the TTAD of DM, starch, crude protein (CP), gross energy and the DE content of the total diet ($P<0·001$), with rice-based diets being more digestible than wheat-based diets, except for CP. Supplementation of 20 g OH/kg reduced the TTAD of DM, CP and gross energy ($P<0·001$). However, the TTAD of starch was not affected by OH supplementation. The interaction between cereal source and OH supplementation for all measurements was not significant (Table 5).

Performance of pigs

Average daily gain ($P<0·05$) and feed conversion ratio (FCR; $P<0·01$) were significantly influenced by cereal source, while intake was not affected. Pigs fed wheat-based diets grew faster than pigs fed rice-based diets ($P<0·05$). Supplementation of 20 g OH/kg did not affect ($P>0·05$) performance. There

Table 2. Effect of cereal source and oat hull (OH) supplementation on incidence of post-weaning diarrhoea (PWD) in replicate 2
(Mean values and pooled standard errors)

Cereal source...	Extruded rice		Wheat			*P**		
OH (g/kg)...	0	20	0	20	SEM	Cereal source	OH	Cereal source × OH
Pigs with diarrhoea (*n*)	5/12	2/12	0/12	1/12				
Incidence of PWD†	12·5[a]	3·6[b]	0·0[b]	1·8[b]	2·03	0·017	0·221	0·069

a,b Mean values within a row with unlike superscript letters are significantly different ($P<0·05$).
* Two-way ANOVA.
† Incidence of PWD observed during 14 d after weaning (*n* 12) is expressed as the mean proportion of days with diarrhoea. Pigs in replicate 1 had no diarrhoea.

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Table 3. Post-weaning β-haemolytic *Escherichia coli* scores and faecal consistency of weaned pigs
(Mean values and pooled standard errors)

Cereal source...	Extruded rice		Wheat			*P**		
OH (g/kg)...	0	20	0	20	SEM	Cereal source	OH	Cereal source × OH
E. coli score†								
Day 0	0·29	0·29	0·54	0·38	0·157	0·292	0·597	0·597
Day 2	0·08	0·21	0·13	0·17	0·095	1·000	0·383	0·662
Day 5	0·29	0·13	0·17	0·13	0·095	0·513	0·277	0·513
Day 6	0·08	0·08	0·21	0·08	0·065	0·338	0·338	0·338
Cumulative score	0·75	0·71	1·04	0·75	0·351	0·503	0·503	0·615
Faecal consistency (%)‡								
Day 2	7	21	33	23	5·5	0·011	0·710	0·038
Day 5	19	21	33	22	5·5	0·175	0·387	0·264
Day 6	22	29	34	22	5·9	0·645	0·640	0·104
Faecal moisture content (g/100 g)§	64	66	68	71	0·8	0·021	0·277	0·781

OH, oat hulls.
* Two-way ANOVA.
† *E. coli* score (*n* 24) in faeces is expressed as a mean score per diet, across both replicates. Higher values are associated with faeces containing the greatest number of viable β-haemolytic *E. coli*.
‡ Faecal consistency was analysed using two-way ANOVA and is expressed as the mean faecal consistency value of pigs within a diet having normal faeces (a value of 0 %), moist faeces (a value of 33 %), wet faeces (a value of 66 %) or diarrhoea (a value of 100 %).
§ Faecal moisture content was measured at days 7 and 14 and analysed using repeated-measure ANOVA.

were significant replicate effects for gain and intake ($P<0·001$) but not FCR because of the unavoidable difference in average starting weights between the two replicates (mean live weight of 5·5 (SEM 0·05) kg for replicate 1 and 4·9 (SEM 0·08) kg for replicate 2) (Table 6).

Discussion

Post-weaning diarrhoea and β-haemolytic Escherichia coli *shedding*

The incidence of natural PWD in the present study was relatively low, with no animals affected in the first replicate and only 17 % of pigs affected in the second replicate. Nevertheless, pigs fed diet RAP showed a higher incidence of PWD than pigs offered diet WAP in this replicate. These data contrast with previous reports from our laboratory, where feeding cooked white rice usually has been associated with reductions in PWD[6–8], although recently we reported that mild PWD occurred in pigs fed diets based on different rice types[9]. Interestingly, and despite more pigs fed diet RAP developing PWD, faeces were drier (in spite of the diet having a higher water-holding capacity; Table 3) and faecal shedding of β-haemolytic *E. coli* was broadly similar between dietary treatments. These results are consistent with those of Callesen *et al.* [26], who found no correlation between faecal shedding of β-haemolytic *E. coli* and the number of required antibiotic treatments for PWD in the first 14 d after weaning in pigs fed either rice- or wheat-based diets. These data suggest that the diarrhoea observed in the present study was not necessarily just of bacterial origin, but may have had a dietary or physiological component in its aetiology.

Previous experiments conducted in our laboratory have used rice cooked in an autoclave, with the rice cooled at 4°C overnight before feeding. Cooking and cooling rice can significantly increase the resistant starch (RS) content by

Table 4. Effects of cereal source and oat hull (OH) supplementation on blood metabolites and faecal excretion of biogenic amines in weanling pigs
(Mean values and pooled standard errors)

Cereal source...	Rice		Wheat			*P**		
OH (g/kg)...	0	20	0	20	SEM	Cereal source	OH	Cereal source × OH
Blood metabolites†								
Urea (mmol/l)	3·5	2·6	2·5	2·7	0·10	0·064	0·160	0·015
Creatinine (μmol/l)	74·1	76·4	69·2	68·4	1·00	0·005	0·729	0·488
Biogenic amines (mg/kg freeze-dried faeces)‡								
β-Phenylethylamine	28	28	41	28	1·9	0·066	0·066	0·077
Putrescine	43	49	177	153	11·2	0·001	0·443	0·225
Cadaverine	353	211	868	730	59·5	0·001	0·104	0·978
Histamine	7	6	64	63	4·7	0·001	0·848	0·926
Total amine	431	292	1150	974	72·4	0·001	0·103	0·843

* Repeated-measures ANOVA.
† Values are least-square means from twelve observations (randomly picked two pigs from each pen).
‡ Values are least-square means from twenty-four observations (biogenic amines were repeatedly measured at week 1 and 2 from six pens). Biogenic amines were determined from freeze-dried faecal samples, which were collected from the pens.

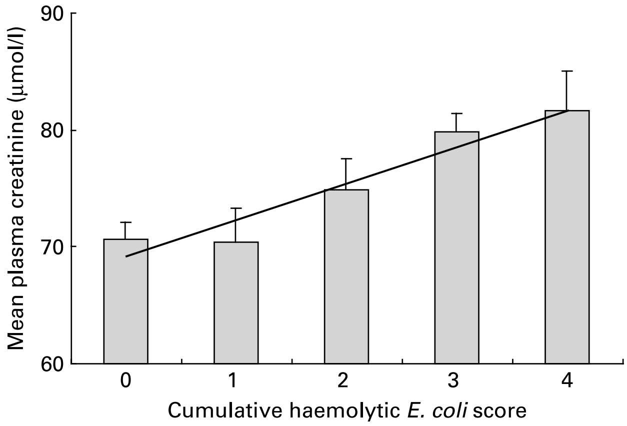

Fig. 1. Relationship between plasma creatinine concentration (mean of two observations on days 7 and 14) and cumulative β-haemolytic *Escherichia coli* score (sum of four observations on days 0, 2, 5 and 6) of pigs during 14 d post-weaning ($P=0.015$). Values are means, with their standard errors indicated by vertical bars. $y = 3.122x + 66.102$; R^2 0.9276.

promoting retrogradation of starch polymers[27]. Indeed, a recent study showed that cooking and then cooling rice increased the RS content twelve-fold (1 *v.* 12 g/kg DM) in the same rice cultivar (*Amaroo*) used in the present study[28]. On the other hand, extrusion of medium-grain rice decreased the RS content[28]. In the present study the rice was extruded, and this physical difference in processing may have contributed to the development of PWD in some pigs. The protection from PWD that is usually seen with cooked rice may be associated with the RS formed during the (autoclave) cooking and cooling of medium-grain rice, which somehow ameliorates proliferation of β-haemolytic *E. coli* and (or) manipulates the hindgut microbiota to indirectly reduce coliform proliferation and shedding in the faeces[13]. The protective effects of the RS, or of other physico-chemical properties of the moist cooked rice, may have been masked when the rice was extruded.

In any case, when the low-fibre, extruded rice-based diet was supplemented with OH at 20 g/kg, the gastrointestinal tract environment was seemingly altered because less PWD occurred. Similarly, it was reported that addition of 20 g OH/kg in a rice-based diet reduced the incidence of PWD between the ages of 21 and 41 d[5]. The basis of this protective effect is still uncertain, but it may be related to changes in the numbers and metabolic activity of selected components of the intestinal microbiota in these young pigs.

Biogenic amines

The biogenic amines putrescine, cadaverine, histamine and β-phenylethylamine are produced in the large intestine from arginine, lysine, histidine and phenylalanine, respectively, by a number of bacterial groups including *Bacteroides*, *Clostridium*, *Enterobacterium*, *Lactobacillus* and *Streptococcus* [14]. Biogenic amines are implicated in the aetiology of PWD, particularly in pigs fed diets containing low dietary fibre and high CP levels[17,18], because they irritate the colonic mucosa and generate high osmotic pressures, thereby producing proteolytic and osmotic diarrhoea[29]. Faecal levels of biogenic amines were lower in diet RAP than in WAP, suggesting that RAP caused lower production of amines, and that the higher incidence of PWD seen in piglets fed diet RAP was unrelated to amine production. Hence, it appears that the type and concentration of amines produced in the large intestine depend on the type of cereal that pigs receive and the amino acid and protein flow into the hindgut.

Due to a lower starch digestibility and higher NSP content in the wheat-based diet, the content and proportion of carbohydrates entering the large intestine is most probably higher than in pigs fed the rice-based diets. For this reason we anticipated lower faecal amine levels in pigs fed the wheat-based diets than in pigs fed the rice-based diets. However, our finding that pigs fed wheat-based diets excreted more amines in their faeces suggests that more fermentable protein was present in the large intestine of pigs fed a wheat-based diet, which probably originated from the lower ileal digestibility and higher endogenous secretion of amino acids owing to the higher NSP content[30].

Our finding that OH tended to decrease faecal protein digestibility and amine excretion is in general agreement with reports by Bolduan *et al.* [17] and Aumaitre *et al.* [18], and implies that OH may have modified the activity of proteolytic microbes in the large intestine. The fact that OH tended to decrease only some amines (decreased β-phenylethylamine and cadaverine but not putrescine and histamine) suggests a suppression of the microbiota specifically metabolising phenylalanine and lysine, but not the microbiota metabolising arginine and histidine. The contents of these four amino acids are generally similar in the endogenous amino acids

Table 5. Effects of cereal source and oat hull (OH) supplementation on total-tract apparent digestibility (TTAD) measured at 1 and 2 weeks after weaning*

(Mean values and pooled standard errors)

Cereal source...	Rice		Wheat			*P*†		
OH (g/kg)...	0	20	0	20	SEM	Cereal source	OH	Cereal source × OH
$TTAD_{DM}$	0.902	0.872	0.856	0.836	0.004	0.001	0.001	0.133
$TTAD_{starch}$	0.999	0.999	0.994	0.995	0.004	0.001	0.486	0.729
$TTAD_{CP}$	0.745	0.712	0.775	0.760	0.006	0.001	0.019	0.371
$TTAD_{GE}$	0.893	0.867	0.843	0.829	0.004	0.001	0.001	0.192
DE (MJ/kg DM)	16.99	16.51	16.28	16.04	0.066	0.001	0.001	0.151

CP, crude protein; GE, gross energy; DE, digestible energy.

* Values are least-square means from twenty-four observations (TTAD was repeatedly measured at week 1 and 2 from twelve pens).

† Repeated-measures ANOVA.

Table 6. Effect of cereal source and oat hull (OH) supplementation on performance indices of weanling pigs
(Mean values and pooled standard errors)

Cereal source...	Extruded rice		Wheat			*P**		
OH (g/kg)...	0	20	0	20	SEM	Cereal source	OH	Cereal source × OH
Pigs (*n*)	24	24	24	24				
Start weight (kg)	5·2	5·2	5·1	5·2	0·08	0·384	0·587	0·599
Finish weight (kg)	9·9	9·8	10·8	11·0	0·30	0·009	0·826	0·680
Gain (g/d)	223	219	271	277	11·6	0·002	0·941	0·764
Intake (g/d)	443	437	423	440	13·0	0·547	0·702	0·428
FCR (g/g)	2·05	2·01	1·58	1·60	0·06	0·001	0·899	0·719

FCR, feed conversion ratio.
* Two-way ANOVA.

determined at the terminal ileum in 20 kg pigs[31]. Higher biogenic amine excretion levels in pigs fed wheat-based diets could have been caused by various reasons including (a) increased endogenous secretions and/or reduced ileal digestibility of dietary amino acids in the WAP diets due to a higher NSP content[30,32,33], (b) reduced digesta retention time in the small intestine[34], and/or (c) increased decarboxylation of N rather than catabolism of amino acids in the WAP diet due to a more favourable environment for microbiota such as *E. coli*, *Proteus* and *Clostridia*[29].

In contrast, Mateos *et al.*[5] postulated that adding OH to a rice-based diet might not influence fermentation in the large intestine due to its highly insoluble and lignified nature. Rather, these authors suggested that supplementation of OH may have influenced the motility and transit time of digesta that, in turn, reduced the availability of substrate for bacterial growth. The finding in the present study that supplementation of OH tended to decrease only cadaverine and β-phenylethylamine but not putrescine and histamine cannot be fully explained by the dilution effect of OH, and suggests that OH influenced the fermentation of dietary and endogenous amino acids in the large intestine indirectly. This notion is supported by a recent study with weaner pigs showing that increasing protein fermentation in the large intestine by feeding fermentable protein (100 g poultry meal/kg) elevated plasma urea content, whilst increasing carbohydrate fermentation in the large intestine by feeding fermentable fibre (50 g beet pulp/kg) decreased biogenic amine levels in the colon[35].

Digestibility and performance data

Diet RAP caused higher total-tract digestibility of all measured dietary components than diet WAP, except for CP. However, pigs fed diet WAP grew faster and had a better feed conversion than their counterparts fed diet RAP. Several reports have shown higher digestibility of dietary components and better performance indices in pigs fed cooked rice-based diets compared with maize-, wheat- and sorghum-based diets[2–5,36]. Pluske *et al.*[9] showed that feeding a medium-grain rice-based diet significantly decreased starch content in the ileal digesta compared with a wheat-based diet (2·5 *v.* 8·5 g/100 g ileal digesta), suggesting a disconnection between the poorer starch digestion in pigs fed the wheat-based diet and the superior performance.

The experimental diets in the present study were lower in isoleucine content that in turn may have limited growth. Nevertheless, the calculated isoleucine:lysine ratio was similar between diets RAP and RAPOH (51 %) and was even lower in the wheat-based diets (45 %), which paradoxically showed better growth performance than the rice-based diets. The ratios between available lysine and other essential amino acids were uniform across all the diets and hence contained a similar pattern of amino acid profiles, which in essence nullifies any between-diet differences. Therefore, and despite lower isoleucine contents in experimental diets, lower daily gains and a higher FCR were probably not associated with amino acid balance, because the level of deficiency was greater in the wheat-based diets than in the rice-based diets.

Rather, the inferior performance shown in piglets fed extruded rice could have been attributable to the use of a lower DE value (14·2 MJ DE/kg) and a higher CP value (80 g/kg) used in the formulation of experimental diets. The DE value determined in a subsequent study with weaner pigs using the same extruded rice samples showed much higher values (15·1 MJ/kg) than the tabulated DE value[28], and hence the dietary DE content of diet RAP was higher than the WAP diet (Table 5). Also, chemical analysis showed that the extruded rice had only 64 g CP/kg (DM), which is much lower than in raw rice (73 g CP/kg DM). Therefore, an unbalanced energy:protein ratio was the most likely cause of the disparity between TTAD and the performance figures. Supplementation of 20 g OH/kg did not influence performance but significantly decreased TTAD of DM, gross energy and dietary DE content, which is in agreement with Lopez *et al.*[37] and was most probably due to the dilution effects of the OH. Considering their low density, it was anticipated that OH might have decreased feed intake through a gut-fill effect. However, our data and those of Mateos *et al.*[38] imply that OH at 20 g/kg is at too low a level to cause this effect, at least in weanling pigs. Several early studies showed that addition of OH at 50–150 g/kg or highly lignified cellulose hulls in diets for growing pigs significantly reduced digestibility of energy and N[39–41]. Also, using pure cellulose at 70 g/kg, Owusu-Asiedu *et al.*[42] demonstrated that cellulose did not affect ileal and total-tract retention time but significantly decreased ileal and faecal digestibility of energy and protein. The extent of the reduction in digestibility of energy and DM in the present study was smaller than in the above-mentioned studies because we used OH at only 20 g/kg in substitution for cereals. Mateos *et al.*[38] also used OH at 20 g/kg and found a non-significant reduction ($P<0\cdot10$) in total-tract nutrient digestibility. However, these authors used cooked OH, and the cooking procedure might have altered the physical properties of OH that in turn caused the tendency for reduced energy digestibility.

Blood metabolites

The concentration of urea in plasma can increase when microbial fermentation of nitrogenous compounds is increased in the large intestine. This results either from increased undigested or endogenous N entering the large intestine, and/or by the proliferation of proteolytic bacteria relative to saccharolytic microbes[43,44]. Catabolism of amino acids by microbes produces NH_3 that diffuses into the portal blood system, where it is converted to urea in the liver. The urea synthesised in the liver is either excreted as urine or diffuses back into the caecum and is incorporated into microbial N[43,44]. Therefore, the higher plasma urea content in pigs fed diet RAP compared with pigs fed diet WAP could be a reflection either of inefficient utilisation of dietary protein or increased protein fermentation due to the proliferation of proteolytic microbes in the pigs. However, the latter is the most likely explanation, as supplementation of OH decreased the plasma urea level in the low-fibre RAP diet, possibly through modification of the large-intestinal microbiota[40,41]. These data concur with the findings of Bolduan *et al.* [17], who reported a linear decline in plasma urea content with increasing crude fibre levels in diets of weaner pigs. Furthermore, pigs fed diet WAP with OH did not show a modified plasma urea concentration, presumably because there was already sufficient dietary fibre from wheat to maintain saccharolytic bacterial activity in the large intestine.

Creatine is synthesised from arginine, glycine and methionine and is used as a high-energy phosphate reserve in muscle. Degradation from creatine and creatine phosphate in muscle is the main source of the creatinine fluxed into the portal blood system. Plasma creatinine levels therefore reflect muscle mass (about 0·3 to 0·5 % of muscle weight)[45]. However, when animals are dehydrated, such as can occur with diarrhoea after weaning[46], plasma creatinine levels can increase due either to simple dehydration of plasma contents or to increased mobilisation of protein reservoirs from muscle (for example, the viscera) to compensate for decreased nutrient intake and/or absorption[47,48]. The positive correlation between plasma creatinine levels and shedding of β-haemolytic *E. coli* (Fig. 1) after weaning is consistent with this possibility, albeit no assessment of total body water content was made in the present study.

Conclusions

Feeding a diet based on extruded rice and animal protein ingredients resulted in an increased incidence of PWD, and enhanced the TTAD of dietary components. Pigs fed diet RAP had lower total biogenic amine concentrations in faeces but had a higher incidence of PWD than pigs fed diet WAP, suggesting PWD seen in piglets fed diet RAP was most probably unrelated to amine production. Addition of OH reduced the incidence of PWD only in pigs fed rice and animal proteins. These data indirectly suggest that using a source of mostly insoluble dietary fibre such as OH may reduce PWD in situations where there may possibly be a misbalance of carbohydrate and proteinaceous material entering the large bowel of the newly weaned pig.

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