

Effects of Particle Size of Rice on Resistant Starch and SCFA of the Digesta in Caecostomised Pigs

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ABSTRACT

A study on the relation between particle size and resistant starch (RS) has been conducted in pigs. The objectives of the study was to investigate the effects of particle size of rice (whole rice and rice flour) on the starch escaping digestion and volatile fatty acids (VFA) concentration in the large bowel of pigs fitted with a caecal cannula.

Four pigs were caecostomised and given 4 diet different in particle size and NSP content *e.i.* Whole brown rice (WB), fine brown rice (FB), whole white rice (WW) and fine white rice (FW) for 8 days. There was a washed-out period of 6 days between changes of diet. Caecal content was taken every 2 hour (2-16 hours and 24 hours after feeding) by withdrawal through a plastic tube. The digesta were analyzed for starch (RS) and VFA.

It was found that concentrations of starch in caecal contents was the same when the animals fed cooked fine white and or fine brown rice diets. The caecal starch concentrations in animals fed the fine product varied from 15 to 44% of the values in animals fed the whole product for brown rice and 18% to 45% for white rice. The presence of NSP was rather less important even though fibre is known to inhibit starch digestion. Differences in the particle size of rice did not affect concentration of the total VFA in the caecal contents. Small differences were found in the molar ratios of the major VFA with the butyrate being highest when pigs fed whole brown rice and lowest when the animals fed whole white rice.

INTRODUCTION

Historically, it was believed that starch; the primary complex carbohydrate and energy source of many plant

foods was completely digested in the small intestine. However, it is now recognized that starch digestion may be incomplete. For example, a significant fraction of starch of potatoes, bananas and legumes (Fleming and Vose, 1979; English and Cummings, 1986; McBurney *et al.*, 1988) and some processed food such as bread and corn flakes (Wolever *et al.*, 1986; Englyst and Cummings, 1985) may escape digestion in the small intestine of humans and other carnivores and enter the large bowel. This fraction is called resistant starch (RS) and is fermented in the colon in exactly the same way as non-soluble polysaccharides (NSP) and with the same end products (Englyst and Cummings, 1987a). The presence of RS manifested the content of fermentative substrate in the food is greater than the NSP content and so-called "carbohydrate gap" (Stephen, 1991).

The presence of RS can be influenced by some factors including processing (cooking and cooling), the type of starch (amylose or amylopectin), the physical state of the starch (degree of hydration, particle size) and the presence of other components (*i.e.* lipids) (British Nutrition Foundation, 1990). Particle size may affect the digestibility of starch through limiting the access of amylase enzymes to the starch granule. Smaller particles have a higher ratio of surface to volume, which therefore increases the access to hydrolytic enzymes and a greater digestibility. Studies on the effects of the particle size of wheat, maize and oats on the rate of starch digestion *in vitro* have shown that hydrolysis of starch by pancreatic amylase was faster with smaller particle size (Heaton *et al.*, 1988). Disruption of cells through grinding or milling of raw beans increases the digestibility of starch (Wursch *et al.*, 1986). Particle size of rice affects the RS which is higher in the whole rice but grinding of roasted chickpeas did not affect the amount of RS (Muir and O'Dea, 1992). It has been reported also that particle size affects transit time in humans

(Heller *et al.*, 1980) or in rabbits (Gidenne, 1992) with larger particles passing more quickly through the gut.

The present studies was investigated the affects of particle size of rice (whole rice and rice flour) on the starch escaping digestion and VFA concentration in the large bowel of pigs fitted with a caecal cannula.

MATERIALS AND METHODS

Diets and its preparation

Rice (*Calrose* brown and white rice) and rice flour (*Sunblend BRF* and *Sunblend 594 RF*) were provided kindly by the Rice Growers' Co-operative (Leeton, NSW). Minced beef and skim milk powder (Bonlac Food Ltd. Victoria) were purchased from local supermarket in Adelaide. Vitamin and mineral mix (*Producta Pig Grower*) was product of Milling Industries Ltd., Adelaide. Composition of pig diets (g/day/pig) was presented in table 1.

Table 1. Composition of pig diets (g/day/pig)

Component	Diet			
	WB	FB	WW	FW
<i>Calrose</i> brown rice	726	-	-	-
Brown rice flour (<i>Sunblend BRF</i>)	-	726	-	-
<i>Calrose</i> white rice	-	-	705	-
White rice flour (<i>Sunblend 594 RF</i>)	-	-	-	705
Minced beef	258	258	359	359
Skim milk powder	103	103	103	103
Sunflower oil	71	71	89	89
Vitamin & mineral mix	2	2	2	2

White rice (705 g) and brown rice (726 g) were cooked in an automatic rice cooker (Sanyo, Model EC 2100, power 600 W and a capacity of 1.8 L) with a 1.6 fold (w/v) of water. After the cooker was turned off the rice was left for 15 min as this is the usual practice during home preparation of rice (Hibi *et al.*, 1990). Technically, it is not possible to cooked fine white and brown rice in the same way as whole rice because during gelatinisation fine rice needs to be stirred continuously. Fine white rice (705 g) and fine brown rice (726 g) were added with boiling water (2 fold, w/v) and stirred well in a plastic bucket (9 L) with a wooden spoon until the flour was homogeneously gelatinized (10 min) and

left for 30 min. The cooked rice was put in plastic bags, sealed and stored at -20°C before used. Fresh minced beef was weighed into portions to provide each animal with its daily ration and stored at -20°C. Oil, skim milk powder and vitamin mixture were weighed prior to feeding.

Animal care

Four pigs of the Large White strain were obtained from the local breeder (Millwood's Piggery, Eudunda). The animals weighed 35-41 kg at purchased. They were housed in individual pen (2 x 2 m) under controlled light (6.00-18.00) and temperature (20±2°C). The pigs were fed 1000g/day of the Pig Grower Pellets. The animals was caecotomized as described by Gooden *et al.* (1993)

Sample preparation

After recovery was complete (5-7 days), the animals were fed one of 4 different diets (whole brown rice, WB; fine brown rice, FB; whole white rice, WW or fine white rice, FW) for 8 days. There was a washout period of 6 days between changes of diet during which time pig pellets were fed. On the 8th day of feeding the experimental diets, 5 g of polyethylene glycol (PEG; Sigma Chemical Co.) in 250 ml of water was given to the pigs along with the feed as a fluid marker to study the passage of digesta into the alimentary tract (Fleming *et al.*, 1989). Caecal contents were taken every 2 hour (from 2 to 16 hours and 24 hours after feeding) by withdrawal through a plastic tube (50 mm internal diameter and 800 mm of length with a metal block at the end and a scalloped hole above the block) with a 20 ml syringe. To prevent blockage of the tube during sampling 20 ml of N₂ was blown into the tube before withdrawing the sample. Samples were taken in duplicate and centrifuged promptly (5500 rpm, 20 min) at 5°C. The supernatant was frozen at -20°C prior to determination of VFA. Samples for total starch determination (4, 6, 8 and 14 h after feeding) were dried immediately in a freeze dryer and the dried contents were ground and sieved (0.5 mm screen) to obtain a representative samples.

Sample analysis

Total starch was measured by a modification of the enzymatic method of Englyst and Cummings (1988).

SCFA were determined by gas-liquid chromatography (Illman *et al.*, 1982). The statistical significance of differences between groups was established by analysis of variance incorporating a calculation of least significant difference (LSD). A value of $p < 0.05$ was taken as the criterion of significance. Calculations were carried out using a Microbits desktop computer (Microbits Ltd., Adelaide) and a commercial statistical program (Statistix Version 3.1, Analytical Software, 1989).

RESULTS AND DISCUSSION

Starch concentrations

On average, starch concentrations of caecal content of pigs fed either whole brown or white rice were higher than in the same animals fed the fine products. These were significantly different at 8 and 14 h after feeding (Table 2). Starch digestibility has emerged as an important issue and over the last two decades, many studies have been directed at establishing the factors, which control the escape of starch into the large bowel. This resistant starch (RS) has been defined as "the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals" (Asp, 1992).

Table 2. Concentrations of total starch in the caecal content of pigs fed whole brown rice (WB), fine brown rice (FB), whole white rice (WW) or fine white rice (FW) diets (mg/g dry matter)

Dietary Group	Hours After Feeding			
	4	6	8	14
WB	29.6 ^{ab}	44.0 ^a	60.6 ^a	44.0 ^a
FB	13.0 ^b	12.6 ^b	10.5 ^c	6.5 ^b
WW	42.6 ^a	41.9 ^a	46.7 ^{ab}	53.6 ^a
FW	9.9 ^b	18.7 ^{ab}	15.8 ^{bc}	9.9 ^b
Pooled SE	13.5	12.2	16.3	15.8

a, b, c Values of any vertical column not sharing the same superscript letter was significantly different ($p < 0.05$)

In rice, as in many other foods, the amount of analytical RS is very low (Muir and O'Dea, 1992; Corujo *et al.*, 1991) and as in those foods may be increased after cooking followed by cooling or freezing. However, it has been reported that RS determined *in vitro* was very

low in both raw and cooked rice and although increased by cooling and/or freezing, the absolute quantities remained low (Marsono and Topping, 1993). The experiments described in this study tested the hypothesis of Muir & O'Dea (1992) who suggested that particle size was a key determinant. Indeed, the data support this view as starch concentrations in caecal digesta were higher in pigs fed whole rice diets (WB and WW) than in those fed the corresponding fine rice diets (FB and FW). These differences are entirely consistent with the suggestion that larger rice particles were less digestible in the small intestine than the fine ones and are in keeping with previous studies with wheat, maize and oats (Heaton *et al.*, 1988) and with beans (Wursch *et al.*, 1986). The reason for the difference is believed to be that smaller particles have a higher ratio of surface to the total volume which increases the accessibility (and digestibility) of the starch granule to amylases. It must be recognized that it is possible also that milling resulted in loss of histological integrity of the granules and so enhanced the degree of hydration and solubilization and consequently digestibility.

It is interesting to note that the starch concentrations in caecal contents taken 4 hours after feeding whole brown rice did not significantly differ from those obtained after feeding the fine product even though concentrations with whole brown rice diet were more than double that of fine brown rice (29.6 mg/g vs. 13.1 mg/g). A similar trend was also found with white rice at 6 h (41.9 mg/g for whole white rice versus 18.7 mg/g for fine white rice). These data reflect the high variability found between individual pigs (as shown by the pooled SE) which prevented the differences achieving significance. Similar variability has been reported also in humans (Stephen *et al.*, 1983) where 2-20% of starch escaped absorption under equivalent conditions.

One possible factor influencing the appearance of starch in the large bowel is fibre. Snow and O'Dea (1981) suggested that fibre could form a physical barrier limiting the access of amylase enzymes to starch granules, inhibiting starch hydrolysis. In the present study, starch concentrations were the same in all diets as fed but the brown rice diets (WB and FB) contained more NSP than the white rice diets (WW and FW) (32.8 g versus 19.5 g). However, starch concentrations in the caecum were higher in the pig fed whole rice diets (WB and WW) than those fed fine rice diets (FB and FW), indicating that in this study NSP content did not affect apparent starch digestibility.

Volatile fatty acids (VFA)

Total VFA are the sum of the major acids (acetate, propionate, and butyrate) and the minor VFA (isobutyrate, valerate and isovalerate). Concentrations of total VFA were not significantly different between the groups at any sampling time (Table 3). With all diets the concentrations were highest at 2 h and were constant at lower values thereafter but the difference was significant only at 4 h and only when the animals were fed FW and FB.

Table 3. Concentrations of total VFA in the caecal content of pigs fed whole brown rice (WB), fine brown rice (FB), whole white rice (WW) or fine white rice (FW) diets (mmol/L)

Hours after feeding	DIET				Pooled SE
	WB	FB	WW	FW	
2	75.0 ^a	96.5 ^a	99.9 ^a	98.5 ^a	30.1
4	60.9 ^a	40.7 ^a	65.9 ^a	57.9 ^a	12.8
6	66.6 ^a	73.5 ^a	71.4 ^a	62.9 ^a	18.4
8	60.0 ^a	70.5 ^a	71.9 ^a	61.0 ^a	12.9
10	64.6 ^a	63.7 ^a	61.4 ^a	57.6 ^a	16.4
12	66.5 ^a	61.9 ^a	69.4 ^a	60.5 ^a	17.6
14	62.8 ^a	63.5 ^a	59.0 ^a	66.9 ^a	18.1
16	54.3 ^a	60.8 ^a	67.7 ^a	60.5 ^a	16.3
24	58.8 ^a	61.1 ^a	87.7 ^a	71.9 ^a	23.7

^aValues of any row sharing the same superscript letter were not significantly different ($p < 0.05$)

There were no significant changes in the proportion of individual VFAs with time so data from all sampling times were combined for each treatment and are presented in Table 4. The proportions of acetate in caecal contents were higher when the pigs ate the FB or WW diets than when they consumed the WB or FW diets. Propionate proportions were lowest after feeding the FB diet. The three other diets gave similar proportions of propionate. The molar contribution of butyrate was significantly higher when the pigs were fed the WB diet than the FB and WW diets but did not differ when they were fed the FW diet.

Concentrations of total VFA in caecal digesta were not significantly different between the groups (Table 3). Although concentrations were lower 4h after feeding, the high variance prevented any differences achieving

Table 4. Molar ratio of the major VFA in the caecal content of pigs fed whole brown rice (WB), fine brown rice (FB), whole white rice (WW) or fine white rice (FW) diets (%)

DIET	Acetate	Propionate	Butyrate
WB	59 ^b	30 ^a	10 ^a
FB	65 ^a	25 ^b	7 ^{bc}
WW	65 ^a	28 ^a	5 ^c
FW	58 ^b	29 ^a	9 ^{ab}
Pooled SE	2	1	1

^{a, b, c} Values of any vertical column not sharing the same superscript letter were significantly different ($p < 0.05$)

significance. As might be expected from their contribution to the total, acetate and propionate (two of the major acids) also were unaffected by diet or time after feeding. Butyrate concentrations were also largely unaffected by diet but in contrast, concentrations of butyrate fell during the day. Of course, the high variance meant that not all time points achieved statistical significance. Nevertheless, butyrate concentrations were lower with WB, FB and WW and only in the case of FW was there no change. These data agree with those showing that VFA concentrations were unchanged in the caecum and proximal colon of pigs fed white rice, brown rice or rice bran (Marsono *et al.*, 1993). In those animals, effects of diet on butyrate were largely in the distal colon with larger pools in animals fed brown rice compared with animals fed white rice. One of the explanations for these data is that effects of diet on colonic FVA may depend on the rate of passage along the large bowel. The weakness in these data has been noted in that only concentrations were measured. The fistulated pig model has great potential from the standpoints of continuous sampling of gut contents but it must be recognized that the VFA pool could easily have changed through variations in the volume of water of the caecum. Thus, there may be major differences in the total VFA available for metabolism in the whole large bowel but with little or no change in concentration in the proximal colon due solely to fluctuations in fluid volume. Fluctuations in VFA concentration throughout the day may reflect the rhythm of digesta movement through the intestine. Kennely *et al.*, (1981) has noted such circadian rhythms and these may have influenced concentrations.

In this study it was not possible to obtain adequate samples at the time of feeding, as there was so little digesta in the caecum. Consistent samples could be obtained at 2 h after feeding so comparisons were made against this value. Overall, total VFA concentrations in the caecum throughout the day were not different between the 4 feeding regimens, and were in agreement with the previous study (Marsono, *et al.*, 1993).

In general concentrations were in the range 54-75 mmol/L, 42-97 mmol/L, 59-100 mmol/L and 58-99 mmol/L with WB, FB, WW and FW diets, respectively (Table 3). These values are in agreement with the results of the previous study (Marsono, *et al.*, 1993) in which pigs were fed similar diets and where values were in the range 70-82 mmol/L. The data also are similar to those in a previous report on pigs fed barley diets wherein the total VFA concentrations varied from 82 to 122 mmol/L (Kenneley *et al.*, 1981). Quite similar values were reported by Farrell and Johnson (1970) who showed that in pigs fed a diet high (26%) or low (8%) in cellulose, concentrations of total VFA in the caecum were 100 mmol/L and 106 mmol/L, respectively. However, Imoto and Namioka (1978) reported higher (approximately 130 mmol/L) concentrations in pigs' 15 h after feeding a meal of rice bran and alfalfa. Kass *et al.*, (1980) reported very much lower concentrations of VFA in the caecum (23.7 mmol/L) and colon (34.0 mmol/L) of pigs fed an alfalfa diet. Clearly, the VFA data in the present experiment are in the range of some literature reports but quite outside others. Since the VFA in the large intestinal tract result from carbohydrate fermentation, the differences in the total VFA concentrations reported in the present and the previous studies could be due to the variability of fermentable carbohydrates available in the caecum, i.e. NSP and any other carbohydrate. Although the dietary intakes of NSP have been quantified in some of the other studies, RS has not and neither have other substrates (e.g. oligosaccharides). Some of the differences between literature reports may be due simply to the fact that the total fermentative substrate has not been reported.

In an *in vitro* study, Goodlad and Mathers (1988) showed that proportion of individual VFA was influenced very much by carbohydrates in the diets. Pectin and cellulose were potent producers of acetate, raffinose was characterized by high propionate and starch resulted in high butyrate levels. They observed similar changes in animals (Goodlad and Mathers, 1990). However, Kim *et al.*, (1978) suggested that the molar proportions of

individual VFA in pigs were similar with high and low carbohydrate diets, with average ratios of 65:25:10 for acetate:propionate:butyrate. Both the total concentrations and the proportions of individual VFA have been investigated by many of researchers and the results fall in between the extremes of Goodlad and Mathers (1988) and Kim *et al.*, (1978). For example, Ehle *et al.*, (1982) reported that feeding coarse wheat bran resulted in a mean ratio of 56:23:12 (acetate:propionate:butyrate) in large bowel digesta. With fine wheat bran the ratio was 52:25:15. Feeding cellulose resulted in high acetate (63%) but low in butyrate (8%). Fleming *et al.*, (1989) reported that in pigs fed wheat bran and red kidney bean diets, caecal VFA ratios were 63:25:10 and 62:30:6, respectively. Kass *et al.*, (1980) reported that in pigs fed alfalfa-based diets, the molar proportions of acetate, propionate and butyrate were 71:21:8 while Kenneley *et al.*, (1981) found that feeding a barley-based diet gave large bowel VFA proportions of 65:27:8. These findings do support the view that the proportion of individual VFA in the caecum of the pigs is affected by the source of dietary carbohydrate in the diet. In fact, work in this laboratory suggests that both this and the contrary view are true. In animals fed wheat and oat bran, total VFA in caecal digesta were raised but with no change in the proportions. In animals fed baked beans, the molar proportions of propionate and butyrate were increased (Topping *et al.*, 1993a).

Based on the starch contents of caecal digesta, it was expected that whole rice diet (WB and WW) might yield a higher proportion of butyrate than fine rice diet (FB and FW). The role of butyrate in enhancing colonic health has been described so the expectation that starch can enhance large bowel butyrate is of some importance. The suggestion comes from the work of Cummings and Macfarlane (1991) who reported that *in vitro* starch was a good precursor for butyrate when incubated with human faecal inocula. This expectation is not supported by animal studies. For example, Fleming *et al.*, (1989) have shown in pigs that feeding legumes (generally high in RS) did not favour increased butyrate in the in the caecum. The same appears to be true with baked beans (Topping *et al.*, 1993a). In the present study it was evident that in the case of brown rice, the proportion of butyrate was higher when WB was fed than with FB. In the case of white rice, the proportion of butyrate was higher when FW was fed than when the animals ate WW. A possible explanation is that in the case of white rice, starch in the FW diet was fermented much

faster than that in WW resulting in higher butyrate levels. If this assumption were correct, the proportion of butyrate with FW diet would be expected to be much lower than with WW diet in the following sections of the colon (not investigated). However, it should be noted that when variations throughout the day were examined, only butyrate showed significant decline against the 2-h value. It appears possible from these data that the passage of butyrate to the distal colon may not be due to excess production in the proximal large bowel but to the actual rates of transit.

CONCLUSIONS

These data show that in fistulated pigs, concentrations of starch in caecal contents was the same when the animals fed cooked fine white and or fine brown rice diets. The major determinant of caecal starch was particle size. For example, when the animals were fed brown rice, the caecal starch concentrations in animals fed the fine product varied from 15 to 44% of the values in animals fed the whole product. Similar variations were found when the animals fed white rice diets (45% at 8 hours after feeding to 18% at 14 hours after feeding). This findings confirm the hypotheses that larger particle increases the starch escaping the small intestinal digestion. The data are rather surprising in that they suggest that the presence of NSP was rather less important even though fibre is known to inhibit starch digestion.

Differences in the particle size of rice did not affect concentrations of the total VFA in the caecal contents. Small differences were found in the molar ratios of the major VFA with the butyrate being highest when pigs fed whole brown rice and lowest when the animals fed whole white rice.

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Evaluation of Organoleptic and Nutritional Characteristics of Traditionally Processed Shrimp Products Based on Its Quality Grade

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ABSTRACT

It is very common that to fit with the market segment, processor produces varied quality grade of product with different prices. However, there is no clue that the quality grade represents the organoleptic and nutritional characteristics of the product. And observation on organoleptic and nutritional characteristics of shrimp crackers (*kerupuk udang*), paste (*petis udang*), and cake (*terasi udang*) obtained from some processor in Sidoharjo, East Java, have been conducted based on product quality grade. Organoleptic characteristics of the three kinds of product in term of product appearance, taste, odor, and texture were assessed by sensory judgement, white measurement of linear expansion was applied only to fish crackers. Protein, carbohydrate, and ash contents were used as parameters for nutritive value. Result of evaluation revealed that for shrimp crackers, there were differences in protein and carbohydrate content between different quality grades and different prices. It seemed that the quality grade, and product price, which is determined by the processors, could represent the nutritive value in term of protein and carbohydrate content. On the contrary, there were no difference on organoleptic characteristics, ash content, and linear expansion of all grades of products. For shrimp paste, there were no significant differences on all quality attributes nor the prices. In this case, the quality grade and price did not represent the organoleptic and nutritional characteristics of the product. As for shrimp cake, differences among the product grade were assessed only on organoleptic characteristics, not the nutritive values. Based on this results, it seems that quality grading by the processor, and so does the product price, did not always represent the organoleptic and nutritional quality of the product. To overcome this,

it is now the time to develop and implement a process and product standardization of traditional processed shrimp, especially shrimp crackers, paste, and cake in Indonesia, as to give assurance to the consumers to get appropriate organoleptic and nutritional quality of the product equal to the amount of money they paid.

INTRODUCTION

Quality is all those attributes which consciously or unconsciously considered by the consumers and buyers should be present (Connel, 1975), while Haryono (1978) describes food quality as a combination of typical characteristics of the product which could differentiate the unit of product and significantly influenced the consumer acceptability. Thus quality will embrace among others, intrinsic composition, nutritive value, degree of spoilage, damage, deterioration during processing, storage, distribution, sale, and presentation to the consumer, hazards to health, satisfaction on buying and eating, and aesthetic consideration.

Since product quality is closely related to the degree of consumer acceptability, product characteristics which highly correlated to consumer acceptance can be used as the quality determinant factors. However consumer's attitude to food quality are not immutable, and it is necessary to be informed of changes in these attributes as they occur. The availability of appropriate quality determinant factors, therefore, will assure the consumers about the quality of the product they are going to buy. Consequently, they will pay more for higher quality product (Heruwati and Saleh, 1997).

Processing of traditional fish or shrimp product such paste, cake, crackers, salted dried, salted boiled, or fermented fish were highly varied in method, formula,