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### **Changes in Carbohydrate and Lipid Metabolism and Gastrointestinal Functional Parameters in Rats Fed Amylase Resistant Corn Starch**

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The effects of feeding amylase resistant corn starch on metabolic and gastrointestinal functional parameters were investigated in rats. Growing rats were either fed a diet containing digestible corn starch (control diet) or digestible corn starch substituted with 18% resistant starch. Resistant starch was produced by autoclaving and cooling cycles. Resistant corn starch feeding significantly reduced the weekly body weight gain ( $p < 0.05$ ) without affecting the rate of feed consumption. The caecal matter to caecal weight ratio was significantly increased ( $p < 0.05$ ) in the experimental group. The caecal matter was significantly ( $p < 0.05$ ) more acidic in the rats fed resistant corn starch diet. Fasting blood glucose concentration was lower in rats fed the resistant starch diet ( $p < 0.05$ ). Serum cholesterol, triglycerides and urea were all significantly decreased. Liver triglycerides and caecal urea were unaffected by feeding resistant starch diet. Chronic feeding of resistant starch diet caused some adaptive changes in the activities of some digestive enzymes. Intestinal sucrase activity was significantly decreased ( $p < 0.01$ ) by the experimental diet, but lipase and amylase activities of the intestine were unaffected. Pancreatic amylase and lipase activities were significantly lower ( $p < 0.01$ ) in rats fed resistant starch diet. It is concluded that resistant starch diet modifies carbohydrate and lipid metabolism and this may be of clinical significance especially in the control of hyperglycaemic and hypertipidaemic conditions.

**Key words:** Resistant starch, lipid metabolism blood glucose, blood urea, caecum

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## **Introduction**

Some dietary polysaccharides such as dietary fibre and resistant starch may escape small intestinal digestion and absorption, but subsequently fermented by the colonic microflora. Resistant starch is defined as the fraction of starch resistant to amylase digestion Asp (1992) or the sum of starch and starch degradation products that, on average reach the human large intestine Englst *et al.* (1996). It is suggested that starch that escape digestion in the small intestine may act similarly to non starch polysaccharides in the colon Young *et al.* (1996). Although dietary fibre and resistant starch are chemically distinct, the products of their microbial breakdown are volatile fatty acids Scheppach (1994), Cummings *et al.* (1996).

Dietary fibre such as gum and pectin have shown to modify carbohydrate metabolism Jenkins *et al.* (1980), Johnson *et al.* (1984), Nunes and Malmloof (1992), Bobboi and Stephens (1996) and lipid metabolism Fuse *et al.* (1989), Jenkins *et al.* (1978), Forman and Schneman (1980), Schninck *et al.* (1991). The volatile fatty acids produced during fermentation of dietary fibre have also been implicated in the altered hepatic carbohydrate and lipid metabolism Chen *et al.* (1984). Some studies on the effects of retrograded starch on lipid metabolism reported varying results Morand *et al.* (1992) showed no significant differences between plasma triglycerides concentration in rats fed digestible starch or resistant diet while de- Deckere *et al.* (1995) reported a decrease. The present studies assess the metabolic and gastrointestinal functional properties of resistant starch. This is to explore the possibility of substituting dietary fibre with resistant starch in clinical situations since most viscous polysaccharides are not tolerated especially at higher doses.

## **Materials and Methods**

### **Isolation of starch and resistant starch preparation**

Starch was isolated from the grains by the method of Szckodrak and Pomeranz (1991). Corn starch was washed with water to recover native starch. This starch was centrifuged to remove starch tailings and air-dried. Starch was mixed with distilled water (20% starch suspension) autoclaved (126°C, 15 psi) for 1 hr followed by cooling at 4°C for 24 hrs for five successive days. This is a prerequisite for resistant starch formation Ranhotra *et al.* (1991). Resistant starch in samples was determined by the method of Berry (1986). The method is based on starch remaining after prolonged incubation of the sample with amylolytic enzyme.

### **Diets**

The control diet differed from the experimental diet only in resistant starch. The treated starch was found to contain 18% resistant starch. The composition of the diets are detailed in Table 1.

### **Animals**

Two groups of male, weaning rats (7 rats per diet) of Wistar Strain were obtained from the animal holdings of the Department of Biochemistry University of Maiduguri, Nigeria. Animals were housed individual in a controlled environment (12-12 h light to dark cycle, temperature 18°C).

Table 1: Composition of diets

Diet (g kg <sup>-1</sup> )	Control diet	Resistant starch diet
Corn Starch	760	580
Resistant Starch	-	180
Albumin	140	140
Soybean oil	50	50
Mineral and Vitamin mix	50	50

\* Anglia Nutrition Products Co. England

Prior to the start of the experiment, the rats had been fed *ad libitum* with a standard rat pellets and drinking water. Daily food consumption and weekly changes in body weight were monitored during the 12 weeks experimental period.

### Sampling and analytical procedures

Towards the close of the experiment, animals were fasted for 18 h and blood collected for glucose analysis. On the last day of fed trial the animals were killed by decapitation and blood collected in a clean centrifuge tube and the serum harvested by centrifugation at 3000 g. The liver, intestine, pancreas and caecum were extracted, blotted and weighted. The intestine was perfused with cold pH 8.0 phosphate-buffered saline (PBS). The intestine was split longitude, then scraped with glass microscopic slide. The mucosa was homogenized in 5 vol. PBS. The homogenate was frozen for subsequent analyses of protein and enzymes activities. A sample of each pancreas was homogenized in 0.04 M Tris buffer pH 8.1 containing 0.01 M CaCl<sub>2</sub>. The caecum was washed in PBS, blotted and weighed. The caecal matter was extracted and the pH determined. The caecum was further washed to remove any remnant of caecal matter and weighed again. Hepatic lipids were extracted in an ethanol ether (3:1) mixture.

Glucose was estimated using the glucose - oxidase - peroxidase Trinder (1969), Blood urea by diacetylmonoxime method (Marsh *et al.*, 1965), cholesterol, Zlatkis *et al.* (1953) and triglycerides Van and Zilversmit (1957) spectrophotometrically. For liver cholesterol and triglycerides 0.5 ml of lipid extract was evaporated to dryness and the residue used. Urea nitrogen in caecal matter was determined in 5 ml suspension after centrifugation at 3000 g for 10 min. intestinal and pancreatic lipase were assayed by a titrimetric procedure Schneeman (1978). Amylase activity was determined by Forman and Schneeman method (1980) and sucrose by Dahlquist method (1969). The protein content of pancreatic and mucosa homogenates were determined by the method of Lowry *et al.* (1951). A lipase unit was defined as the amount of 0.05 N NaOH required to neutralize the free fatty acids released by 1 ml of enzyme extract in 24 h at 37°C. Specific amylolytic activity was expressed as amount of glucose released from starch h<sup>-1</sup> mg<sup>-1</sup> protein at 37°C. The specific sucrase activity was expressed as the amount of glucose released from sucrose by 1 mg protein from mucosal homogenate.

### Statistical analysis

Values were presented as means with their standard error of 7 replicate determinations. Means were statistically compared for significance by the student's t-test.

**Results**

The processing of the corn starch to produce resistant starch did not adversely affect the rate of consumption of feed as there was no significance difference in the daily feed intake (Table 2) of the groups of rats. Consumption of the experimental diet however significant ( $p < 0.05$ ) reduced the weekly (%) gain in body weight of rats. The caecal matter was significantly ( $p < 0.05$ ) increased in the experimental group. Similarly the caecal matter was significantly ( $p < 0.05$ ) more acidic in the rats fed resistant starch diet than in those fed the control diet.

Table 2: The effects of feeding resistant starch diet on body, caecal and caecal matter weights of rats

Parameters	Normal starch diet	Resistant starch diet
Feed intake (g/ 100g/day)	8.15±1.13	7.14±1.16
Weekly percentage weight gain (%)	15.36±4.60	10.63±3.75a
Caecal: Body weight ratio (g)	3.30±0.84	4.00±0.34
Caecal matter: Caecal weight ratio	2.71±0.25	3.40±0.76a
pH of caecal matter	7.4±0.21	6.00±0.14b

All values are mean±SEM of 7 replicate determination comparison was done between groups (a =  $p < 0.05$ , b =  $p < 0.01$ )

Table 3: Effects of feeding resistant starch diet on some biochemical parameters of rats

Parameter	Normal starch diet	Resistant starch diet
Fasting Blood Glucose (mmol L <sup>-1</sup> )	3.60±0.06	2.27±0.49a
Serum Triglycerides (mmol L <sup>-1</sup> )	5.20±0.45	4.14±0.54a
Serum cholesterol (mmol L <sup>-1</sup> )	1.47±0.17	0.99±0.08a
Serum Urea (mmol L <sup>-1</sup> )	5.34±0.24	4.19±0.23a
Liver triglycerides (mmol L <sup>-1</sup> ) Triolein/g wet weight)	2.47±0.34	2.68±0.07
Liver cholesterol (mmol g <sup>-1</sup> . wet weight)	2.00±0.31	3.00±0.38a
Caecal urea (mmol L <sup>-1</sup> )	4.29±0.055	5.00±0.80
Specific Amylolytic activity	10.05±0.57*	9.88±0.54
	72.36±12.59*	34.11±3.99a
Specific sucrase activity (mg glucose/h/mg protein)	10.05±0.73	4.16±0.82a
Specific lipase activity	0.084±0.003*	0.079±0.007
	0.37±0.04*	0.22±0.03

All values are mean + SEM of 7 replicate determination

Comparison were done between groups (a =  $p < 0.01$ )

\* values for instinal enzyme extract

\*\* values for pancreatic enzyme extract

The fasting blood glucose was significantly ( $p < 0.05$ ) lowered by the long term feeding of the resistant starch diet (Table 3). Serum triglycerides, cholesterol and urea were all significantly

( $p < 0.05$ ) reduced by the chronic feeding of the experimental diet to rats, while liver cholesterol was significantly ( $p < 0.01$ ) increased. Liver triglycerides and caecal urea were unaffected by resistant starch diet. Chronic feeding of resistant starch diet by rats caused some adaptive changes in the activities of some digestive enzymes. Instinal sucrase activity was significantly ( $p < 0.01$ ) decreased by the experimental diet but lipase and amylase activities of the intestine were unaffected. The pancreas of the animals in the experimental group had significantly ( $p < 0.01$ ) lower levels of amylolytic and lipase activities than the rats maintained on the control diet.

### **Discussion**

Heat processing of starchy foods normally increases the digestibility, however, some fraction may be rendered resistant to mammalian digestive enzymes and escape digestion. Thus amylase resistant 6 starch is termed resistant starch. X - ray diffraction studies indicates that retrograded amylase is mainly responsible for the generation of resistant starch and represents non-covalently bonded crystallites within the amylase component of starch Sievert *et al.* (1991). With five days heating and cooling cycles we isolated 18% resistant starch. The formation of resistant starch is strongly influenced by the number of autoclaving - cooling cycles Szczodrak and Pomeraz (1991).

Resistant starch shares some of the properties of dietary fibre in that they both escape small intestinal digestion and undergo microbial fermentation in the colon to produce volatile fatty acids especially acetate propionate and butyrate Cummings *et al.* (1996).

Although there was variation in feed intake, the animals group maintained on resistant starch diet exhibited low body weight gain. It is evident that the digestibility of diets varies and therefore differences in energy supply. A significant increase in caecal matter was noted in the case of animals fed resistant starch preparation. This suggests that significant amount of indigestible starch is reaching the caecum intact. The observed lowering of caecal pH with resistant starch diet may arise from generated short chain fatty acids from bacterial fermentation. Berggren *et al.* (1993) showed a significant correlation between caecal concentration of short chain fatty acids and pH of difference dietary fibre and oligosaccharides. The volatile fatty acids produce during dietary fibre fermentation have been implicated in altered hepatic carbohydrate and lipid metabolism Chen *et al.* (1984). However, experiments have indicated that starch fermentation produced more butyrate than the fermentation of fibre Englsts and Macfarlane (1986), Weaver *et al.* (1992). The resistant starch fed animal group showed lower fasting blood glucose concentrations and this could be due to an adaptive response to the carbohydrate passing through the gut undigested. Alternatively, the resistant starch affect the rate of starch digestion in the small intestine as demonstrated with polysaccharides Jenkins *et al.* (1980). Higher rates of glucose uptake by the liver in rats fed digestible than resistant starch was reported Morrand *et al.* (1992). Liver being the site of glycogenolysis is expected to reflect the low glucose uptake since low fasting blood glucose was observed in the resistant starch diet animals group. Propionate, one of the volatile fatty acids product of microbial fermentation is glucogenic and is expected to contribute to the circulating blood glucose.

Feeding resistant starch depressed the serum triglycerides. This was reflected by high liver lipogenesis. Serum cholesterol concentration was also depressed. Low availability of carbohydrate was shown to inhibit plasma cholesterol synthesis and enhance bile acid excretion Sacquet *et al.* (1983). Propionate was reported to have hypocholesteromic effect (Chen *et al.*, 1984; Anderson and Bridges, 1984). Similarly propionant was shown to inhibit cholesterol synthesis *in vitro* Bush and Milligan (1971). Insulin may also play a role in cholesterol regulation. Insulin was shown to increase cholesterol synthesis Flodin (1986). Reduction in availability of carbohydrate is expected to reduce the level of insulin and hence cholesterol concentration.

Animals fed resistant starch diet exhibited lower plasma urea nitrogen. The urea nitrogen flux to the caecum was increased. Diets rich in fermentable carbohydrates tends to enlarge the caecum and hence greater surfaces Younes *et al.* (1995). The caecal enlargement and elevated blood flow promotes flux of urea towards the caecum and concomitantly lowers the ammonia concentration in the digestive content. Faecal nitrogen excretion by resistant starch may also arise from an accelerated rate of bacteria protein synthesis. Feeding fibre diet to rats enhanced faecal excretion of nitrogen with development of caeca flora Eggum *et al.* (1984).

The intestinal amylase and lipase contents were determined to evaluate if changes in the activities of these enzymes in the pancreas would be reflected in the intestine. Animals maintained on resistant starch diet showed parallel lower levels of amylase and lipase in the pancreas and the intestine. It appears that the composition of the secreted juice was not altered. Forman and Schneeman (1980) reported that feeding dietary pectin to rats increased the small intestinal amylase and lipase activities without affecting the pancreatic enzymes. The changes in the amylase and lipase content of the pancreatic juice was associated with increased material in the small intestine. Pectin a soluble viscous dietary polysaccharide may behave differently from resistant starch, a product of retrogradation.

In this study intestinal specific sucrase activity was also depressed by feeding resistant starch. Rats fed semi-purified diet with cellulose produced lower intestinal activities of trypsin, chymotrypsin, amylase and lipase Schneeman and Gallaher (1980). Similarly, Johnson *et al.* (1984) showed that the addition of guar gum to a semi-synthetic rat diet stimulated the growth of mucosa but appeared to reduce the activities of mucosal enzyme cellular levels. Increased cellular proliferation can give rise to shorter villus transit time and hence reduction in the average life span of the mucosal cells. Studies in rats also showed cellulose, guar gum, pectin and resistant starch changed the epithelial structure and proliferative activity (Brunsgaard and Eggum, 1995).

Feeding resistant starch diet significantly reduced the specific amylolytic and lipase activities. Some investigators have reported that pancreatic amylase and lipase activities respond to changes in the carbohydrate and lipid content of the diet (Forman and Schneeman, 1980; Calvert *et al.*, 1985). The low availability of carbohydrate in resistant starch diet could lead to adaptive changes earlier mentioned with resultant lower amylase activity. Similarly, reduced blood lipids may lead to decreased lipase activity. There is also the possibility that resistant starch feeding influences secretion of intestinal hormones through a negative feedback mechanism.

It is concluded that resistant starch can modify some aspects of carbohydrate and lipid metabolism and may have significant clinical implications, especially in the control of hyperglycaemic and hyperlipidaemic conditions. Gastrointestinal adaptive response to the diet is suggested to be a mechanism.

### References

- Asp, N.G., 1992. European Flair Concerted Action. No. 11 on physiological Implications of the consumption of resistant starch in man. *European J. Clinical Nutr.*, 46: 31.
- Anderson, J.W. and S.C. Bridges, 1984. Short chain fatty acid fermentation products of plant fibre affect glucose metabolism of isolated rat hepatocytes. *Proceedings of the Society of Exp. Biol. and Med.*, 177: 372.
- Bobboi, A. and A.G. Stephens, 1996. The effects of electrolytes and hydrogen ion concentrations on guar gum and glucose tolerance following intraduodenal administration. *Nutr. Res.*, 16: 1403-1409.
- Berry, C.S., 1986. Resistant starch: Formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fibre. *J. Cereal Sci.*, 4: 301-314.
- Berggren, A.M.I.M.E. Bjork, E.M.C.L. Nyman and B.O. Eggum, 1993. Short chain fatty acid content and pH in caecum of rats given various sources of carbohydrate. *J. Sci. Food and Agric.*, 63: 397-406.
- Bush, R.S. and L.P. Milligan, 1971. Study of the mechanism of inhibition of ketogenesis by propionate in bovine liver. *Canada J. Animal Sci.*, 51: 121-125.
- Brunsgaard, G. and B.O. Eggum, 1995. Small intestinal tissue structure and proliferation as influenced by adaptation period and indigestible polysaccharides. *Comparative Biochem. and Physiol.*, 112A: 365-377.
- Cummings, J.h., E.E. Beatty, S.M. Kingman, S.A. Bingham and H.N. Englst, 1996. Digestion and Physiological properties of resistant starch in the human large bowel. *Br. J. Nutr.*, 5: 733-747.
- Chen, W.L., J.W. Anderson and D. Jennings, 1984. Propionate may mediate the hypocholesterolemic effect of certain soluble plant fibres in cholesterol fed rats. *Proceedings of the Society of Experimental Biol. and Medicine*, pp: 175-215.
- Calvert, R.B.O. Schneeman, S. Satchithanandam, M.M. Cassidy and G.V. Vahouny, 1985. Dietary fibre and intestinal adaptation: effects on intestinal and pancreatic digestive enzyme activities. *Am. J. Clinical Nutr.*, 41: 1249-1256.
- de-Deckere, E.A., W.J. Hloots and J.M. Van-Amelsvoort, 1995. Both raw and retrograded starch decrease serum triacylglycerol concentration and fat accretion in the rat. *Br. J. Nutr.*, 2: 287-298.
- Danhlquist, A., 1969. Determination of sucrase activity enzymes. In: *Clinical Chemistry*. Ruysern V. and Elsevier. Eds. Amsterdam, pp: 136-137.
- Englst, H.M., S.M. Kingman, G.J. Hudson and J.H. Cummings, 1996. Measurement of resistant starch *in vitro* and *in vivo*. *Br. J. Nutr.*, 5: 749-755.



- Englyst, H.N. and G.T. Macfarlane, 1986. Breakdown of resistant and readily digestible starch by human gut bacteria. *J. Sci. Food and Agric.*, 37: 699-706.
- Eggum, B.O., R.M. Beames, J. Woolstrup and K.E. Bach Knudsen, 1984. The effect of protein quality and fibre level in the diet and microbial activity in the digestive tract on protein utilization and energy digestibility in rats. *Br. J. Nutr.*, 51: 305-314.
- Fuse, F., T. Bamba and S. Hosoda, 1989. Effects of pectin on fatty acid glucose absorption and on thickness of unstirred water layer in rat and human intestine. *Digestive Disease and Sci.*, 7: 1109-1116.
- Forman, L.P. and B.O. Schneeman, 1980. Effects of Dietary pectin and fat on small intestinal contents and exocrine pancreas of rats *J. Nutr.*, 10: 1992-1999.
- Flodin, N.W., 1986. Atherosclerosis: An insulin dependent disease. *J. Am. Coll. Nutr.*, 5: 417.
- Jenkins, D.J.A., A.R. Leeds, B. Slavin, J. Mana and B.I.M. Jepson, 1980. Dietary and blood lipids: Reduction of serum cholesterol in type II hyperlipidemia by guar gum. *Am. J. Clinical Nutr.*, 32: 16-20.
- Johnson, I.T., J.M. Gee and R.R. Mahoney, 1984. Effect of dietary supplements of guar gum and cellulose on intestinal cell proliferation enzyme levels and sugar transport in the rat. *Br. J. Nutr.*, 52: 477-487.
- Jenkins, D.J.A., T.M.S. Wolever, A.R. Leeds, M.A. Gassaul, P. Haisman and J. Dilawari, 1978. Dietary fibres, fibre analogues and glucose tolerance: Importance of viscosity. *Br. Med. J.*, 1: 1392-1399.
- Lowry, O.H, N.J. Rosebrough, A. Lfarr and R.J. Randle, 1951. Estimation of proteins. *J. Biol. Chem.*, 133: 265-300.
- Morrand, C., C. Remesy, M. Levrat and C. Demigne, 1992. Replacement of digestible wheat starch by cornstarch alters splanchnic metabolism in rats. *J. Nutr.*, 122: 345-354.
- Marsh, D.J.B. Fraiser and J. Decter, 1965. Measurement of urea concentrations in nanoliter specimens of renal tubular fluid and capillary blood. *Analytical Biochem.*, 11: 73-80.
- Nunes, C.S. and K. Malmloof, 1992. Effect of guar gum and cellulose on absorption hormonal release and hepatic metabolism in the pig. *Br. J. Nutr.*, 68: 69-700.
- Ranhotra, G.S., J.A. Belroth, K. Astroth and G.J. Elsenbrun, 1991. Effect of resistant starch on intestinal responses in rats. *Cereal Chem.*, 68: 130-132.
- Scheppach, W., 1994. Effects of short chain fatty acids on gut morphology and function. *Gut.*, 31: 35-38.
- Schninck, F.L., R. Matheus and S. Ink, 1991. Serum cholesterol reduction by oats and other fibre sources. *Cereal Foods World*, 36: 825-821.
- Szczodrak, J. and Y. Pomeranz, 1991. Starch and enzyme-resistant starch from high-amylose barley. *Cereal Chem.*, 68: 589-596.
- Schneeman, B.O., 1978. Effect of plant fibre on lipase, trypsin and chymotrypsin activity. *J. Food Sci.*, 43: 634-636.
- Sievert, D.Z. Czuchajowska and Y. Pomeranz, 1991. Enzyme resistant starch. III X - ray diffraction of autoclaved amylo maize VII starch and enzyme resistant starch residues. *Cereal Chem.*, 68: 86-91.

- Sacquet, E., C. Leprince and M. Riottor, 1983. Effect of amylo maize starch on cholesterol and bile acid metabolism in germ-free (axenic) and conventional (hokoxenic) rats. *Reproduction Nutr. and Develop.*, 23: 783-792.
- Schneeman, B.O. and D. Gallaher, 1980. Changes in small intestinal digestive enzyme activity and bile acids with dietary cellulose in rats. *J. Nutr.*, 110: 584-590.
- Trinder, P., 1969. Use of glucose-oxidase-peroxidase and O-dianisidine in determination of blood and urinary glucose. *Am. J. Clin. Biochem.*, 6: 24.
- Van Handle, C. and D.B. Zilversmit, 1957. Triglycerides estimation. *J. Lab. Clinical Med.*, 50:152.
- Weaver, G.A., J.A. Krause, T.L. Miller and M.J. Wolin, 1992. Corn starch fermentation by the colonic microbial community yield more butyrate than does cabbage fibre fermentation; corn starch fermentation rates correlate negatively with methanogenesis. *American J. Clinical Nutr.*, 55: 70-77.
- Young, G.P., A. McIntyre, Y. Albert, Muir and P.R. Gibson, 1996. Wheat bran suppresses potato starch potentiated colorectal tumorigenesis at the aberrant crypt state in rat model *Gastroenterol.*, 110: 508-514.
- Younes, H., C. Demigne, S. Behr and C. Remesy, 1995. Resistant starch exerts a lowering effect on plasma urea by enhancing urea in transfer into the large intestine. *Nutr. Res.*, 15: 1199-1210.
- Zlatkis, A., B. Zak and G.J. Boyle, 1953. Estimation of cholesterol in serum and tissues. *J. Lab. Clinical Med.*, 41: 486-492.