



# Journal of Medical Sciences

ISSN 1682-4474

**science**  
alert

**ANSI***net*  
an open access publisher  
<http://ansinet.com>

**JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.**

**For further information about this article or if you need reprints, please contact:**

Aminu Bobboi  
Department of Biochemistry,  
University of Maiduguri,  
PMB 1069, Nigeria

## ***In vitro* Starch Digestibility and *in vivo* Glycaemic Responses of Different Varieties of Wheat in Normal Rats**

Aminu Bobboi, Ismaila Umar and Abel Ajiga

The *in vitro* digestibility of the starch in different varieties of wheat by amylase and the glycaemic responses of rats to the starch in the wheat varieties were investigated. There were significant ( $p < 0.05$ ) differences in the *in vitro* digestibility of the starch in the different varieties of wheat with veery 601 and Induc 66 varieties recording the lowest digestibility and chre. 5386 the highest. The digestibility was inversely correlated with the proximate protein ( $r = -0.736$ ), fat ( $r = -0.709$ ) and crude fibre ( $r = -0.807$ ) content in the wheat. 'veery 601 and Induc 66 varieties elicited the lowest glycaemic responses in rats as compared to other varieties. The glycaemic responses elicited by the various variety of wheat was negatively correlated with the proximate protein and fibre contents of the varieties of wheat. The glycaemic response was also positively correlated with the *in vitro* digestibility of the starch content ( $r = 0.533$ ). It is therefore concluded that the differences in the macromolecular composition of the varieties of wheat are significant in determining the rate of *in vitro* digestibility of and glycaemic responses to the starch content. This may be important in formulating wheat-based diets for special conditions such as diabetes.

**Key words:**Wheat starch, proximate composition, digestibility, blood glucose response

## INTRODUCTION

Early studies showed that starchy carbohydrate foods have very different effects on postprandial blood glucose and insulin responses in healthy and diabetic subjects, depending on the rate of digestion (Jenkins *et al.*, 1981, 1982). A range of factors besides carbohydrates may influence digestibility (Thorne *et al.*, 1983; Jenkins *et al.*, 2002). These factors include the interaction of starch with fibre, antinutrients such as phytate (Yoon *et al.*, 1983), the association between starch and protein (Anderson *et al.*, 1981; Jenkins *et al.*, 1987). The nature of starch (Behall *et al.*, 1988) and its physical form, that is whether the food is raw or cooked, ground or whole (O'Dea *et al.*, 1980; Collier and O'Dea, 1982; Colling *et al.*, 1981) also affects digestibility. Addition of fat to a carbohydrate meal was shown to decrease postprandial glucose response (Collier and O'Dea, 1983; Normand *et al.*, 2001). Starch lipids can complex with amylose and contribute to retrogradation (Russel and Juliano, 1983). Retrograded starch is highly resistant to pancreatic amylase digestion. Diets containing a debranched amylopectin to lipid complex (V-complex) have been shown to exhibit limited digestibility (Murray *et al.*, 1998).

Many grain legumes have genetic variations in their seed contents and the composition of starch they contain (Wang *et al.*, 1998). Mutation can alter the shape of the seed and directly affect the starch content, its polymer composition, amylose to amylopectin ratio and the physical structure of the starch granule (Wang *et al.*, 2003). Blood glucose and insulin responses to ingested rice with varieties differences was attributed to difference in amylograph paste consistency and amylose content (Juliano and Goddard, 1986). Some work suggests that the interactions between nutrients from its composition are not a good predictor of glycaemic response (Sud *et al.*, 1988). In this study, we examined the *in vitro* digestion and *in vivo* blood glucose responses to varieties of wheat and attempt was made to correlate with their macromolecular composition. This will limit some of the variables that may arise from the nature of food source. Genetic manipulation of starch content, composition and granule structure that will give low glycaemic index may be potentially beneficial in the prevention and treatment of chronic disorders such as obesity and diabetes mellitus.

## MATERIALS AND METHODS

**Animals:** Forty-six male white strain Wister rats weighing averagely 100 g were used for the *in vivo* glycaemic response experiments. The rats were maintained on a standard diet (Pfizer, Nigeria Ltd.) and water *ad libitum* except on days when the rats were used for the glycaemic

response experiments. Prior to each day of experimentation the rats were fasted for 18 h; thereafter 0.1 mL of blood was collected from the tail for the estimation of fasting blood glucose concentration Colorimetrically (Trinder, Sigma Inc). Groups of rats were then either given the aqueous suspension of the powder of any of the varieties of wheat, pure potato starch (M and B, Dagenham, England), or glucose, on different occasions and the glycaemic responses determined. Post-prandial blood samples were collected at 30 min intervals for 3 h and blood glucose determined. The wheat powder was administered by gavage as an aqueous suspension 2 g kg<sup>-1</sup> body wt. As standard, an aqueous suspension of pure potato starch containing the same amount of starch as in 0.2 g of the wheat powder was intragastrically administered to the rats on a separate occasion. As control, an aqueous solution of glucose containing the glucose equivalent of the starch was also administered.

**Wheat samples:** The different wheat varieties were obtained from Lake Chad Research Institute, Federal Ministry of Agriculture, Maiduguri, Nigeria. The varieties obtained, were the sompi Afan-TI, veery 601 chre S386 and induc 66. The wheat was dehusked, sun-dried and ground to a powder which was then sifted with a 0.3 mm pore-size sieve to obtain a finer powder, which was used for both the *in vitro* and *in vivo* studies.

**Proximate analysis:** The crude wheat powder was used for the proximate analysis. The assays of the proximate moisture, ash, crude proteins, fat and crude fibre content of wheat varieties was done as described by the AOAC Subtracting the sum of the percentage composition of all other components 100% gives the proximate carbohydrate and soluble sugar content.

***In vitro* digestibility of wheat starch:** *In vitro* digestibility of wheat starch was carried out according to Lee *et al.* (1985) with minor modifications. Briefly, 0.5 g of the powder of each wheat variety was placed in a test tube containing 10 mL of phosphate buffer (pH 7.0) and shaken vigorously. The suspension was warmed for 2 min in a boiling water bath and then cooled before addition of 1 mL of 1% diastase enzyme (M and B, Dagerham, England) solution and incubating at 37°C for 1 h. A control for each sample was ran simultaneously by mixing 0.5 g of wheat powder, 10 mL phosphate buffer and 1 mL of enzyme solution in a test tube and heating the test tube in a boiling water bath for 10 min. After cooling, the mixture was incubated (37°C) for 1 h. At the end of the 1h incubation, the test reaction mixture was heated in boiling water bath for 10 min, cooled and centrifuged. The controls were also centrifuged. For a standard, an equivalent amount of pure potato starch (M and B,

Dagerham, England) was treated as the wheat samples. One milliliter aliquots of the supernatants were taken and 1 mL of 1 M HCl added to each and then incubated at 75°C for 1 h. The reaction mixtures were then neutralized with 1 mL of 1 N NaOH. The neutralized solution was used in determining the amount of glucose released following hydrolysis.

**Analysis of data:** For the *in vivo* study the post-prandial blood glucose concentrations were plotted against the sampling time and the area under the curve (AUC) and peak blood glucose concentration (PBC) above the fasting level attained calculated. The AUC and peak concentration were correlated using linear regression with the *in vitro* digestibility of the wheat starch and the percentage compositions of protein, fat, fibre and carbohydrates in the wheat varieties.

To calculate the percentage *in vitro* digestibility of the wheat starch the amount of glucose released from the wheat starch was expressed as a percentage of glucose released from pure potato starch under the same reaction conditions. The amount of glucose released from the wheat starch was correlated with the proximate percentage protein, fat fibre and carbohydrates contents in the wheat varieties. All comparison of means was done by the student t-test.

**RESULTS**

Table 1 shows that the carbohydrate contents of the different wheat varieties ranged from 65.7 to 68%, while their fibre content ranged from 2.2 to 3.0%, in both cases induc 66 recorded the lower limit, while chre. S386, had the upper limit. Induce 66 however, had the highest fat content amount of 8.9% and cher. S386 the lowest (7.6%). The lowest amount of crude protein was recorded with sompi and the highest with veery 601.

The results of the *in vitro* digestibility analysis are presented in Table 2 and they show that the starch in all the varieties of wheat was significantly (p<0.05) less digestible by diastase than pure potato starch. The starch in chre. S386, sompi and Afan-T1 had similar digestibility.

Table 1: The proximate composition of different varieties of wheat

Wheat variety	Proximate composition (%)					
	Moisture	Ash	Crude protein	Fat	Crude fibre	Carbohydrates*
Chre. S386	11.50	2.80	7.8	7.55	2.20	67.99
Sompi	12.35	2.25	7.56	8.25	2.30	67.29
Afan-T1	12.60	1.90	8.40	7.65	2.25	67.20
Veery 601	12.30	2.00	8.82	8.15	2.50	66.23
Induc 66	11.35	2.65	8.40	8.95	3.00	65.65

\* These values were determined by difference

Table 2: *In vitro* digestibility of starch in different varieties of wheat

Wheat variety (n = 4)	Amount of glucose released by diastase (mm)* Mean±SEM	Percentage digestibility (%)**
Pure starch	11.83±1.12 <sup>a</sup>	100.0
Chre s386	8.46±0.7 <sup>b</sup>	71.5
Sompi	7.80±0.90 <sup>b</sup>	65.9
Afan-TI	7.17±0.97 <sup>ab</sup>	60.6
Veery 601	6.51±0.42 <sup>b</sup>	55.0
Induc 66	6.22±0.60 <sup>c</sup>	52.5

\* These values are means with standard error of means of four determinations and values with different superscripts are significantly different (p<0.05),

\*\* These values are the amounts of glucose released from the wheat starch expressed as percentages of the amount released from an equivalent quality of pure potato starch under identical conditions

Table 3: The glycaemic indices of rats intubated with different varieties of wheat samples (n = 6)

Intubated sample	Area under the curve (mm min <sup>-1</sup> ) Mean±SEM	Peak change in concentration (Mm) Mean±SEM
Glucose	382.60±57.60 <sup>a</sup>	4.93±0.80 <sup>a</sup>
Pure starch	338.10±31.70 <sup>a</sup>	4.28±0.86 <sup>a</sup>
Sompi	312.40±87.60 <sup>a</sup>	3.38±0.50 <sup>ab</sup>
Chre. s386	207.53±66.15 <sup>b</sup>	2.93±0.88 <sup>b</sup>
Induc 66	316.10±62.00 <sup>a</sup>	2.83±0.70 <sup>b</sup>
Afan-TI	235.50±54.20 <sup>c</sup>	3.10±0.16 <sup>c</sup>
Veery 601	109.35±48.90 <sup>cd</sup>	2.60±0.70 <sup>b</sup>

\* Glycaemic index, These values were calculated from blood glucose response curves after incubations of the samples and they are means with SEM of six animals. Comparison was done down the columns and values with different superscript are statistically different (p<0.05)

The digestibility of veery 601 and induc 66 starch was significantly (p<0.05) less than that of the starch in other varieties. The *in vitro* digestibility of the wheat starch was significantly (p<0.05) negatively correlated with the percentage protein (r = -0.736), fat (r = -0.709) and fibre (r = -0.807) contents of the wheat varieties and positively correlated with the percentage carbohydrate (r = 0.995) content.

Table 3 presents the glycaemic indices calculated from the glucose response curves of rats intubated with the wheat samples, pure starch, or glucose. There was no significant difference in the parameters when the rats were intubated with the different varieties of wheat. Correlation studies however showed that both AUC and PBC attained were significantly (p<0.05) negatively correlated with the proximate protein (AUC, r = -0.644; PBC, r = -0.850) and fibre (AUC, r = -0.625; PBC, r = -0.843) contents of the wheat varieties. While AUC was positively, though not significantly correlated with percentage fat (r = 0.467) and PBC was negatively correlated (r = -0.381). A significant positive correlation (p<0.05) existed between proximate carbohydrate (r = 0.620) content of the wheat and PBC, but not significant with the AUC. Similarly, a positive correlation was observed between PC and the *in vitro* digestibility of the wheat starch (r = 0.533).

**DISCUSSION**

The starch content of the various wheat varieties were significantly (p<0.05) less digestible than pure

potato starch. This may have been due to the presence in the wheat varieties of non-starch components, which interfered with the diastase hydrolysis. The presence of fibre (Throne *et al.*, 1983) and proteins (Anderson *et al.*, 1981; Jenkins *et al.*, 1987) in foods reduces digestibility of the starch contents of such foods. The veery 601 and Indus 66 varieties were the least digestible of all the varieties and this was attributed to the fact that these two varieties contained the least amounts of carbohydrates and highest amounts of proteins, fats and fibre than other varieties; factors which lead to reduced digestibility of starch contents of foods. This was further confirmed by the negative correlation observed between *in vitro* digestibility of the starch content and the proximate protein, fat and fibre. Fibre reduces the extent of contact between enzyme and substrate (Bjorck *et al.*, 1994), thereby showing down the rate of digestion. While proteins are known to form starch-protein complexes in foods, these complexes are much more resistant to amylase hydrolysis than pure starch (Anderson *et al.*, 1981; Van Loon *et al.*, 2000).

The glycaemic responses of rats to veery 601 and induce 66 were the least amongst the varieties of wheat indicating that *in vivo* glycaemic response may be positively correlated with *in vitro* digestibility of the cereal starch. This was confirmed by correlation studies, which showed that the area under the glycaemic response curve (AUC) and the peak change in blood glucose concentration (PBC), were positively correlated with the *in vitro* digestibility. These findings also confirm earlier reports (Jenkins *et al.*, 1980; O'Dea *et al.*, 1981) that *in vitro* digestibility studies may be significant in predicting the *in vivo* glycaemic response to foods. Much like the *in vitro* digestibility, the *in vivo* glycaemic response was negatively correlated with proximate protein, fat and fibre contents and positively correlated with carbohydrate contents. The fibre in the original food not only slows down digestion of starch but also reduces the rate of absorption of the digesta from the intestine by reducing the rate of gastric emptying and mixing of carbohydrate contents all of which lead to reduced glycaemic response. Protein in food affect digestibility of the starch content and glycaemic response. The formation of less digestible protein-starch complexes in foods have been reported (Anderson *et al.*, 1981; Jenkins *et al.*, 1987) and oral administration of amino acids or proteins have been shown to stimulate insulin secretion (van Loon *et al.*, 2000) which affects AUC and PBC as observed in this experiment.

The co-ingestion of fat and carbohydrates led to flattening of postprandial blood glucose response (Collier and O'Dea, 1983) and the effect was ascribed to delay gastric emptying and potentiation of insulin release.

Similarly, starch lipids are known to complex with amylase leading to retrogradation (Murray *et al.*, 1998). Interactions between starch and fatty acids have also been shown to contribute in resistant starch formation and particularly the amylose fraction (Crowe *et al.*, 2000). Retrograded products are more resistant to amylase digestion.

It was thus concluded that the differences in proximate composition of different wheat varieties affects both the *in vitro* digestibility of the starch constituent and the *in vivo* glycaemic response elicited by the starch. The higher the protein, fat and fibre content of the wheat the lower the *in vitro* digestibility of the starch constituent and the *in vivo* glycaemic response elicited by the starch component of the wheat. This may be useful in formulation of wheat-based diets for the management of carbohydrate disorders such as diabetes. It would seem that diets containing the veery 601 and Indus 66 varieties of wheat would be better for such purposes than those containing any of the other varieties investigated in this study.

## REFERENCES

- Anderson, I.H., A.S. Levine and M.D. Levitt, 1981. Incomplete absorption of the carbohydrate in all-purpose wheat flour. N. Eng. J. Med., 304: 891-892.
- Behall, K.M., D.J. Scholfield and J. Canary, 1998. Effect of starch structure on glucose and insulin responses in adults. Am. J. Clin. Nutr., 47: 428-432.
- Bjorck, I., Y. Granfeldt H. Liljeberg J. Tovar and N.G. Asp, 1994. Food properties affecting the digestion and absorption of carbohydrates. Am. J. Clin. Nutr., 59: 6995-7055.
- Collier, G. and K. O'Dea, 1982. Effects of physical form of carbohydrate on the postprandial glucose, insulin and gastric inhibitory polypeptide response in type 2 diabetes. Am. J. Clin. Nutr., 36: 10-14.
- Collier, G. and K. O'Dea, 1983. The effect of congestion of fat on the glucose, insulin and gastric inhibitory polypeptide responses to carbohydrate and protein. Am. J. Clin. Nutr., 37: 941-944.
- Colling, P., C. Williams I. MacDonald, 1981. Effect of cooking on serum glucose and insulin responses to starch. Br. Med. J., 282: 1032.
- Crowe, T.C., S.A. Seligman and L. Copeland, 2000. Inhibition of enzymatic digestion of amylase by free fatty acids *in vitro* contributes to resistant starch formation. J. Nutr., 130: 2006-2008.
- Jenkins, D.J.A., T.M.S. Wolever, R.H. Taylor and A.I.J. Ghafari *et al.*, 1980. Rate of digestion of foods and postprandial glycaemia in normal and diabetic subjects. Br. Med. J., 281: 14-17.

- Jenkins, D.J.A., D.M. Thomas and T.M.S. Wolever, 1981. Glycaemic index of foods: A physiological basis for carbohydrate exchange. *Am. J. Clin. Nutr.*, 34: 362-364.
- Jenkins, D.J.A., R.H. Taylor and T.M.S. Wolever, 1982. The diabetic diet, dietary Carbohydrate and differences in digestibility. *Diabetologia*, 23: 477-484.
- Jenkins, D.J.A., M.J. Thorne, T.M.S. Wolever and A.L. Jenkins Rao *et al.*, 1987. The effect of starch-protein interaction in wheat on the glycaemic responses and rate of *in vitro* digestion. *Am. J. Clin. Nutr.*, 45: 946-951.
- Jenkins, D.J.A., C.W.C. Kendall, L.S.A. Augustin and S. Franceschi *et al.*, 2002. Glycaemic index: Overview of implications in health and disease. *Am. J. Clin. Nutr.*, 76: 266-273.
- Juliano, B.O. and M.S. Goddard, 1986. Causes of varietal differences in insulin and glucose responses to ingested rice. *Qual. Plant Foods Hum Nutr.*, 36: 35-41.
- Lee, P.C., S. Brooks L.A. Heitlinger and E. Lehenhel, 1985. Digestibility of native and modified starches: *In vitro* studies with human and rabbit pancreatic amylases and *in vivo* studies in rabbits. *J. Nutr.*, 115: 93-103.
- Murray, S.M., A.R. Patil, G.C. Fahey and N.R. Merchen *et al.*, 1998. Apparent digestibility of a debranched amylopectin-lipid complex and resistant starch incorporated into enternal formulas fed to ileal-cannulated dogs. *J. Nutr.*, 128: 2030-2035.
- Normand S., Y. Khalfallah and C. Louche-Peliissier, 2001. Influence of dietary fat on postprandial glucose metabolism (exogenous and endogenous) using intrinsically <sup>13</sup>C-enriched durum wheat. *Br. J. Nutr.*, 86: 3-11.
- O'Dea, K., P.J. Nestle and L. Antionoff, 1980. Physical factors influencing postprandial glucose and insulin responses to starch. *Am. J. Clin. Nutr.*, 33: 760-765.
- O'Dea, K., P. Snow and P. Nestel, 1981. Rate of starch hydrolysis *in vitro* as a predictor of metabolic responses to complex carbohydrate *in vivo*. *Am. J. Clin. Nutr.*, 34: 1991-1993.
- Russel, P.L. and B.O. Julinano, 1983. Differential scanning calorimetry of rice starch. *Starke*, 35: 382-386.
- Sud, S., A. Siddhu, R.L. Bijlan and M.G. Kammarkar, 1988. Nutrient composition is a poor determinant of the glycaemic responses. *Br. J. Nutr.* 59: 5-12.
- Thorne, M.J., L. Thompson and D.J.A. Jenkins, 1983. Factors affecting starch digestibility and the glycaemic response with special reference to legumes. *Am. J. Clin. Nutr.* 38: 481-488.
- Van Loon, L.J., W.H. Saris, H. Verhagen and A.J. Wagenmakerers, 2000. Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate. *Am. J. Clin. Nutr.*, 22: 96-105.
- Wang, T.L., T.Y. Bogracheva and C.I. Hedley, 1998. Starch as simple as A,B,C.? *J. Expt. Bot.*, 49: 481-502.
- Wang, T.I., C. Domoney, C.L. Hedley, R. Casey and M.A. Grussak, 2003. Can we improve the nutritional quality of legume seeds? *Plant Physiol.*, 131: 886-891.
- Yoon, J.H., I.U. Thompson and D.J.A. Jenkins, 1983. The effect of phytic acid on *in vitro* rate of starch digestibility and blood glucose response. *Am. J. Clin. Nutr.*, 38: 835-842.