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The role of resistant starch in human nutrition

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Abstract. In this paper, we examine the role and effect of resistant starch (RS) in human nutrition; further, the structure and properties of RS, the food sources based on resistance to digestion in the colon, and the physiological effects of RS are described. The nutritional value of RS, the effect of RS on short-chain fatty acid (SCFA) production, the relationships between RS and colon function, and the relationships between food starch, dietary fibre, and RS content and colon cancer development are reviewed. It has been shown that the use of RS in foods may have some benefits. Resistant starch, digestion of resistant-starch-containing foods have a number of health benefits for colon function but appear to have less effect on lipid-glucose metabolism. It has a positive effect on colon bacterial activity, promotes the growth of beneficial microbes, and reduces the activity of enzymes that are harmful to the digestive system. Under the influence of RS, increased SCFA production lowers the pH of the colon and stimulates bile acid secretion. The decreased pH protects against colon cancer and inhibits the conversion of primary and secondary bile acids, which are cytotoxic to intestinal cells. At the end of the review article, the relationships between RS and the colon microflora, its use as a prebiotic, and the relationship between RS and glucose metabolism are analysed. It was found that the use of RS in the diet might have benefits as it shortens the time it takes food to pass through the colon and increases the amount of stool. It was also found that the physicochemical properties of foods can directly affect the amount of RS and thereby the blood glucose levels and insulin response.

Keywords and phrases: resistant starch, human nutrition, short-chain fatty acid, blood sugar level, insulin response, prebiotic, probiotic bacteria

1. Effects of resistant starch in the human body

Nutritional science has recognized from the earliest years of its appearance that the body is incapable of utilizing all the nutrients in the food it consumes. An increasing number of evidence suggests that only a portion of the total nutrients consumed is available, and the term "utilization" is used to quantify this portion (*Southgate*, 1989). Nutrients measured by chemical analysis are not fully utilized, mainly due to indigestible cell walls, bulky or denser structures, low solubility, digestive inhibitors, and specific constituents (inhibitors, dietary fibre, phytic acid, and tannic acid) of foods of plant origin that can significantly reduce the absorption and utilization of certain nutrients (*Rosado et al.*, 1987).

During food processing, the ingredients are transformed, and cross-links may be formed, making them inaccessible to degrading enzymes. These types of nutrients are also "unusable" by the human body (*Erbersdobler*, 1989).

Starch is the most common storage of polysaccharides in plants and the main source of dietary carbohydrate. It can be found in the chloroplast of green leaves and the amyloplast of seeds and tubers as granules (*Ellis et al.*, 1998). Recently, it has been found that the partial digestion and absorption of starch in the small intestine is a normal phenomenon associated with indigestible starches (*Englyst & Cummings*, 1991; *Englyst et al.*, 1992). These are called resistant starch (RS). All starches and starch breakdown products that are not absorbed in the small intestine of a healthy person are classified as RS (*Nugent*, 2005). Extensive studies have shown that their physiological functions are similar to those of dietary fibre (*Asp*, 1994; *Eerlingen & Delcour*, 1995). Resistant starch (RS) is fermented by microbes in the colon, leading to the production of short-chain fatty acids (SCFAs) (*Topping & Clifton*, 2001; *Bird et al.*, 2007).

Short-chain fatty acids lower the pH in the colon, preventing the excessive growth of pathogenic bacteria (*Roy et al.*, 2006). Acetic acid, propionic acid, and butyric acid are the main SCFAs produced in the colon, and the last of these has the most health benefits. The health-promoting effects of butyric acid are prevention and inhibition of carcinogenesis in the colon, protection of the mucosa from oxidative stress, and strengthening the barrier of the colon. Butyric acid also has anti-inflammatory properties (*Hamer et al.*, 2008). Propionic acid can lower blood cholesterol levels (*Hosseini et al.*, 2011). In addition, short-chain fatty acids are thought to play a role in the development of satiety (*Sleeth et al.*, 2010). RS contributes to health protection by promoting the formation of short-chain fatty acids (*Haenen et al.*, 2013).

2. Characterization of starch and resistant starch

Chemically, starches are polysaccharides in which the glucose molecules are linked together by α-1-4 and/or α-1-6 bonds. There are two main structural types of starch: amylose, which is a linear α-1-4 molecule and typically accounts for 15–30% of starch, and amylopectin, which is a larger branched chain – α-1-4 and α-1-6 molecules that also contain bonds – and accounts for 70–85% of starch.

In vivo studies of dietary-fibre-like non-starch polysaccharides by *Englyst et al.* (1992) revealed that certain starches remain after enzymatic hydrolysis. Follow-up studies with a healthy ileostomy (an operation in the abdominal wall through which a small section of the small intestine (ileum) is passed to the body surface) confirmed the presence of similar starches that resisted digestion in the small intestine (*Cummings et al.*, 1996).

RS is classified into five general types, numbered from RS1 to RS5 (*Englyst et al.*, 1992; *Brown et al.*, 1995; *Asp et al.*, 1996; *Nugent*, 2005; *Sajilata et al.*, 2006; *Fuentes-Zaragoza et al.,* 2011). *Table 1* gives an overview of the RS types, the different classification criteria, and the food origin.

Table 1. Types of resistant starch and their food sources

RS type	Description	Food sources
RS1	Physically protected starch	Wholly or partly ground cereals and seeds, legumes, pasta
RS2	Non-gelatinized resistant starch granules with type B crystallinity, which can be slowly hydrolysed by α-amylases	High amylose starch, some legumes, raw potatoes, and green bananas
RS3	Retrograded starch	Boiled and chilled potatoes, bread, cornflakes, long-lasting and/or repeated wet heat-treated foods
RS4	Chemically modified starches (with ether, ester groups, other chemicals)	Some fibre drinks, foods in which modified starch is used, in some breads and cakes
RS5	Amylose-lipid complexes	Stearic acid-complexed high-amylose starch

Sources: *Nugent*, 2005; *Sajilata et al.*, 2006; *Lunn & Buttriss*, 2007; *Sharma et al.*, 2008; *Birt et al.*, 2013; *Lockyer & Nugent*, 2017; *Metzler-Zebeli et al.*, 2019; *Gutiérrez & Tovar*, 2021
RS, resistant starch

In RS1, starch is physically inaccessible to digestion because the intact cell walls in grains, seeds, or tubers prevent their digestion. RS2 are native starch granules, which contain granular starch that is resistant to digestion because of the conformation or structure of the granules. RS3 represents non-particulate starch-derived substances that are resistant to digestion. RS4 is a group of starches containing chemically modified starch that is etherified, esterified, or cross-linked with chemicals in a way that reduces the digestibility of the starch (*Brown*, 2004; *Fuentes-Zaragoza et al.*, 2011). RS5 is a group of starches that contain an amylose-lipid complex (*Birt et al.*, 2013; *Lockyer & Nugent*, 2017; *Metzler-Zebeli et al.*, 2019; *Gutiérrez & Tovar*, 2021).

Resistant starch can be found in a variety of everyday foods. However, depending on the degree of processing or cooking, as well as the length and circumstances of storage to which the starch is subjected, these levels can be variable and unexpected (*Brown*, 1996). Phosphate and other extreme additions can adhere to starch, making it more or less sensitive to breakdown (*Niba*, 2003). Physiological factors can also have an impact on the amount of RS in foods. Increased chewing reduces particle size (smaller particles are easier to digest in the gut) although individual differences in transit time and biological factors also play a role (*Nugent*, 2005). It is currently unknown how different types of RS4 affect digestion in vivo.

RS is a type of dietary fibre that has a number of nutritional benefits, including lowering blood sugar and insulin levels, reducing calorie intake, increasing faecal excretion and decreasing faecal transit time, promoting the growth of beneficial intestinal bacteria and the colonic production of short-chain fatty acids (SCFA), etc. (*Zhao et al.*, 2018).

The many definitions of dietary fibre derive from the method of its definition, i.e. dietary fibre is defined as a plant component or as a chemical (*Champ et al.*, 2003a). In 2000, the American Association of Cereal Chemists (AACC) defined dietary fibre as edible components that resist digestion and absorption in the human small intestine and are fully or partially fermented in the large intestine. Dietary fibres include oligosaccharides, polysaccharides, lignin, and other plant compounds. These dietary fibres have beneficial physiological effects such as lowering blood glucose and blood cholesterol levels, assisting bowel movements and defecation (*Jones*, 2000).

The National Academies' Institute of Medicine's Food and Nutrition Board recently issued a definition of dietary fibre that includes RS. The same definition is being worked on by the Codex Alimentarius Commission. In the UK, the definition of dietary fibre is based on the method developed by *Englyst et al.* (1992); it does not include RS (*EFSA*, 2017) and applies only to non-starch polysaccharides and lignin. Manufacturers use the method developed by the Association of Official Analytical Chemists (AOAC) to measure dietary fibre, so food tables used in practice for nutritional purposes for all foods in the UK continue to include data according to the *Englyst* principle.

When RS is found naturally in food, it is classified as dietary fibre. If it is produced artificially, such as through physical, enzymatic, or chemical means, it must give physiological benefits to be deemed dietary fibre (*Dai & Chau*, 2017).

3. Physiological effects of resistant starch

A number of physiological effects are attributed to RS and are listed in *Table 2*.

Table 2. Physiological effects of resistant starch

Possible physiological effects	Circumstances in which they are required
Improves glycaemic and insulin responses	Diabetes, decreased glucose and insulin response, metabolic syndrome
Normalization of intestinal function	Colorectal cancer, ulcerative colitis, inflammatory bowel disease, colonic abscess, constipation
Better lipid profile	Cardiovascular disease, lipid metabolism, metabolic syndrome
Prebiotics and intestinal flora protective components	Colon dysfunction
Increased feeling of satiety and decreased energy intake	Obesity
Increased micronutrient absorption	Increased mineral absorption, osteoporosis
Oral rehydration therapies	Cholera treatment, chronic diarrhoea
Synergistic interactions with other dietary components, e.g. dietary fibre, protein, lipids	Improved metabolic control and increased intestinal health
Thermogenesis (heat generation)	Obesity, diabetes

Source: *Brown*, 2004; *Champ*, 2004; *Bindels et al.,* 2017; *Snelson et al.*, 2019; *Tian & Sun*, 2020

As it passes through the small intestine, RS interacts weakly with digestive enzymes in the upper gastrointestinal tract and ferments strongly in the colon to produce fermentation products such as carbon dioxide, methane, hydrogen, and organic acids (lactic acid and SCFAs). However, it is believed that RS results in little gas production compared to other indigestible polysaccharides (fructooligosaccharides, lactulose) (*Christl et al.*, 1992). Fermentation products, such as butyric acid, acetic acid, and propionic acid, are thought to contribute to the exertion of the physiological effects of RS (*Topping et al.*, 2003).

Dietary fibre is divided into two types: insoluble and soluble. Insoluble fibre resists fermentation, but soluble fibre is easily digested by gut bacteria (*Singh et al.*, 2018). The consumption of soluble fibre may be beneficial in cardiovascular diseases as it affects both lipid and glucose metabolism. RS shares certain characteristics with soluble fibre in that it is poorly digested in the small intestine and highly digested and metabolized (fermented) in the large intestine, which releases SCFA. However, unlike soluble fibre, the RS fraction is not viscous in the large intestine, can be easily incorporated into most starchy foods in the diet, and is considered much more palatable (*Demigne et al.*, 2001).

Other beneficial physiological effects of RS have also been demonstrated in studies on rats, showing an effect on lipid metabolism, where a large reduction of fat in the body was observed. In these studies, reductions in plasma cholesterol levels of 22–32% and plasma triglyceride levels of 29–42% were observed. According to *Younes et al.* (1995), RS has been shown to be effective in lowering plasma cholesterol and triglyceride levels with cholestyramine (a bile acid sequestering agent). In genetically obese lean (*Mathé et al.*, 1993) and diabetic rats, RS has been demonstrated to be beneficial in decreasing plasma cholesterol levels (*Kim et al.*, 2003).

4. Analytical methods for the measurement of resistant starch

The methods are based on the principle of enzymatic digestion and indicate the amount of starch resistant to enzymatic digestion at 37 °C. The first step in any method for measuring the resistant starch content of food is to remove all digestible starch from the product using thermostable α-amylases (*McCleary & Rossiter*, 2004).

In the United States, Japan, and Australia, the AOAC method 985.29 is used for the determination of Total Dietary Fibre (TDF) in food (*Prosky et al.*, 1985). After an enzymatic treatment that mimics human digestion, the amount of dietary fibre is determined gravimetrically. The method is generally used to measure total digestible dietary fibre (*Devries*, 2004). This method measures only some forms of RS (RS3, the retrograded portion, and RS2, found in high amylose corn) that appear as part of the total fibre. Therefore, additional methods are needed to quantify other forms of RS (*Champ et al.*, 2003b).

After extensive interlaboratory evaluation, AOAC methods 2009.01 and 2011.25 and AACCI methods 32-45.01 and 32-50.01 were adopted as AOAC methods 2009.01 and 2011.25 and AACCI methods 32-45.01 and 32-50.01, respectively, for measuring dietary fibre that is generally consistent with the Codex definition (*McCleary et al.*, 2010). Following that, various flaws were discovered in the procedure, the most notable of which was the use of a 16-hour incubation period, which was correctly deemed physiologically irrelevant. To preserve consistency with the Official Method for measuring resistant starch, an incubation time of 16 hours was adopted in the development of AOAC methods 2009.01 and 2011.25. (AOAC Method 2002.01; AOAC, 2012). Application of the method to different food samples and individual food ingredients revealed certain limitations. One weakness of the method was that the incubation time with the pancreatic-α-amylase/amyloglucosidase (PAA/AMG) mixture was 16 hours, whereas the transit time to the human small intestine was probably only about 4 hours. In response to this limitation, to address it, the integrated Total Dietary Fibre (TDF) method was modified by reducing the PAA/AMG incubation time from 16 to 4 hours and increasing the enzyme concentrations accordingly, ensuring that the resistant starch values obtained for a variety of reference materials were consistent with those obtained using AOAC methods 2002.02 (*McCleary et al.*, 2002) and 2009.01 and ileostomy data (*Champ et al.*, 1999). This update (*McCleary et al.*, 2015) successfully underwent interlaboratory evaluation under the auspices of AOAC International and ICC to become AOAC Method 2017.16 and ICC Method 185 (*McCleary et al.*, 2018). The method is directly applicable to various foods and food ingredients and is significantly faster than the previously used method (*McCleary et al.*, 2015). The developed method for resistant starch is an update of current procedures and incorporates incubation conditions with pancreatic α-amylase and amyloglucosidase (AMG) equivalent to those used in AOAC Method 2017.16 for total dietary fibre (*McCleary et al.*, 2019).

5. The nutritional value of resistant starch

Under experimental conditions, the energy value of RS is about 8 kJ/g (2 kcal/g). This is significantly lower than that of fully digestible starch, which is 15 kJ/g (4.2 kcal/g) (*Liversey*, 1994). Rapidly digestible starch leads to a rapid increase in blood glucose and insulin concentrations (*Englyst et al.*, 1999), while slowly digestible starch leads to moderate glycaemic responses. The same results were observed in pigs (*Van Der Meulen et al.*, 1997a; *Noah et al.*, 2000). It was found that within 4 hrs of a meal, blood glucose and insulin levels were higher in pigs consuming a rapidly digestible starch-containing diet than in diets containing corn starch and RS (*Van Der Meulen et al.*, 1997b; *Noah et al.*, 2000).

6. Short-chain fatty acids and resistant starch

Short-chain fatty acids (SCFAs) are metabolites of anaerobic bacterial fermentation and are formed during the degradation of polysaccharides, oligosaccharides, proteins, peptides, and glycoproteins in the colon. Substrates include those derived from dietary fibre and RS (*Andoh et al.*, 2003). The major SCFAs are butyric acid, propionic acid, and acetic acid although other SCFAs are also produced in smaller amounts (*Macfarlane & Macfarlane*, 2003). SCFA is a nutrient for intestinal epithelial cells in the colon, increasing blood flow, lowering pH, and helping to prevent the development of abnormal colon cell populations (*Topping & Clifton*, 2001). SCFAs are mainly found in the proximal colon, where fermentation intensity is the highest and their amount depends on the carbohydrate content of the diet (*Topping et al.*, 2003). The concentrations of SCFAs decrease as they pass through the colon due to absorption and utilization by colonocytes and bacteria. Any diet, any nutrient that increases the amount of SCFAs in the colon is beneficial to colon health, wherefore SCFAs are commonly used as markers of fermentation and colon health. The time spent in the colon and the composition of the diet have the greatest impact on the concentration and composition of SCFAs in the colon. Longer transit time increases the concentration of protein-derived SCFAs due to protein breakdown (*Macfarlane & Macfarlane*, 2003), while dietary fibre and the content of RS can alter the amount of SCFAs in the colon and stool (*Bird et al.*, 2000).

Butyric acid has been observed to inhibit inflammation directly by affecting the central regulation of many immune and inflammatory responses (*Segain*, 2000).

Resistant starch can increase SCFA production and thus improve intestinal function. Animal studies in pigs and rats showed that feeding RS increased total SCFAs and the concentrations of propionic acid, butyric acid, and acetic acid (*Ferguson et al.*, 2000; *Henningsson et al.*, 2003).

Following dietary supplementation with RS, most human research found increased faecal excretion and/or higher faecal concentrations (*Phillips et al.*, 1995; *Silvester et al.*, 1995; *Cummings et al.*, 1996; *Birkett et al.*, 2000; *Muir et al.*, 2004). Experiments show that RS2 (from raw potato starch) increases butyric acid concentration in humans and rats (*Cummings et al.*, 1996; *Ferguson et al.*, 2000; *Martin et al.*, 2000; *Henningsson et al.*, 2003), while the presence of RS3 (retrograded starch) increases the concentration of acetic acid in pigs (*Martin et al.*, 2000) but has no effect on the human body (*Cummings et al.*, 1996). It has also been observed that the composition of SCFAs changes only when microbes are given sufficient time to adapt during RS feeding (*Topping & Clifton*, 2001).

SCFAs stimulate the production of mucus as well as the pace of blood flow. They also provide acetyl-CoA, which is necessary for lipid biosynthesis and cell membrane formation as well as maintaining mucosal integrity. SCFAs appear to be important mediators of the positive effects of the intestinal microbiota, according to research. SCFAs also have a direct and indirect impact on risk factors for cardiovascular diseases through a range of tissue-specific pathways linked to intestinal barrier function, glucose homeostasis, immunological modulation, appetite regulation, and obesity (*Chambers et al.*, 2018).

7. Functions of resistant starch and colon

The role of starch, and colon cancer

Several studies examined the potential benefits of dietary fibre and starch in the fight against colon cancer, but there is little information on the effect of RS. Results suggest that RS enhances the beneficial effects of fibre on colorectal tumours. A large study in Europe found that in populations where fibre intake had doubled, the risk of colorectal cancer was reduced by up to 40% (*Bingham et al.*, 2003). *Cassidy et al.* (1994) found a strong negative association between starch intake and colorectal cancer in an international comparative study. Non-starch polysaccharides showed a significant positive correlation only in combination with starch. The authors hypothesized that 5% of starch is resistant and this RS contributes to its protective effect. This actually means that a significant amount of RS reaches the colon, as starch enters the digestive tract in amounts 8–10 times higher than non-starch polysaccharides (*Cassidy et al.*, 1994).

Through the creation of metabolites such as butyrate, a combination of living microbes and prebiotics has been proven to have cancer-preventive properties. Butyrate is produced when galacto-oligosaccharides are fermented (*Ambalam et al.*, 2016; *Thilakarathna et al.*, 2018), which inhibits metastasis and promotes death in colon cells. It is also known to increase the expression of enzymes involved in carcinogen inhibition (*Fernández et al.*, 2018). The clinical studies have shown the potential of synbiotics in reducing the proliferation rate, inflammatory state, and the use of antibiotics to prevent the occurrence of cancer (*Polakowski et al.*, 2019).

Resistant starch and colon functions

In animals and humans, the effect of RS on colon function has been studied. These studies focused on two main areas: the outcome of colorectal neoplasia and markers of bowel function, and colorectal cancer. Measurable features of colorectal neoplasia include tumour formation, tumour size and incidence of new diseases, cell proliferation, DNA adduct formation, presence of abnormal cryptocytes, and apoptosis. Maintenance of epithelial mass is important for the regulation of normal colon function and hyperproliferation (cellular overgrowth), which may increase the risk of developing colon cancer. Epithelial cell proliferative activity is considered an intermediate risk indicator for colorectal tumours (*Van Gorkom et al.*, 2002), but the exact utility of RS for colonic cell function is unclear, and results are often difficult to interpret. Other measurable markers of colorectal tumorigenesis and colonic function include production of SCFAs, particularly butyric acid, faecal pH, ammonia and phenol concentrations, faecal mass and yield, secondary bile acid excretion, faecal water volume, transit time, and activity of bacterial enzymes and microbial populations.

Increased SCFA production generally improved colonic function due to decreases in pH, ammonia and phenol production and secondary bile acid excretion, decreases in faecal water volume and transit time, and changes in bacterial activity. Low pH is expected to lower primary and secondary bile acid conversion rates as well as their carcinogenic effects. Low pH paired with high SCFA concentrations is thought to inhibit pH-sensitive pathogenic bacteria from overgrowing (*Topping & Clifton*, 2001). Phenol and ammonia are products of protein fermentation, and their reduced concentrations suggest that RS reduces protein degradation in the colon and possibly shortens transit time (retention time). The inhibition of certain bacterial enzymes (e.g. β-glucuronidase) may reduce the formation of toxic and carcinogenic metabolites from food and endogenous compounds (*Young & Le Leu*, 2004; *Yang et al.*, 2017).

The effects of resistant starch on colon function in animal studies

Animal studies thus suggest that RS has a protective effect on colon function, increases SCFA concentration, and lowers pH. The results are less clear in terms of tumour formation, size, cell proliferation, and DNA damage. The different results may be partly due to the animal models and the carcinogens used, but the results may also have been influenced by different types of RS (mainly RS2 or RS3) or even different diets. The effect of RS on colon function and colon cancer development in animals has been studied in pigs, mice, and rats, using previously experimentally induced colon cancer (mostly using dimethylhydrazine, azoxymethane) or colitis (dextran sodium sulphate) and genetic models of colon cancer. Rats and mice are more commonly used than pigs to study gut function, but it should be kept in mind that in mice genetically susceptible to colorectal cancer, the cancerous areas are predominantly in the small intestine rather than the large intestine, but the maximal benefits of RS fermentation are in the large intestine (*Young & Le Leu*, 2004; *Shen et al.*, 2017).

Dietary RS2 effectively lowers digesta pH throughout the colon and increases lactic-acid-producing bacteria in swine faeces, which may limit the growth of opportunistic pathogens in the hindgut (*Metzler-Zebeli et al.*, 2019).

The ability of resistant starch type 2 (RS2) – a dietary fibre made entirely of glucose – to promote metabolic and systemic health has been widely explored in human trials and animal models. These studies frequently incorporate assessments of RS2-mediated changes in gut microbiome composition and function since the health-modulatory effects of RS2 and other dietary fibres are assumed to be caused by changes in the gut microbiota (*Bendiks et al.*, 2020).

Table 3 shows the effects of resistant starch on colon function in animal studies. The protective effect of RS aberrant crypt foci (ACF) was observed in two animal studies (*Thorup et al.*, 1995; *Cassand et al.*, 1997). However, according to *Young et al.* (1996), raw potato starch (RS2) containing 20% carbohydrate (14.4 g/100 g diet) increased ACF density. This effect disappeared when RS was mixed with wheat bran (*Young et al.*, 1996).

Table 3. Animal intervention studies examining the effects of resistant starch on colonic function

Animal model studied	**Intervention**	**Measured parameter**	**Result**
Wistar rats (azoxymethane) (*Thorup et al.*, 1995)	The carbohydrate content of the meal can be replaced by: sucrose, corn starch, or RPS (RS2, 67g/100 g)	ACF	All RPS decreases and ACF increases
Sprague-Dawley rats (*Caderni et al.*, 1996)	Sucrose, glucose, fructose, corn starch, or HYLON VII (RS2)	Cell proliferation Bladder pH SCFA concentration in the caecum	NSD Decrease Decrease
Sprague-Dawley rats (*Sakamoto et al.*, 1996)	3 or 10 g/100 g of cellulose or 3 or 10 g/100 g of RS3 (high amylose-containing, hydrolysed corn starch pancreatin)	Occurrence of the tumour SCFA and butyric acid production Stool excretion	NSD Increased Increased
Sprague-Dawley rats (*Young et al.,* 1996)	Low RS, lower diet or 14.4 g/100 g dietary RPS (RS2) or 14.4 g/100 g RPS and 14.4 g/100 g wheat bran	Occurrence of the tumour Tumour size and variety ACF Cell proliferation Stool excretion	NSD It is growing Density increase It is growing
In mini-mice (*Pierre et al.*, 1997)	RS-free diet (2% cellulose without RS) or wheat bran (18.8 g/100 g) or RS3 (high amylose containing corn starch, 18.8 g/100 g)	Occurrence of the tumour	NSD

Animal model studied	Intervention	Measured parameter	Result
Sprague-Dawley rats (*Mazière et al.*, 1998)	RS-free and trend (2% cellulose) or 15 g/100 g RS3 (high amylose corn starch)	ACF Bladder pH Stool volume and efficiency Bacterial enzyme activation	Decrease Decrease growing β-glucuronidase activity is growing
Sprague-Dawley rats (*Cassand et al.*, 1997)	Retrograded high amylose-containing corn starch (RS3)	ACF Stool excretion Stool pH SCFA	Decrease It is growing Decrease Increased total and butyric acid content
Wistar rats (*Kleessen et al.*, 1997)	RPS or retrograded potato starch (RS2) 10 g/100 g	SCFA	SCFA is growing Butyric acid is growing
Wistar rats (*Ebihara et al.*, 1998)	Potato starch or CMS	Stool excretion Appendix SCFA Appendix bile acids	It is growing Decreases the butyric CMS and increases the CMS
Fisher rats (*Silvi et al.*, 1999)	RS- and cellulose-free food (2.1%) or retrograde amylose starch (15 g/100 g)	Appendix SCFA Bacterial enzyme activation Ammonia production Cell proliferation	Butyric acid is growing Decreased β-glucuronidase activity Decrease NSD
Mini-mouse (*Williamson et al.*, 1999)	RS- and NSP-free diet or 1:1 RPS (RS2) and high amylose corn diet (RS3)	Tumour presence	It is growing
Pigs (*Bird et al.*, 2000)	Brown rice or white rice and bran	SCFA selection Mass digested in the colon	It is growing It is growing
Wistar rats (*Ferguson et al.*, 2000)	RS- and NSP-free diet, or potato starch, or high amylose corn starch, or α-amylase-treated Hi-corn (35 g/100 g)	Stool excretion SCFA Transit time	It is growing It grows, including butyric acid It is growing treated with potato starch and α-amylase Hi-maize dosing
Sprague-Dawley rats (*Le Leu et al.*, 2003)	High amylose content of corn starch	pH	Decrease

Animal model studied	Intervention	Measured parameter	Result
Sprague-Dawley rats (*Conlon & Bird,* 2003)	10 g/100 g of fish oil or sunflower oil and 10 g/100 g of dietary fibre (wheat bran or cellulose) or 10 g/100 g of RS (hybrid or NOVELOSE)	Colon DNA damage	There is less DNA damage with RS / sunflower oil than with RS / fish oil combinations
Sprague-Dawley rats (*Toden et al.*, 2005)	15 or 25 g/100 g of casein 48% Hi-maize or without	DNA damage Thinning of the mucosal layer	Decrease Decrease

Source: *Young & Le Leu* (2004) and *Nugent* (2005)

Notes: ACF, aberrant crypt foci; CMS, chemically modified starch; RS, resistant starch; RPS, raw potato starch; SCFA, short-chain fatty acid; NSD, no significant difference.

Various forms of RS (especially RS2 and RS3) continuously increase the number of defaecations and stool weight (*Cassand et al.*, 1997; *Ebihara et al.*, 1998; *Mazière et al.*, 1998; *Ferguson et al.*, 2000; *Bird et al.*, 2000), decrease faecal pH and/or appendix pH (*Caderni et al.*, 1996; *Cassand et al.*, 1997; *Mazière et al.*, 1998; *Le Leu et al.*, 2003), reduce ammonia levels (*Silvi et al.*, 1999), and have a positive effect on bacterial enzyme activity (*Mazière et al.*, 1998; *Silvi et al.*, 1999). Feeding RS had no effect on tumour incidence in four animal experiments (*Young et al.*, 1996; *Sakamoto et al.*, 1996; *Pierre et al.*, 1997; *Mazière et al.*, 1998), decreased tumour incidence in other experiments, and resulted in an increase in tumour size, with increased cell proliferation also observed (*Young et al.*, 1996; *Williamson et al.*, 1999), while *Silvi et al.* (1999) reported no such effects.

It appears that feeding RS in combination with macronutrients can directly influence outcomes. According to *Conlon & Bird* (2003), it provided protection against DNA damage in male Sprague-Dawley rats fed RS (Hi-maize or Novelose) supplemented with 10% sunflower oil and 10% fish oil. Toden et al. (2003) showed that when male Sprague-Dawley rats were fed a high protein (15 or 25% casein) and RS (48% Hi-corn) diet, the diet containing RS alleviated colonic damage and thinning of the colonic mucosa in a high-protein diet.

8. Resistant starch and the colonic microflora; prebiotics and probiotics

Prebiotics were defined by *Gibson & Roberfroid* (1995) as "growth substrates" for potentially beneficial bacteria in the colon. Prebiotics are non-digestible food components that promote the growth and/or activity of bacteria in the colon, enhancing the health of the host (*Topping et al.*, 2003). By this definition, a resistant starch is a prebiotic that promotes the growth of probiotic bacteria when co-administered with synbiotics (*Brown et al.*, 1997; *Wang et al.*, 1999). Studies in humans and pigs showed that high RS food intake causes a time-dependent shift in the SCFA profile of faecal and colonic content, suggesting a change in the original (autochthonous) microbial population, and that RS can interact with gut bacteria (*Topping et al.*, 2003). RS probably functions differently from known prebiotics (e.g. fructooligosaccharides). When co-administered with fructooligosaccharides, faecal bacteria growth was higher than when administered alone (*Brown et al.*, 1997).

RS may act as a nutrient for *Bifidobacteria* in vitro (*Wang et al.*, 1999) and may also provide protection for these bacteria in vivo as they pass through the upper gastrointestinal tract (*Wang et al.*, 1999). In vitro studies have also demonstrated that different types of RS (RS2 and RS4) can nourish the *Bifidobacterium* strain (*Brown et al.*, 1997), which protects them from the physical effects of food preparation and storage (*Brown et al.*, 1997) and as they pass through the gastrointestinal tract (*Wang et al.*, 1999). Because of these protective effects, RS can be considered an adjuvant of intestinal flora (*Conway*, 2001). To exploit this effect, RS was combined with *Bifidobacteria* in yogurt (*Crittenden et al.*, 2001). RS may provide physical protection and slow down the rate of bacterial excretion as long as they are consumed. RS in combination with fructooligosaccharides was not observed to reduce the local number of bacteria (*Brown et al.*, 1997). Therefore, *Topping et al.* (2003) concluded that probiotics should not be consumed as frequently in combination with foods rich in RS or fructooligosaccharides.

Because of these prebiotic effects, RS also appears to exert other health-promoting effects in the intestinal tract. In the context of hydration therapy with RS, it has been observed to reduce fluid loss and halve recovery time when fed to people with cholera-induced diarrhoea, for example (*Ramakrishna et al.*, 2000). Similar effects were observed after feeding green bananas following diarrhoeal illness in children (*Rabbani et al.*, 2001).

It is believed that the use of RS has a greater benefit by improving fluid absorption due to higher SCFA production (*Topping et al.*, 2003). SCFA stimulates the absorption of water and cations (sodium, potassium, and calcium) in the proximal colon and may directly reduce the severity of diarrhoea by increasing muscle activity and stimulating blood flow in the colon. The beneficial effect may be triggered by the fact that RS impairs the viability of cholera bacteria in the intestine as these microbes, such as *Bifidobacteria*, can adhere to RS and thus be excreted in the faeces (*Topping et al.*, 2003).

9. Resistant starch and glucose metabolism

The hormone insulin regulates the uptake of glucose into muscle and fat cells, thereby lowering blood glucose levels. It inhibits the breakdown of body fat and can affect appetite and satiety. Glucose is released slowly from foods rich in RS, reducing the insulin response, aiding the breakdown of fat deposits, and satisfying hunger. These effects may help with metabolic diseases such as treating diabetes and decreased glucose tolerance, but probably also obesity. Several studies have been conducted on the effects of various forms and doses of RS on glucose concentration and insulin response, but a consensus on the exact effects of RS is still pending. In diabetic patients, improvement after consumption of a diet rich in RS was reported in 15 cases, while in 10 cases there was no effect, or the effect was physiologically irrelevant. On a positive note, however, no RS-induced adverse insulinaemia and glycaemic response has been reported to date. In general, beneficial effects occurred within a short period of time, after the first 2–8 hrs of ingestion, following consumption of foods high in RS (*Higgins et al.*, 1996).

Consumption of RS appears to reduce postprandial glycaemia only slightly, but is associated with a significant reduction in postprandial insulinaemia. It has been concluded that the proportion of RS should be at least 14% to show a beneficial effect on glycaemic or insulin response (*Higgins et al.*, 1996; *Behall & Hallfrish*, 2002; *Brown et al.*, 2003).

Intravenous glucose tolerance studies on rats have shown that digestible starch causes insulin resistance during 16 weeks of feeding, whereas feeding RS caused no such problems (*Higgins et al.*, 1996; *Byrnes et al.*, 1995; *Wiseman et al.*, 1996; *Snelson et al.*, 2019).

10. Conclusions

RS-related studies have been conducted on healthy animals (mainly on animal models such as pigs). The use of RS as a dietary ingredient may have beneficial effects on digestive system function by shortening the transit time of food in the colon and increasing the volume of stool. It has a positive effect on the bacterial activity of the colon by promoting the proliferation of beneficial microbes and reducing the activity of certain enzymes (β-glucuronidase).

RS has an indirect effect through SCFAs, which are important in maintaining colon function by regulating colonocyte gene expression, cell cycle, and apoptosis. Increased SCFA production lowers colon pH and stimulates bile acid secretion. Because secondary bile acids are cytotoxic to colon cells, the lower pH protects against colon cancer and slows the conversion of primary and secondary bile acids. SCFA acetates inhibit the breakdown of cholesterol and may reduce the bioavailability of free fatty acids because high concentrations of free fatty acids are harmful to the body since they reduce insulin activity.

Starchy foods significantly affect metabolism, blood glucose levels, and insulin response. Resistant starch, digestion-resistant starch, and starchy foods have several health benefits for gut function but appear to have less effect on lipid-glucose metabolism. Further studies are needed to understand the responses of RS to insulin and glucose in pigs. Most of the effects of RS are mediated by SCFAs, but the use of RS as a prebiotic has also become a focus of interest.

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