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Supplementation of prebiotics to a whey-based beverage reduces the risk of hypercholesterolaemia in rats

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ABSTRACT

Fructooligosaccharide-supplemented whey drinks may have potential as an interventional strategy for lowering cholesterol and triglyceride levels in dyslipidaemic conditions. A bioefficacy study was conducted in normal, hypercholesterolaemic and hyperglycaemic Sprague Dawley rats, running two consecutive trials for validity of results. Control drink (T_0), whey drink (T_1) and whey drink supplemented with 1.5% fructooligosaccharides (T_4), were given to each group. The functional drink caused a significant reduction in serum total cholesterol, low density lipoprotein (LDL) and triglycerides, and an increase in high density lipoprotein. The greatest decline in total cholesterol was induced by T_4 (10.9% and 12.0% in hypercholesterolaemic rats and 7.0% and 7.7% in hyperglycaemic rats, for trials 1 and 2, respectively). The decrease in total cholesterol by T_4 was mainly due to a pronounced decrease in LDL cholesterol (14.7% and 16.1% in hypercholesterolaemic rats and 7.4% and 8.1% in hyperglycaemic rats for trials 1 and 2, respectively).

1. Introduction

Fructooligosaccharides (FOS) are extensively used as prebiotics, synonymously known as oligofructose or oligofructan. These are the derivatives of inulin or polyfructose, a polymer of D-fructose linked by β (2 → 1) bonds with a terminal α (1 → 2) D-glucose. Hypercholesterolaemic individuals can be treated by cholesterol lowering drinks supplemented with FOS (Roberfroid, 2007). Numerous cohort studies have suggested that prebiotics possess hypocholesterolaemic properties. Many studies have also been performed in monogastric rodents owing to their resemblance with humans in terms of cholesterol and bile acid metabolism, circulating plasma lipoproteins and regulation of hepatic enzymes (Lin et al., 2000; Patterson, Lei, & Miller, 2008). The lipid lowering potential of prebiotics has convincingly been observed in an array of animal and human studies. These investigations have demonstrated that inulin type fructans decrease both fasting and postprandial plasma triglyceride levels, mainly due to decreases in very low density lipoprotein (VLDL) and triglyceride concentrations in the post-absorptive state (Busserolles, Gueux, & Rock, 2003). The principal hypotriglyceridaemic mechanism is reduction in liver lipogenesis through increased short chain fatty acids production in the bowel, primarily acetate, propionate and butyrate. The acetate and propionate arrive in the liver after absorption by the colonic mucosa. Acetate is used as a substrate for de novo cholesterol and triglyceride synthesis, whilst propionate exerts antagonistic effects and inhibits expression of lipogenic enzymes involved in cholesterol synthesis (Hosono et al., 2003). Therefore, the propionate/acetate ratio formed during the fermentation of prebiotics is important in determining their cholesterol lowering capacity (Pereira & Gibson, 2002).

Previously, whey was largely regarded as waste stream produced during cheese manufacture; however, its nutrition profile has now been well recognised. Recent investigations have explored whey protein as a source to reduce blood cholesterol level in rodent model studies. Increased low density lipoprotein (LDL) and triacylglyceride levels are likely to increase the risk of atherosclerosis; nevertheless, there is an inverse relationship between HDL cholesterol and atherosclerosis (Sharpe, Gamble, & Sharpe, 1994). Earlier, a study conducted on albino rats depicted beneficial effect of lactose-hydrolysed condensed whey on cholesterol levels (Beena & Prasad, 1997). In a similar investigation, it was observed that whey protein lowered the liver cholesterol level and also reduced

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plasma cholesterol concentration. Additionally, whey protein in comparison with casein appreciably reduced plasma cholesterol level (Groziak & Miller, 2000). The hypocholesterolaemic effect induced by supplementing whey protein in the diet is due to decreased LDL fractions. Whey peptides also hold antioxidant activity thereby improving coronary health (Harper, 2000).

Considering the above, the present research aimed to investigate the health claims of prebiotics, i.e., FOS supplementation in whey based functional drink through experimental animal model.

2. Methods

2.1. Procurement of raw material

Sweet liquid whey (Cheddar cheese) was procured from Noon Pakistan Limited (Sargodha, Pakistan). Refrigeration conditions were maintained during storage to ensure whey quality The whey was assessed for pH (5.42 ± 0.24) acidity (0.29 ± 0.01%), total solids (6.49 ± 0.31%), fat (0.25 ± 0.01%) protein (0.81 ± 0.03%), ash (0.56 ± 0.02%), and lactose (5.26 ± 0.26%) content, as previously described (Yasmin, Butt, Sameen, & Shahid, 2013). FOS were obtained from Organic Herbs (Changsha, China). For biological assay, diagnostic kits were purchased from Sigma–Aldrich, Bioassay (Bioassays Chemical Co., Munich, Germany) and Cayman Chemicals (Cayman Europe; Tallinn, Estonia).

2.2. Development of functional drinks

Whey drinks were formulated as described by Yasmin, Butt, Yasin, and Qaisrani (2014). The whey was first clarified through cheese cloth to eliminate curd particles, followed by the addition of citric acid, carboxymethyl cellulose (CMC) and aspartame (sweetener) were added. The resultant mixture was pasteurised at 65 °C for 30 min in a water bath. Next, preservative (sodium benzoate), food grade colour (orange red) and orange flavour were added to enhance the hedonic response of the product. Afterwards, the heated whey drink was cooled and homogenised at 7000 rpm with a laboratory scale homogeniser: this was designated as T_1. For the preparation of the functional drink, T_4, FOS was added at 1.5% to the formulated whey based drink. Likewise, control (T_0) was prepared following the same procedure except for the addition of whey and FOS (Yasmin et al., 2014).

2.3. Study design

The hypocholesterolaemic efficacy of three types drinks, i.e., control (T_0), whey-based drink (T_1) and whey drink supplemented with 1.5% FOS (T_4) was assessed in duplicate according to a completely randomised design. For the purpose, 100 male Sprague Dawley rats were procured from the National Institute of Health (Islamabad, Pakistan) and housed in the animal room of NIFSAT, University of Agriculture (Faisalabad, Pakistan). The experimental rats were acclimatized by giving basal diet for one week. The environmental conditions (temperature 23 ± 2 °C and humidity 55 ± 5%) were maintained throughout the study with 12 h light–dark periods. Before the start of the trial, some rats were sacrificed to obtain baseline values. Three studies were carried out in parallel using normal (study I), hypercholesterolaemic (study II) and hyperglycaemic (study III) rats. Each study was comprised of 30 rats, divided in three equal groups. In study I, rats were kept on their normal diet, whereas in study II and III, high cholesterol and high sucrose diets were given to the rats for 8 wk (Table 1). In addition, three types of drinks, i.e., control (T_0), whey-based drink (T_1) or a whey drink supplemented with 1.5% FOS (T_4) were given to groups of 10 rats in each study. The effect of the drinks on the blood lipid profile of each group of rats was evaluated at the end of the 8 wk intervention. Overnight fasted rats were decapitated and blood was collected in EDTA-coated tubes. The whole trial was repeated (2 trials were conducted) for the validation of results.

Table 1
Composition of experimental diets.

Ingredients (%)	Diet		
	Normal	High cholesterol	High sucrose
Corn oil	10	10	10
Corn starch	66	64.5	26
Casein	10	10	10
Cellulose	10	10	10
Salt mixture	3	3	3
Vitamins	1	1	1
Cholesterol	–	1.5	–
Sucrose	–	–	40

2.4. Serum lipid profile

Lipid profile, including total cholesterol, LDL, HDL and triglycerides, was determined with commercially available kits. Serum total cholesterol level was measured using the CHOD–PAP method, according to the protocol of Lee (2006). The HDL level was estimated by the cholesterol precipitant method, as described by Alshatwi et al. (2010), whereas LDL in serum samples was calculated from total and HDL cholesterol following the protocol of Lee (2006). Triglycerides were measured by the liquid triglycerides (GPO–PAP) method, as described by Kim, Seo, Kim, and Paik (2011).

2.5. Statistical analysis

The data were subjected to statistical analysis with statistical software Cohort version 6.1 (Co-Stat, 2003). Analysis of variance (ANOVA) was applied to compare the mean responses with the different drinks per trial for each of the studies (Steel, Torrie, & Dickey, 1997).

3. Results and discussion

In the present research, the impact of FOS supplementation was evaluated in normal, hypercholesterolaemic and hyperglycaemic rats with special reference to lipid profile, i.e., total cholesterol, LDL, HDL and triglycerides. There was no significant difference in total cholesterol between drinks in normal rats, whereas, significant differences were observed in hypercholesterolaemic and hyperglycaemic rats (Table 2). Means for cholesterol in hypercholesterolaemic rats exhibited the highest value for T_0 (control; 149.5 mg dL^{-1} in trial 1; 146.5 mg dL^{-1} in trial 2), whereas for T_1, slightly, but non-significantly lower (145.6 and 142.5 mg dL^{-1}) were observed. For T_4 (whey drink supplemented with FOS), significantly lower total cholesterol levels were observed (133.3 and 129.0 mg dL^{-1}) respectively. Likewise, in for hyperglycaemic rats, total cholesterol did not differ significantly between T_0 (98.0 and 96.0 mg dL^{-1} in trial 1 and 2, respectively) and T_1 (96.2 and 94.5 mg dL^{-1}), but was significantly lower for T_4 (91.2 and 88.9 mg dL^{-1}).

Statistical analysis indicated a significant effect of drink on LDL in hypercholesterolaemic and hyperglycaemic rats, with the FOS-supplemented drink (T_4) resulting in significantly lower LDL levels than the control (T_0) and whey (T_1) drink; however, only non-significant variations were noted in normal rats (Table 3). For hypercholesterolaemic, LDL values decreased from 62.4 to 60.1 mg dL^{-1} for T_0 and 60.5 and 58.2 mg L^{-1} for T_1, to 53.2 and