Efficacy assessment of prebiotic enriched camel milk along with various prebiotic combinations against metabolic syndrome using animal model

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Abstract
Metabolic syndrome (MetS) is a public health concern even in apparently healthy individuals. This study was conducted with the main objective to evaluate the potential role of prebiotic enriched camel milk (PECM) as a functional food, against different biomarkers of MetS in comparison to fresh camel milk (CM) and other prebiotic sources, i.e., chicory root powder (CRP) and galacto-oligosaccharides (GOS) in rat’s model. The MetS was induced through a high-fat diet and streptozotocin. The PECM, CM, CRP, and GOS were fed to rats for 8 weeks, and different biochemical parameters were measured on baseline (0th day), 20th day, and 40th day. Prebiotics were able to significantly reduce the concentration of low-density lipoprotein, triglycerides, total cholesterol, and glucose compared to the control group with PECM being the most effective of all groups, whereas liver enzymes (ALT, AST, and AP) were significantly reduced and antioxidants improved by CM and PECM than other prebiotics and control. Histopathology results revealed improvement in degeneration and hydropic atrophies in the hepatocytes of the treatment group. It is concluded that PECM is effective in the management of MetS biomarkers and can improve the parameters of hyperlipidemia, liver enzymes, antioxidants, and glycemic control.

Keywords: metabolic syndrome; prebiotic; antioxidants; hyperlipidemias; glycemic control.

Practical Application: Lactose in camel milk was converted to prebiotic galacto-oligosaccharides through an enzyme, and the novel product is effective in the management of MetS.

1. Introduction
The traditional way to manage diseases especially non-communicable diseases (NCDs) by using allopathy is fading away due to huge cost, side effects, as well as the rise of functional foods and nutraceutical concept. The functional food and nutraceuticals provide extra benefits to the host beyond basic nutrition (Roberfroid et al., 2010). Being organic and natural in origin, they can provide benefits in the prevention and management of various medical conditions without causing side effects. Therefore, the idea of nutraceutical and functional foods being novel can lead us to a new era of medicine and health (Sreeramya et al., 2018).

Metabolic syndrome (MetS) is a public health concern around the globe and is characterized by increased waist circumference, triglycerides, high blood pressure, fasting blood glucose, and decreased high-density lipoprotein cholesterol (McCracken et al., 2018). Major risk factors for MetS include age, gender, family history, genetics, menopausal status, and dietary and lifestyle factors. In developing countries, like Pakistan, it is highly prevalent even in apparently healthy individuals (Adil et al., 2023). If not addressed, it can lead to the development of cardiovascular diseases (CVDs), hypertension, and diabetes. Improved dietary approaches, including functional foods and nutraceuticals, can be introduced to manage the abovementioned syndrome (Pandey et al., 2019; Roberfroid et al., 2010).

Camel milk (CM) contains a high amount of iron, salts, immunoglobulins, and insulin-like factors. People with anemia, immunity issues, diabetes, and hypertension can consume the CM for all the aforementioned medical conditions (Sakandar et al., 2018). Various clinical trials have been performed to assess the effectiveness of CM consumption for the prevention of diabetes (Mohamad et al., 2009), hyperlipidemia (Elayan et al., 2008), and liver disorders. The protein content of CM ranges between 2.15 and 4.9%, fat 1.2 and 6.4%, lactose 2.4 and 5.8%, and overall mineral content 0.6 and 0.9%. The CM is also rich in vitamins A, D, and E and contains higher amounts of cyanocobalamin, folic acid, and pantothenic acid compared to bovine milk (Haddadin et al., 2008) as well as high amounts of immunoglobulins and lacto-peroxidases (Konuppayeva, 2020). This extraordinary composition makes CM a functional food. For lactose-intolerant individuals, it is very difficult to consume milk and other dairy products due to gastrointestinal problems. However, bioconversion of lactose to prebiotic galacto-oligosaccharides (GOS) using β-galactosidase (Iqbal et al.,...
2010; Iqbal et al., 2011; Raza et al., 2018) will not only reduce problems of lactose intolerant individuals but would also increase its nutritional value, and prebiotic enriched camel milk (PECM) may further improve the functional properties of the CM. Furthermore, the efficacy of prebiotic GOS depends on the degree of polymerization and complexity of glycosidic bonds in the product. The enzyme used in this study is sourced from Kluyveromyces lactis which produces $\beta$-(1-4) and $\beta$-(1-6) linkage while commercially available GOS mainly have $\beta$-(1-4).

Prebiotics are the non-digestible oligosaccharides that provide beneficial effects to the host when consumed in daily life by improving the growth of beneficial intestinal microbiota. Among prebiotics, GOS, fructo-oligosaccharides (FOS), and inulin have received much attention in the last few decades. The prebiotics provide benefits to the host in terms of immunity, anti-pathogenic activities, and prevention against various NCDs, including cancer, diabetes, and CVDs (Hill et al., 2014; Roberfroid et al., 2010). Probiotics, prebiotics, and synbiotics provide immunity and anti-diabetic, anticancer, anti-hypertensive, anti-inflammatory effects via the regulation of various neurological and immunological pathways (Pandey et al., 2019; Quigley, 2019). They are also involved in the production of short-chain fatty acids (SCFAs), including butyrate that can be used in gut remodeling providing regulation of gut barrier and facilitates the exclusion of pathogens (Hill et al., 2014). The results of trials suggested that prebiotics are effective in reducing the lipid profile (i.e., low-density lipoprotein (LDL), triglycerides, and cholesterol), markers of inflammation (i.e., nitric oxide, interleukins, tumor necrotic factor, etc.), and MetS (Quigley, 2019; Roberfroid et al., 2010).

The natural diet, mainly plant-based foods, provides fiber that is a rich source of prebiotics and can enhance the growth of probiotics and healthy gut bacteria. On the contrary, animal-based foods like milk are poor sources of fiber; however, milk sugars can be converted to prebiotic GOS. The transgalactosylation of the CM can also enhance the prebiotic value and can add more benefits to the consumer. Keeping in mind the high prevalence of MetS and the importance of functional foods like prebiotics, this study was conducted to answer the question that whether prebiotic GOS produced through transgalactosylation of lactose in CM are effective in the management of MetS biomarkers in comparison to other prebiotics in the animal model.

2. Materials and Methods

All chemicals, reagents, and standards were purchased from Sigma-Aldrich, Darmstadt, Germany, otherwise stated. Fresh CM was obtained from the institute research farm, while the ingredients for rat feed were purchased from the local market. Chicory roots were obtained from the local market followed by drying in an oven at 70°C and grinding in a kitchen grinder.

2.1. Animal trial

Male albino Wister rats of 6–7 weeks of age were purchased from the animal breeding center of the University of Veterinary and Animal Sciences, Lahore, and kept in an animal room at a controlled temperature (23±2°C), under a 12-h light and dark cycle. For the induction of MetS in rats, a high-fat diet (HFD) was prepared with 40% saturated fats, 10% protein (casein), 5% vitamin and mineral mixture, 15% sugar, 15% corn flour, and 15% maize starch. The rats were kept on HFD for 4 weeks, and afterward, a low dose of streptozotocin (45 mg/kg body weight) was administered intraperitoneally for the induction of diabetes.

2.2. Confirmation of metabolic syndrome

Anthropometric assessment (i.e., weight and length), blood chemistry analyses (i.e., blood glucose and lipid profile), and liver histopathology were randomly performed on six rats to confirm the induction of MetS before starting the intervention.

2.3. Prebiotic product development

The CM was transgalactosylated to produce PECM by using $\beta$-galactosidase enzyme from K. lactis at specific pH, temperature, and time. In enzymatic transgalactosylation, the enzyme transfers a nucleophile mainly a monosaccharide to lactose, resulting in a trisaccharide and so on. After pasteurization at 85°C for 15 s, milk was cooled to 45°C followed by the addition of 5 mL of the enzyme to start transgalactosylation, and the process was continued for 60 min with continuous agitation at 300 rpm in a closed container. After 1 h, the milk was heated to 90°C for 3 min for enzyme deactivation and again cooled to 4°C. A detailed scheme is mentioned in our previous study (Raza et al., 2018). In this study, ~70% lactose was transgalactosylated to produce ~10 g of GOS per liter of CM.

2.4. Study design

All rats with MetS were divided into five different groups (18 in each)—Group 1 (Control) with no intervention, Groups 2 and 3 received 6.30 mL/kg body weight of plain CM (CM group) and PECM, respectively, and Groups 4 and 5 received 10 g/kg body weight of chicory roots powder (CRP) and prebiotic GOS (Bimuno Daily by Bimuno, Berkshire, UK), respectively. All the rats received ad libitum basal diet and water throughout the study. The basal diet was prepared following the instruction described by Miller and Bender (1955). The doses for GOS, CRP, CM, and PECM were calculated using the human equivalent dose formula (HED (mg/kg)): Animal dose (mg/kg) × [(Animal Weight (kg)/Human Weight (kg))$^{0.13}$] (Reagan-Shaw et al., 2008). All the doses were updated weekly as per the mean weight of each group. The doses of interventions of Groups 2 and 3 were provided orally by tube-feeding methods for 6 weeks, whereas CRP (Group 4) and GOS (Group 5) were mixed in a basal diet. To equalize the stress due to dose administration, the control group and Groups 4 and 5 were administered with equal amounts of plain water throughout the trial. The blood chemistry analysis, lipid profile, and histopathology were performed at baseline (0th day), 20th day, and 40th day of intervention for all groups using six rats from each group.

2.5. Blood chemistry analysis

The blood was taken from rats through the heart puncture technique (Hoggatt et al., 2016) and collected in the yellow
clotting activator vacutainers for plasma and blood chemistry parameters. For blood chemistry analysis, glucose, insulin, liver enzymes, lipid profile including triglyceride, HDL, cholesterol, and LDL, antioxidants including catalase, glutathione peroxidase (GPx), and superoxide dismutase (SOD), kidney function tests, urea, and creatinine were analyzed using Human diagnostic kits (USA) following the methods given in the manual. The microplate reader (Synergy HTX Multi-mode reader; Company; Biotek, Country; USA) and the 96-well plate were used to measure the absorbances of plasma samples against various blood chemistry parameters.

2.6. Histopathology of multiple organs

After dissection of the rat, major organs, including intestine, liver, and pancreas, were separated and stored in neutral buffered formalin. Dehydration form of various concentrations of alcohol and pure xylene was used for tissue clearance. After clearing, the tissues were impregnated in paraffin wax. Tissue sectioning was performed using a microtome with a 5 mm thick blade, and then tissues were placed on a transparent glass slide after slight warming in a water bath. Hematoxylin and eosin staining was performed following the instructions of Bancroft and Gamble (2008). The critical evaluation of histopathology images was done by an experienced clinical pathologist, and the interpretations were recorded for all the groups with the intervention as well as the control groups.

2.7. Ethical consideration

All the methods and procedures opted in this study were approved by the animal ethical committee of the institute prior to conducting the trial, and an ethical certificate was issued.

2.8. Statistical analysis

All the collected and recorded data through the trial was entered and analyzed using Statistical Package for Social Sciences (SPSS; version 25, SPSS Inc., Chicago, IL, USA). Data are presented as mean and standard deviation using tables and figures. Complete factorial analysis was run against each parameter of blood chemistry analysis and anthropometric assessment to assess the effect of different interventions (treatment group), duration of the intervention (days), and their combination (treatment*days). The level of significance was set at 5%. For the significant parameters, Duncan's multiple range test was performed to assess the differences between the groups and days as a whole.

3. Results

3.1. Lipid profile of various groups at different time intervals

The HFD consumption disturbed the lipid profile: total cholesterol, triglyceride, and LDL were increased, while HDL was decreased (Table 1). The triglycerides, total cholesterol, and LDL levels were significantly reduced in all treatment groups as compared to control at the 20th and 40th days with maximum reduction in the PECM group for all three above-mentioned parameters followed by CM, prebiotic GOS, and CRP. At the end of the trial, HDL levels were significantly higher in the PECM group as compared to all other treatment and control groups (Table 1).

3.2. Glucose and insulin levels of rats fed with prebiotic combinations on different intervals

The day-wise data state a decreasing trend in glucose from the 0th day to the 20th and 40th days (p<0.05) in all treatment groups except the control. On the 40th day, a maximum reduction in blood glucose level was observed in the PECM group compared to the control and treatment groups, whereas GOS, CRP, and CM groups had significantly the same level of glucose on the same day but less than the control group (Table 2). The concentration of insulin was significantly increased with respect to different intervals of the trial as evidenced by p-value<0.05. The day-wise data showed an increasing trend in insulin from the 0th day to the 20th and 40th days. In the case of treatment groups, insulin levels were significantly higher in the CM group followed by the PECM and prebiotics groups. The control group without any treatment was having significantly lower levels of insulin (Table 2).

3.3. Catalase, GPx, and SOD of rats fed with prebiotics combinations on different intervals

The concentrations of catalase, GPx, and SOD were significantly increased in all treatment groups in comparison to the control.
control group on the 20th and 40th days of the trial. However, among all treatment groups, CM and PECM performed best and significantly improved all antioxidants at all study intervals after baseline, whereas other prebiotics CRP and GOS were less effective as evidenced by p-value<0.05 (Table 3).

### 3.4. Liver enzymes and kidney function parameters of different study groups

The ALT, AST, and AP levels (U/L) were significantly decreased with respect to different study intervals of the trial as evidenced by p-value<0.05. After 40 days of treatment with different prebiotic sources, ALT was significantly lower in the CM group followed by PECM, GOS, and CRP as compared to the control group. An exactly similar pattern was observed for the AST enzyme. However, in the case of AP, the GOS performed best, resulting in the lowest level followed by other treatments in comparison to the control at the completion of the study (Table 4). The urea and creatinine levels (Table 4) were significantly decreased due to treatment of various prebiotic sources and CM on the 20th and 40th days in comparison to the control group evidenced by p-value<0.05. Maximum reduction in urea on the 40th day was observed in the CM group while creatinine was observed in the PECM group.

### 3.5. Histopathology of hepatocytes, pancreas, and intestine of different treatment groups

Severe degeneration and hydropic atrophies can be observed in the hepatocytes of the control group. Treatment of various prebiotics showed improvements, while the PECM was most effective among treatment groups. Infiltration of inflammatory cells can be observed in the portal vein of hepatocytes from the control group which became attenuated in the other treatments best, resulting in the lowest level followed by other treatments.

#### Table 2. Glycemic control of rats fed on various treatments*

<table>
<thead>
<tr>
<th></th>
<th>0th Day</th>
<th>20th Day</th>
<th>40th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>159.2±2.09</td>
<td>188.6±2.9</td>
<td>230.8±4.55</td>
</tr>
<tr>
<td>CM</td>
<td>158.5±1.14</td>
<td>109.8±1.8</td>
<td>97.5±2.16</td>
</tr>
<tr>
<td>PECM</td>
<td>158.9±1.92</td>
<td>114.9±2.0</td>
<td>92.3±2.55</td>
</tr>
<tr>
<td>CRP</td>
<td>158.4±3.49</td>
<td>116.7±2.2</td>
<td>96.7±2.01</td>
</tr>
<tr>
<td>GOS</td>
<td>158.1±4.06</td>
<td>124.1±1.9</td>
<td>95.4±2.63</td>
</tr>
</tbody>
</table>

#### Table 3. Antioxidant levels of rats fed on various treatments*.

<table>
<thead>
<tr>
<th></th>
<th>0th Day</th>
<th>20th Day</th>
<th>40th Day</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.5±0.06</td>
<td>4.2±0.03</td>
<td>4.0±0.1</td>
</tr>
<tr>
<td>CM</td>
<td>4.5±0.08</td>
<td>7.4±0.19</td>
<td>10.1±0.16</td>
</tr>
<tr>
<td>PECM</td>
<td>4.5±0.06</td>
<td>6.8±0.03</td>
<td>9.4±0.24</td>
</tr>
<tr>
<td>CRP</td>
<td>4.4±0.10</td>
<td>5.2±0.04</td>
<td>7.2±0.04</td>
</tr>
<tr>
<td>GOS</td>
<td>4.4±0.09</td>
<td>6.5±0.12</td>
<td>7.2±0.09</td>
</tr>
</tbody>
</table>

#### Table 4. Liver enzymes and kidney function parameters of rats fed on various treatments*.

<table>
<thead>
<tr>
<th></th>
<th>0th Day</th>
<th>20th Day</th>
<th>40th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>121.9±1.74</td>
<td>122.5±1.65</td>
<td>131.1±2.86</td>
</tr>
<tr>
<td>CM</td>
<td>121.6±1.66</td>
<td>90.6±1.56</td>
<td>67.7±2.15</td>
</tr>
<tr>
<td>PECM</td>
<td>121.7±1.68</td>
<td>92.1±1.85</td>
<td>70.1±2.17</td>
</tr>
<tr>
<td>CRP</td>
<td>121.6±1.67</td>
<td>105.6±1.90</td>
<td>97.2±2.13</td>
</tr>
<tr>
<td>GOS</td>
<td>121.5±1.67</td>
<td>101.1±2.95</td>
<td>89.3±4.48</td>
</tr>
</tbody>
</table>

*Data are expressed as mean±standard deviation. Mean values having different superscript CAPITAL letters in a column on a specific day and small letters in a row for treatment are statistically different; N=6 for each treatment as well as for each day.

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prebiotic supplementation groups along with the prebiotic enriched CM group (Figures 1A–1E). Islets cells of the diseased control group (Figure 2A) were irregular in shape with unclear margins and few endocrine cells, while the prebiotic treatment groups showed normal round with clear boundaries and densely distributed endocrine cells (Figures 2B–2E). Several short, blunt-shaped villi and epithelial damage were seen in the small intestine due to MetS. There were intense fibrosis, infiltration, and cell inflammation in mucosal degeneration areas. Due to the preventive treatment of prebiotics, mucosa became smooth having long thin villi and Lieberkühn crypts on its bases. The villi surface was seen in normal histological appearance, and among them, prebiotic enriched CM was the most effective group (Figures 3A–3E).

4. Discussion

This study was conducted to assess the impact of fresh CM and various sources of prebiotics, including PECM-containing GOS, inulin-containing CRP, and commercially available GOS on the parameters of lipid profile, glycemic control, liver enzymes, antioxidants, and kidney function tests using an animal model of MetS. As the conventional methods for managing and preventing diseases, e.g., the use of drugs and medicinal agents comes with unwanted side effects, and therefore the idea of using natural bioactive components is gaining attention all over the world.

The results from this study revealed that fresh CM and transgalactosylated CM are very much effective in the reduction of triglycerides, total cholesterol, and LDL and can improve HDL and even perform better than pure prebiotic sources (Table 1). This is due to the hypolipidemic potential of CM (Isa et al., 2019), while newly produced prebiotic GOS have further added to it. This shows that CM consumption can be vital in delaying the progression of coronary heart disease.

A number of free oligosaccharides are available in mammalian milk which are not only important for the growth and development of offspring but also provide various benefits beyond basic nutrition. CM oligosaccharides named “medalose” are proven to be effective in resolving and managing dry piles, anemia, asthma, and diabetes (Gangwar et al., 2018). The results of this study showed that fresh CM consumption only is effective in the management of MetS biomarkers. Similar results were observed in a double-blind clinical trial conducted in the MetS adolescents where fermented CM consumption was found effective against glycemic control, hyperlipidemia, and insulin resistance without any significant change in inflammation biomarkers (Fallah et al., 2018a). This is most probably due to the presence of bioactive ingredients in fresh CM mainly immunoglobulins, pro-active proteins, and lacto-peroxidases which are heat labile and destroyed during the heat processing of CM (Konuspayeva, 2020).

The prebiotic GOS are fermented by lactobacilli and bifidobacteria, resulting in the production of SCFAs which reduces the pH of the gut and help in the growth of beneficial bacteria in the colon. These play crucial roles in decreasing blood glucose levels, mitigating insulin resistance, inflammation reduction, and stimulating the secretion of glucagon-like peptide 1 in the host, and this accounts for the observed decline of metabolic diseases. The daily consumption of prebiotics in a designed diet has a major effect on gut microbiota by reducing gut permeability, bacterial translocation, and reducing LPS-induced inflammation. This diet increases SCFAs and gut bifidogenicity.
and lowers TC levels, LDL, triglycerides, and adiposity, eventually resulting in lowering risk factors of MetS (Megur et al., 2022). Furthermore, the presence of insulin-like factors in CM and the low glycemic nature of GOS also help in the reduction of blood glucose and higher insulin level as compared to other treatment groups (Table 2).

Naturally, lipid-based encapsulated insulin, high minerals, and other functional ingredients in CM make it a potential therapeutic food against diabetes, dyslipidemia to cancer (Abrhaley & Leta 2018). In this study, the consumption of CM and PECM significantly reduced the concentration of glucose in the blood as well as increased the circulating insulin, especially fresh CM (Table 2), resulting in an improvement of the glycemic response of the MetS animal model.

The findings of a double-blind clinical trial conducted to assess the effects of fermented CM on lipid parameters and glycemic control revealed positive results against the parameters. The consumption of fermented camel has shown to reduce the liver enzymes, lipids, as well as weight of the participants in the clinical trial compared to the control group (Fallah et al., 2018b).

The supplementation of CM and PECM was associated with increased activity of antioxidants such as SOD, catalase, and GPX (Table 3) even after 20 days of treatment in comparison to the control and other prebiotic groups. It is proven that higher levels of reactive oxygen species are strongly related to cellular dysfunction, resulting in several chronic diseases, including diabetes, MetS, and hypertension (Kargar et al., 2021).

In another study, the hypolipidemic and antioxidant potential of CM was assessed using hyperlipidemic animals. The results revealed a significant reduction in liver enzymes (i.e., ALT, AST, and AP) as well as in urea and creatinine levels (Isa et al., 2019). In this study, fresh CM was the most effective group in lowering liver enzymes mainly ALT and AST in the MetS rat model after 40 days of study (Table 4).

Functional foods (e.g., pre- and probiotics and CM) enhance health effects, diminish the danger of a few illnesses (e.g., cholesterol-bringing down items), and could even be utilized for the management of severe chronic diseases such as obesity, heart diseases, diabetes type 2, and MetS. The CRP and pure GOS were also effective to reduce the parameters of MetS effectively compared to the control group; however, the effect of CM and PECM was more prominent. This might be due to the presence of other bioactive compounds in CM, glycosidic linkage, and degree of polymerization of GOS in PECM. The use of prebiotics and probiotics is becoming a pharmaco-nutrition-therapeutic target.

Prebiotics are beneficial in the rehabilitation of a healthy microbiome disturbed by dysbiosis related to the progression of MetS (Quigley, 2019). The prebiotics not only modulates the intestinal microbiota to healthy one, especially bifidobacteria, increases the production of anti-hypertensive peptides, SCFAs, and immunoglobulins, and increases fecal excretion of bile salts and cholesterol through physical binding but also decreases the production of various interleukins, mainly IL-6, IL-8, and tumor necrosis factor-α. All these factors improve satiety, appetite, and glycemic control and reduce insulin resistance and obesity, resulting in the management of MetS (Xavier-Santos et al., 2020).

The prebiotic and probiotic help in moderate pathological alterations in hepatic cells, renal tissues (cortex and medulla), and minimal histology changes of the intestinal mucosa with occasional areas of degeneration of villi and necrosis with loss of villi (Shahzad et al., 2022). This study also revealed that the consumption of CM and PECM reduces the fatty vacuoles and inflammation in hepatocytes as well as in pancreas and intestine (Figures 1–3).

5. Conclusion

The consumption of prebiotics, probiotics, such as GOS, FOS, and chicory roots, and dairy sources, including CM plain or after prebiotic enrichment, has a preventive effect on cholesterol, LDL, triglyceride, ALT, AST, AP, urea, and creatinine. Consumption of these functional foods can reduce the risk of various chronic non-communicable diseases, which can lead to a better and healthy increase in life expectancy and a decline in medical and hospital costs due to these diseases.

References


