Fermented Camel Milk Reduces Inflammation in Rats Fed a High-Fat Diet

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ABSTRACT

Fermented camel milk (FCM) is a probiotic with unique antibodies and medicinal properties. Regular consumption of a high-fat diet might lead to obesity. Obesity is associated with inflammation through the increased macrophage infiltration in adipose tissue along with production of inflammatory adipokines. Changes in dietary habits could reverse the proinflammatory changes in obesity. FCM could provide a beneficial effect on the inflammation associated with obesity.

Aim: This study examined the effect of camel milk fermented with two types of bacteria strains on inflammation associated with ingestion of a high-fat diet.

Methods: Twenty eight albino rats were randomly divided into 4 equal groups: G1: fed a normal diet (ND), G2: fed a high-fat diet (HFD), G3: fed a HFD and FCM with Lactobacillus acidophilus (ACID), and G4: fed a HFD and FCM with a mixture of Bifidobacterium bifidum and Streptococcus thermophilus (BIF), for 6 weeks. Body weight was determined weekly. Fasting blood samples were collected at the beginning and end of trial to determine the cytokines TNF-α, IL-1ß, IL-10, and C-reactive protein (CRP).

Results: Body weight significantly increased in both treatment groups compared to ND control group. CRP and TNF-α were significantly less than the level of the HFD group. No difference was observed in IL-1ß, IL-10 levels compared to HFD group.

Conclusion: Despite no decrease in BW was observed in treatment groups compared to HFD, a decrease in the inflammatory biomarkers CRP and TNF-α, might indicate a beneficial effect of FCM with the ACID, and BIF strains.

Keywords: probiotics, inflammation, camel milk, high-fat diet

INTRODUCTION

The interest and scope for research in the field of camel milk has significantly widened in recent years. Camel milk is extremely popular and is widely consumed in Saudi Arabia both as fresh raw milk and as soured milk. Saudi Arabia is rated the second world’s biggest camel milk producer after Somalia, with a yearly production of 89,000 tonnes. [1] Camel milk is slightly saltier than cows’ milk, three times as rich in Vitamin C and is known to be rich in iron, unsaturated fatty acids and B vitamins. [1] Moreover, the nonprotein-bound amino acids in camel milk are easily digested by microorganisms; therefore, camel milk has a higher metabolic activity when used as a starter culture preparation. [2]
Many beneficial properties are attributed to camel milk and to its fermented product called “shubat”. It is traditionally used for the treatment of tuberculosis, gastroenteritis, and many infections, and is also drunk as a tonic.\[3\] Research is also ongoing into the role claimed for camel milk in reducing diabetes and coronary heart disease.\[1\]

A high fat diet such as the Western diet has been shown to trigger inflammation by increasing the number of macrophages, cells associated with inflammation in the colon, as well as several proteins associated with inflammation.\[4\] A body of evidence suggests the presence of an overall, low-grade inflammation in obesity, with altered levels of several circulating factors such as an increase in the plasma levels of C-reactive protein (CRP), tumor necrosis factor-a (TNF-α), interleukin-6 (IL-6), and other biological markers of inflammation.\[5\] Changes in dietary habits could reverse the proinflammatory changes in the small intestine and the progression to obesity.\[6\]

Recent evidence suggests a compelling role for probiotics in their ability to moderate immune response and aid in decreasing inflammation. Fermented dairy products are generally considered to be one of the most suitable vehicles for the administration of an adequate number of probiotic microorganisms to the consumer.\[7\] Fermented camel milk is a probiotic rich dairy product. Probiotics are nonpathogenic live microorganisms that are believed to confer health benefits to the host when ingested.\[8\] Researchers have suggested weight loss and/or anti-obesity effects are among these benefits.\[9\] In the light of the link between the gut microbiota, metabolism, and immunity, the use of dietary strategies to modulate microbiota composition is likely to be effective in controlling metabolic disorders.\[10\] The anti-obesity effect of Lactobacillus rhamnosus (L. rhamnosus) PL60, a bacterium of human origin that produces conjugated linoleic acid was studied in diet-induced obese mice.\[11\] Hence, treatment of obesity might be achieved through modifying gut microbiota through the use of Probiotics. It is hypothesized that FMC could provide anti-inflammatory effects in rats fed high-fat diets. This study aims to examine the role of camel milk fermented with two types of bacteria strains, Lactobacillus acidophilus, or Bifidobacterium bifidum and Streptococcus thermophilus combination on body weight and obesity-induced inflammation resulting from ingestion of a high-fat diet.

**MATERIALS AND METHODS**

**Materials**

**Bacterial cultures**

Lyophilized cultures Lactobacillus acidophilus and a mixture of Bifidobacterium bifidum and Streptococcus thermophilus were purchased from Hansen laboratories, Denmark.

**Preparation of fermented camel milk**

Fresh camel milk was fermented according to the methods in references.\[12,13\]

**Diets**

The components of the normal and high-fat diets were locally purchased. The normal diet components percentage weight were as follows: Casein (20%), sucrose (50%), Corn Starch (15%), powdered cellulose (5%), corn oil (5%), mineral mix (3.5%), vitamin mix (1%), DL- Methionine (0.3%), Choline bitartrate (0.2%). The high-fat diet (HFD) components percentage weight were as follows: Casein (20%), L- Cystine (0.3%), Corn Starch (7.28%), Maltodextrin (10%), Sucrose (17.28%), powdered Cellulose(5%), Corn oil (2.5%), Beef fat (17.75%), mineral mix (1%), vitamin mix (1%), Dicalcium phosphate (1.3%), Calcium carbonate (0.55%), Potassium citrate 1 H₂O (1.65%), and Choline bitartrate (0.2%).
Biochemical tests

The kits for IL-1 ß, IL-10, C-reactive protein (CRP), and TNF-α were purchased from RayBiotech Inc. (Georgia, USA).

Animals

Twenty-eight adult male albino rats weighing (200-250g) were purchased from the Agricultural Research Center (Cairo, Egypt). Experimental procedures were conducted and approved by the Ethical Committee of the Agricultural Research Center. Seven rats were randomly chosen as a control group and were only fed a normal diet (ND) throughout the experiment. The remaining twenty-one rats were adapted on a high fat diet for 2 weeks. Those rats were then randomly divided into 3 groups (n=7 each) and fed the high fat diet for 42 days according to the following scheme: G2: fed a high-fat diet (HFD) without fermented milk and represented the positive control group (PO), G3: fed a HFD with camel milk fermented by Lactobacillus acidophilus (ACID), and G4: fed a HFD with camel milk fermented by a mixture of Bifidobacterium bifidum and Streptococcus thermophilus (BIF). Each group of rats was kept in a separate cage. The two types of fermented camel milk were administered daily (1 ml) via a gavage tube. All rats were allowed access to feed and water ad libitum. Rats were kept in the animal house at the center in polypropylene cages (six rats/cage) in a temperature-controlled room at 23 ± 1°C and relative air humidity 55–60% with 12\12 hour light-dark cycle. Food and tap water were provided ad-libitum. Rats’ body weights were recorded every week throughout the experiment. The weights of liver and intestines of each rat were determined at the end of the experiment.

Blood Analysis

Fasting blood samples (5ml) were withdrawn at baseline and at the end of the experiment. Rats were deprived from food overnight (12 hours) on the day before excision. They were anesthetized using diethyl ether. Blood was withdrawn from the abdominal aorta with capillary tubes. Serum was separated by centrifuge at 3000 r.p.m for 15 minutes and aliquots were stored at – 80°C until biochemical analysis.

Biochemical analysis

Serum concentrations of the interleukins IL-1 ß, IL-10, as well as C-reactive protein and TNF-α were determined by kits using enzyme-linked Immuno-absorbent assay (ELISA) according to the method reported in the manufacturer’s kit.[14] Briefly the assay for these kits employs an antibody specific for the rat cytokines: IL-1 ß, or IL-10, or TNF-α, or CRP coated on a 96-well plate. Standards and samples are pipetted into the wells and the cytokine present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-rat cytokine antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a 3,3’,5,5’- tetramethylbenzidine (TMB) in buffer solution is added to the wells and color develops in proportion to the amount of cytokine bound. The Stop solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Statistical Analysis

Data analysis was performed using the statistical package (SPSS) version 16. The mean ± standard deviation (SD) was determined for body weight and each inflammation biomarker. The Analysis of Variance (ANOVA) test was used to determine the difference between groups at the end of the experiment. The least significant difference (LSD) post-hoc test followed the ANOVA test to determine the probability of difference between body weight of the treatment and control groups, as well as the probability of difference in
body weight between the treatment groups themselves. The paired sample t-test was performed for each biomarker to determine the difference between pre-treatment and post-treatment concentration levels. Statistical significance was based on a two tailed P value (P<0.05).

RESULTS

Effects on body weight

The mean ± standard deviation (SD) of body weight (BW) over the six week period of the experiment is indicated for each group of rats in table (1). As the table indicates, BW increased significantly in the HFD control group (HFD) only compared to the normal diet control group ND after the first week (P= 0.05). Body weight also increased significantly after the second week in both the ACID and HFD groups (P=0.004) compared to the normal-diet control group (ND). After 3 and 4 weeks of intervention BW increased significantly in both ACID (P=0.006) and BIF (P=0.03) groups compared to the normal-diet control group (ND), but not to the HFD group. After the fifth week, no significant differences were observed between groups. At the end of the experiment, the increase in BW was significant in both ACID (P=0.02), and BIF (P=0.01) treatment groups compared to the normal-diet control group (ND) but not to the HFD group. As for organ weights, the ANOVA and LSD post-hoc test indicated a significant difference between the mean weight of intestines of the HFD (P=0.044) and BIF (P= 0.049) groups compared to the normal-diet control group (data not shown). No significant difference was observed for the mean weight of the liver among groups. The data in table (2) show the mean serum concentrations of the cytokines IL-1β, CRP, IL-10, TNF-α in rat groups at baseline and at the end of the experiment. To determine the variance between groups at the end of the experiment, the analysis of variance (ANOVA) test was performed, followed by Tamhane and LSD post-hoc tests to determine which groups showed significant differences compared to the control groups or if differences existed between groups. The mean difference was considered significant at (P≤0.05).

The ANOVA test, as shown in table 2, indicates that the TNF-α level significantly increased in the HFD control group compared to the BIF (P≤0.001), and the ACID groups, as well as to the normal diet control group. On the other hand, no significant difference was observed in the levels of interleukins IL-1β and IL-10 among all groups at the end of the experiment (figure 1). Also, as shown in table 2, the ANOVA test indicated a significant reduction in CRP level (P=0.001).
in the BIF, ACID, and the normal diet control group (ND) compared to the HFD control group (figure 2).

Table 2. Mean serum Cytokine levels in rat groups at the end of the experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>IL-1β (ng/ml)</th>
<th>CRP (pg/ml)</th>
<th>IL-10 (ng/ml)</th>
<th>TNF-α (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND</td>
<td>1025.25±242.40</td>
<td>6.50±4.32* (p&lt;0.001)</td>
<td>557.00±214.30</td>
<td>88.5±41.48* (p&lt;0.001)</td>
</tr>
<tr>
<td>HFD</td>
<td>1022.50 ±315.12</td>
<td>21.65 ± 7.49* (p&lt;0.001)</td>
<td>513.25±249.28</td>
<td>323.0±53.87* (p&lt;0.001)</td>
</tr>
<tr>
<td>ACID</td>
<td>702.75±112.96</td>
<td>6.28±1.94* (p&lt;0.001)</td>
<td>254.25±167.32</td>
<td>111.7±43.12* (p&lt;0.001)</td>
</tr>
<tr>
<td>BIF</td>
<td>718.00±278.14</td>
<td>7.05±1.76* (p&lt;0.001)</td>
<td>396.50±167.18</td>
<td>76.7±33.82* (p&lt;0.001)</td>
</tr>
</tbody>
</table>

Data represent Mean ± SD. * Mean difference is significant at (p<0.05) compared to the control group (HFD) as determined by ANOVA at the end of the experiment. Abbreviations: NA= normal-diet fed (n=7), HFD = HFD control (n=7), ACID= Lactobacillus acidophilus (n=7), BIF= bifidium+ strep.therm (n=7)

Figure (1) Mean serum concentrations of TNF-α, IL-1B, IL-10 for the negative control (NA, n=7), (PO, n=7) positive control, (ACID, n=7) Acidophilus treatment, and (BIF, n=7) bifidiobacteria treatment groups as determined by ANOVA test. * represents significant difference (p<0.001) compared to HFD control group at the end of the experiment. Abbreviations: NA= normal-diet fed (n=7), HFD = HFD control (n=7), ACID= Lactobacillus acidophilus (n=7), BIF= bifidium+ strep.therm (n=7)

To determine if treatments resulted in a significant change in the inflammation biomarkers at the end of the experiment compared to baseline, the dependent sample t-test was performed for each group. The CRP level increased significantly in the HFD (p=0.024) control group at the end of the experiment. Whereas, in both the ACID and BIF groups, CRP level significantly decreased (p=0.01, p=0.02) respectively at the end of the experiment compared to baseline (figure 3).

Figure (2) Mean serum CRP levels for the negative control (NA, n=7), (PO, n=7) positive control, (ACID, n=7) Acidophilus treatment, and (BIF, n=7) bifidiobacteria treatment groups as determined by paired t-test. Abbreviations: NA= normal-diet fed (n=7), HFD = HFD control (n=7), ACID= Lactobacillus acidophilus (n=7), BIF= bifidium+ strep.therm (n=7)
TNF-α significantly increased (p=0.001) only in the HFD control group at the end of the experiment compared to baseline. However, no significant change was observed in the BIF and ACID treatment groups or in the normal diet control group (ND) compared to baseline (figure 4). Also, IL-1β significant decreased in both the BIF and ACID groups (p=0.02) at the end of treatment compared to baseline level (figure 5). For, IL-10, the concentration significantly decreased in the ACID treated group (p=0.035), and the HFD control group (p=0.05) at the end of the experiment compared to baseline concentration levels. However, no change occurred in the BIF treatment group, or in the normal diet control group (ND) (figure 6).

**DISCUSSION**

This study aimed to examine the role of fermented camel milk, enriched with two types of probiotics, in the modulation of body weight, some organ weights, and inflammation biomarkers associated with high-fat diet.

Evidence suggests that the metabolic activities of the gut microbiota facilitate the extraction of calories from ingested dietary...
substances and help to store these calories in host adipose tissue for later use.\[15\] Manipulation of the gut microbiota by diet, antibiotics, or probiotics could promote, prevent, or reverse the development of specific diseases, including obesity.\[16\] It is worth to note that most previous studies have focused on camel milk, while intervention studies using fermented camel milk is lacking.

The high-fat diet administered in this study resulted in increased body weight. In accordance with this result, the study of Serino et al., (2011) \[17\] indicated that a high-fat diet fed to mice caused a modulation in gut microbial profile which was associated with increased gut permeability linked to increased endotoxemia and to a dramatic increase in cell number in the stroma vascular fraction from visceral white adipose tissue. The effects of probiotics vary from one strain and another. Among commensal bacteria, bifidobacteria is one of the most numerous probiotics in the mammalian gut and are a type of lactic acid bacteria.\[18\] The administration of FCM with the Lactobacillus strain (ACID) and streptothermophilus-bifidium combination (BIF) resulted in a significant increase in body weight compared to that of the normal diet group after three, four, and six weeks of intervention. This is not in accordance with the findings of previous studies which reported anti-obesity effects of some bacterial strains such as Lactobacillus spp. and Bifidobacterium spp.\[11,18\] Takemura et al., (2010) reported that Lactobacillus plantarum strain No. 14 (LP14) exert a beneficial effect on the onset of diet-induced obesity by reducing the cell size of white adipose tissues of female C57BL/6 mice fed either normal, or high-fat diet.\[18\] Another probiotic strain, bifidobacterium breve B-3, administered to mice fed a high-fat diet supplemented with at 10(8) or 10(9) CFU/d for 8 weeks had been suggested to be effective in reducing the risk of obesity.\[19\] The production of conjugated linoleic acid (CLA) by a metabolically active Bifidobacterium strain in the mammalian gut has been shown to be associated with alterations in fatty acid composition of other regions in the body, including the liver and adipose tissue.\[20\] However, despite that the probiotic strains used in this study did not lead to reduction in body weight, the mean BW of the treatment groups did not differ from that of the HFD control group, and did not exceed it. The ineffectiveness of FCM treatments to reduce body weight of rats might imply that the dose administered might have not been sufficient to exert weight reduction and probably treatment with a higher dose might be more effective. The studies where probiotics exerted weight reduction might be attributed to the use of a higher dose, or to the difference in types of strains used, or to the duration of trial. One limitation of this study is that it did not compare FCM with fermented bovine milk using the same dose and strains of probiotics.

In their study to assess the anti-obesity and lipid-lowering effects of Bifidobacterium spp. on high fat diet-induced obese rats, An et al., (2011) showed that administration of LAB for 5 weeks reduced body and fat weights suggesting that Bifidobacterium spp. used in their study may have beneficial anti-obesity effects.\[21\] Tanida et al., (2008) showed that Long-term ingestion of the lactobacillus strain Lactobacillus paracasei ST11 (NCC2461) reduced body weight and abdominal fat weight in high-fat fed rats.\[22\] Researchers suggested that the NCC2461 affects autonomic nerves, enhances lipolysis, and reduces body weight. Although the majority of the studies published so far have assessed the effects of dietary fat, additional studies are necessary
to deepen the understanding of how the amount, the quality and the structure of the fat may affect endotoxemia.\[^{23}\]

A strong association between obesity and inflammation has been identified a little more than a decade ago when it was first discovered that TNF-\(\alpha\) inflammatory cytokine is overexpressed in the adipose tissues of rodent models of obesity.\[^{24}\] Also, in obese humans, circulating and adipose derived cytokines such as tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) or interleukin-6 (IL-6) have been shown to be elevated.\[^{25,26}\] Probiotics have been shown to modulate gut bacteria and thus enhance the anti-inflammatory effects of high-fat diet. In this study, the significant reduction observed in CRP and TNF-\(\alpha\) levels after the administration of both ACID and BIF enriched FCM at the end of the experiment, indicates a beneficial effect of these strains in reducing inflammation induced by the high-fat diet. TNF-\(\alpha\) has been shown to be over-expressed in white adipose tissue (WAT) from different animal models of obesity, and is considered to be a molecule that makes a link between inflammation and obesity.\[^{5}\] Finding of this study is consistent with studies where diverse strains of probiotics administered to animals and humans have been shown to cause alterations in body fat as well as on inflammation through modulation of gut microbiota.

In the small intestine, bacteria and high-fat diet interact to promote proinflammatory changes, which precede weight gain and obesity and show strong and significant associations with progression of obesity and development of insulin resistance.\[^{27}\] The manipulation of the gut microbiota by diet, antibiotics, or probiotics could promote, prevent, or reverse the development of specific diseases, including obesity.\[^{16}\] It has been postulated that imbalances in gut microbiota and increased bacterial lipopolysaccharide derived from the intestinal microbiota may act as a triggering factor linking inflammation to high-fat diet-induced metabolic syndrome.\[^{10}\] Probiotics have been shown to modulate gut bacteria and thus enhance the anti-inflammatory effects of high-fat diet. This has been supported by the idea that a selective increase of \textit{Bifidobacterium} spp. reduces the impact of high-fat diet-induced metabolic endotoxemia and inflammatory disorders.\[^{29}\] This might explain why treatment with BIF in this study resulted in a reduction in both TNF-\(\alpha\) and CRP levels. Despite that no change in IL-10 level occurred with the administration of ACID and BIF treatments in this study, Chen et al., (2010), in contrast to this result, demonstrated that consumption of milk fermented with Lactobacillus helveticus R389 was able to attenuate the symptoms of acute inflammation and increased regulatory cytokine IL-10-producing cells, leading to desirable changes of the intestinal microbiota.\[^{30}\] HF feeding of rats has been shown to cause significantly high levels of the plasma lipopolysaccharide, interleukin-1\(\beta\) and intestinal myeloperoxidase, as well as intestinal inflammatory activity index. These parameters were normalized to the control levels in high-fat-\textit{Bifidobacterium longum}-treated rats compared with the normal chow-fed control rats.\[^{30}\]

**CONCLUSION**

The administration of fermented camel milk enriched with \textit{Lactobacillus Acidophilus} or \textit{Bifidobacterium bifidum} and \textit{Streptococcus thermophilus} combination strains did not result in weight reduction compared to the control group fed a HFD alone. However, both FCM treatment groups caused a significant decrease in TNF-\(\alpha\) and CRP levels compared to the HFD control group. This indicates a beneficial effect since these two cytokines are associated...
with increased inflammation in case of obesity induced by a high-fat diet.

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