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Feeding Bifidus Milk Beverage: Effects on Albino Rats after Ketogenic Diet

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KETOGENIC diet is one of the most famous diets due to its ability to reduce weight quickly. With the high cost of healthy fats, there is a tendency to use more harmful fats in what is known as the dirty ketogenic diet. This study was conducted to evaluate the role of *Bifidobacteria* in reducing the potential negative effects of the ketogenic diet. Thirty-five experimental male albino rats were acclimated for two weeks and then divided into 5 equal groups. Groups were fed as follows: G1 negative control, a healthy balanced diet for 12 weeks; G2: positive control, ketogenic diet (3 fat: 1 protein) for 8 weeks and then a balanced diet for 4 weeks; G3, G4 and G5: fed as G2 plus 2 mL Bifidus milk inoculated with *Bifidobacterium angulatum* 2238, *Bifidobacterium animalis* subsp *lactis* BB12 and *Bifidobacterium bifidum* LMG 10645, respectively. G2 showed a decrease in weight gain and an increase in liver index compared to G1. Rat groups fed with Bifidus milk (G3, G4 and G5) triggered an improvement in liver index. G4 showed significant recovery in liver enzymes, and antioxidant biomarkers, as well as lipid profile, and showed a lower atherogenic index. In conclusion, consumption of Bifidus milk during and after the ketogenic diet become a more secure way to apply this diet with low liver index, liver enzymes, and lipid profile.

Keywords: Bifidus milk, Ketogenic diet, Lipid profile, Liver enzymes, Atherogenic index

Introduction

The ketogenic diet (KD) is a diet that focuses on significantly increasing fat intake and reducing carbohydrate intake, leading to a state of ketosis. This diet has gained great popularity due to its effects on promoting weight loss, reducing dyslipidemia and increasing insulin sensitivity (Attaye et al., 2022). KDs are widely utilized as long-standing methods of attenuating a variety of medical problems (Gasior et al., 2006). In addition, it could have the ability to inform future therapies for various human diseases, including pathologies associated with both metabolic disorders and autoimmunity (Le and Johnson, 2020). For example, KD is indicated as a first-line therapy for children with pyruvate dehydrogenase deficiency or glucose transporter deficiency (GLUT-1). In these cases, the usage of energy sources other than glucose, for brain metabolism, can prevent seizures by providing acetyl-CoA directly into the tricarboxylic acid (TCA) cycle without prior glycolysis. In these conditions, the diet is not only beneficial but also lifesaving (Sinha and Kossoff, 2005).

The widespread use of high-fat KD in the treatment of obesity and the role of nonalcoholic fatty liver disease (NAFLD) and hepatic insulin resistance in promoting type 2 diabetes possibly be linked to the rise in energy expenditure, which results in weight loss. Obese patients on such diets could lose weight but develop NAFLD and hepatic insulin resistance, which could be attributed to the increase in hepatic diacylglycerol content, leading to protein kinase Cε activation

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and subsequent impaired insulin signaling (Jornayvaz et al., 2010).

Furth et al. (2000) and Kossoff et al. (2002) reported that 5 to 6% of children have the incidence of renal calculi on the ketogenic diet. At the same time, the diet can cause urine acidification, hypercalciuria, and hypocitraturia, increasing the risk of uric acid and, less commonly, calcium phosphate and oxalate stones (Sinha and Kossoff, 2005). Also, hyperlipidemia is another known side effect of KD (Sinha and Kossoff, 2005). Where a study of children on the classic KD diet showed significant elevations of total cholesterol, triglycerides, and atherogenic apolipoprotein B containing lipoproteins (LDL and VLDL) (Sinha and Kossoff, 2005). Also, bacteria taxa, richness and diversity are strictly influenced by the KD in the human gut (Paoli et al., 2019).

In a previous study on epileptic children supplemented with KD, authors found a significant diminishing of the beneficial bacteria like *Bifidobacteria*, *E. rectale* and *Dialister*, which are linked with health-promoting benefits such as irritable bowel syndrome, the prevention of colorectal cancer, and necrotizing entercolitis (O'Callaghan and van Sinderen, 2016). In the same study, the authors observed a relative abundance of *Actinobacteria* and *Escherichia coli* (Lindefeldt et al., 2019), which its expansion in patients during KD might be of concern for general gut health because of its association with a variety of chronic intestinal diseases, including inflammatory bowel disease (IBD) (Singh et al., 2015). Also, studies have shown that ketone bodies directly inhibit the growth of gut bacteria (Ang et al., 2020). In this case, Tagliabue et al. (2017) suggested using additional supplementation containing prebiotics or probiotics to maintain the "ecological balance" of the gut microbiome. The use of probiotics during the ketogenic diet may be a proper suggestion for maintaining a healthy gut microbiota. Many researches confirmed their positive benefits, e.g., improving immune function at intestinal and peripheral sites in aging (Finamore et al., 2019) and reducing seizure-induced neurological disorders (Bagheri et al., 2019). Since *Bifidobacterium* shows the greatest reduction in KD (Ang et al., 2020), hence the suggestion was to study the use of Bifidus milk in this experiment.

This biological experiment was conducted to evaluate the role of feeding Bifidus milk inoculated with three different strains of *Bifidobacteria* in reducing the potential negative effects of the ketogenic diet on a group of rats and to study the extent of how they affect and to know their health indicators.

Materials and Methods

Materials

Startres

Freeze-dried cultures of *Bifidobacterium* were obtained from MIRCEN (Ain Shams University, Cairo, Egypt). The strains were *Bifidobacterium angulatum 2238, Bifidobacterium animalis* subsp *lactis BB12* and *Bifidobacterium bifidum LMG 10645*.

Chemical composition of milk

Fresh cow milk was collected from the farm of the Faculty of Agriculture, Minia University. Moisture, fat, protein, specific density, solid-not fat (SNF), total solids (TS) contents, titratable acidity and pH were determined according to AOAC (2016).

Manufacture of Bifidus milk

Bifidus milk was manufactured according to the method as described by Nagawa et al. (1988). The different strains of *Bifidobacterium* were added to the milk as 3%. The mixture was then incubated at 37 ºC for 24 hr.

Methods

Growth media, activation and counting of Bifidobacteria

For activation of *Bifidobacteria sp.* strains, MRS broth (Biolife Italiana, Milano, Italy) supplemented with 0.05% (w/v) L-cysteine-HCL as a reducing agent was used. For counting bifidobacteria in Bifidus milk, the same media was used with the addition of agar at 18 g/L (EL-Saleh et al., 1998). The inoculated tubes and plates were incubated at 37 º C for 48 hr at anaerobic conditions.

Experimental design and feeding treatments

According to OIE regulations for using animals in research, the ethical committee of the Faculty of Agriculture at Minia University in Minia, Egypt, accepted this work. The ethical approval numberis (MU/FA 0160523). Male Sprague-Dawley rats (170 to 200 g) were purchased from the Egyptian company for drugs and veterinary vaccines (Vacsera, Helwan, Egypt). The rats were in the growth phase. They were kept under normal healthy conditions (17-22 °C) and fed on rat chow for two weeks as an acclamation period. After adaptation, thirty-five rats were randomly divided

equally into five groups, G1 group was the negative control which fed on a healthy balanced diet for 12 weeks. G2 group was the positive control which fed on the ketogenic diet for 8 weeks and turned into a balanced diet (composed of 30 percent of the metabolizable energy from fat) for 4 weeks. G3, G4 and G5 were fed as G2 plus a dietary supplementation of Bifidus milk once daily (2 mL of Bifidus milk, 10^{10} CFU/mL of Bifidobacteria). The strains of Bifidobacteria given to the rat groups were *Bifidobacterium angulatum2238, Bifidobacterium animalis* subsp *lactis BB12* and *Bifidobacterium bifidum LMG 10645* for G3, G4 and G5, respectively. Water and diets were presented to the rats *ad libitum*. The ketogenic diet composed of 1:1 saturated fat to unsaturated fat), and 70% of the metabolizable energy was from fat (3 fat, 1 protein). Body weight was recorded at the end of the experimental period.

Collection of samples

At the end of the experimental feeding period, animals were fasted for 16 hours and then sacrificed under deep anesthesia. Blood samples were collected individually from the jugular vein of each rat in tubes containing 6% EDTA and empty tubes, the plasma and serum fractions respectively were immediately collected and stored under frozen at -20˚C until it was assayed. The liver was collected from each rat, weighed, and stored at –80˚C.

Blood analysis

Lipid profile, Serum total cholesterol was determined according to Allain et al. (1974), high-density lipoprotein (HDL) was determined according to the methods of Wieland and Seidel (1983) and triglycerides (TG) was determined according to Fossati and Prencipe (1982), Very low-density lipoprotein-cholesterol (VLDL-c) was estimated by Friedewald et al. (1972) as follows:

$$\text{VLDL-c} = \text{Triglycerides} / 5.$$

The liver enzyme activities, alanine aminotransferase (ALT) (U/l) and aspartate aminotransferase (AST) (U/l) were determined according to Alan (2006). Catalase and super oxidase dismutase (SOD) were estimated as described by Aebi (1984) and Nishikimi et al., (1972) as oxidative stress biomarkers following the manufacturer instructors provided by Biodignostic chemical company (Egypt).

Liver index was calculated as liver weight / final body weight × 100. Atherogenic index was calculated according to Zhu et al. (2018) with the following equation:

$$\text{The atherogenic index} = \text{Log10}\left(\frac{\text{Triglycerides}}{\text{HDL- cholesterol}}\right)$$

The tail vein blood glucose levels were measured using a portable device (Accu-Chek Aviva Nano System, Roche Farma, S.A., Barcelona, Spain).

Statistical analysis

Each evaluation was carried out a minimum of three times. Means and standard errors were calculated. Data were analyzed by one-way ANOVA analysis followed by the Tukey test for multiple comparisons as a post-test using GraphPad Prism 5 analysis software (Motulsky, 1999).

Results and Discussion

Many people who follow the ketogenic diet resort to using harmful fats in their daily diet, either due to the lack of availability of healthy fats or their high cost. These fats are characterized by a high percentage of saturated fatty acids, and this system is known as the dirty ketogenic diet. A high proportion of saturated fatty acids in diets negatively affects the gut microbiota and decreases bacteria richness (Paoli et al., 2019). The use of probiotics is an appropriate suggestion for maintaining a healthy gut microbiome during the ketogenic diet. Probiotics are live bacteria (especially from the genera *Bifidobacterium* and *Lactobacillus*) that show a positive effect on human health when consumed in adequate amounts; they are usually served as fermented dairy products e.g., yoghurt and Bifidus milk. Foods rich in these microorganisms can restore and improve the intestinal microflora, reaching the state of eubiosis that defined as balance in the microbiota community which is the opposite of dysbiosis (Tagliabue et al., 2017).

The chemical composition of milk used in the study

Data in Table 1 presents the chemical composition of milk used in making Bifidus milk. The average values of the composition of milk was in the normal renge for cow milk. Similar results reported by Hamad and Baiomy (2010) who found that the pH values of fresh cow milk ranged from 6.41 and 6.79 with an average of 6.65±0.191, and the specific density renged from

1.028 to 1.034 with an average of 1.0312±0.0008. Also, they found that SNF was 8.53±0.8% and TS was 12.81±0.24%.

The chemical composition of Bifidus milk

Figure 1 presents the pH values of different Bifidus milk types with three strains of *Bifidobacterium* sp.after 24 hr of inoculation. The results showed that the value of pH was higher in the milk with *Bif. angulatum* (5.47) compared to the Bifidus milk made with *Bif. animals* and *Bif. bifidum* which were 4.02 and 4.67, respectively. Regarding the composition of the Bifidus milk, the values of pH in different Bifidus milk types in this study were in the normal range as obtained in previous studies. Slačanac et al. (2005) found a higher rate of acidification resulted in a faster decrease in pH value in cow milk as a result of the growth of *Bifidobacterium longum* Bb-46 in the milk.

Glucose level of the experimental groups of rats

Results in Fig. 2. showed that there were no differences (P>0.05) between the experimental groups in the levels of glucose. All the values were in the normal range, however, the higher value (79.33±3.48 mg/L) appeared inG3 that fed on a Ketogenic diet and then supplemented with *Bifidobacterium angulatum.* The lower value (70.0 ±2.89 mg/L) appeared in G1, the negative control group. The results for G2, G4 and G5 were 77.67 ±1.202, 73.67 ±1.856 and 78.67±1.453mg/L, respectively. Several studies suggested the positive role of *Bifidobacterium* in the improvement of blood glucose levels. These may refer to the modulation of intestinal microbiota and restoring microbial balance or the enhancement of the inflammatory state (Esposito et al., 2009). The absence of differences in glucose levels in our study between treatments was similar to a previous study by Van Syoc et al. (2023) who noticed that *Bifidobacterium* alone may have minimal effects on glycemic control, however, this effect may be more effective when combined with multiple probiotic species, or may be more effective in conditions of hyperglycemia rather than elevated fasting blood glucose concentrations. In addition, the lack of fasting hyperinsulinemia or hyperglycemia in rats fed the keto diet would indicate increased whole-body insulin sensitivity, which explains the role of this diet in reducing blood glucose in general (Jornayvaz et al., 2010).

Impact on body weight and liver index of rats

Results in Table 2 present the body weight and the liver index of rat groups. As expected, there were no differences (P>0.05) between rat groups for the initial weight at the beginning of the experiment, on the other hand, significant differences (P≤0.05) appeared by the end of the experiment for the positive control group (G2) which had the lower body gain (0.782±0.071%). By supplementation with Bifidus milk, there were significant differences (P≤0.05) for the G4 and G5 groups (1.092±0.081 and 1.143±0.054%, respectively) compared to the G2 group, however, G3 (0.911±0.023%) did not show a difference (P>0.05) in weight gain in comparison with G2. A recent trial in obese patients reported that following a very low-calorie ketogenic diet for four months has the potential to induce rapid weight loss (Ferraris et al., 2021). Additionally, this diet was linked to a rise in the diversity of the gut microbiota, a decrease in Proteobacteria, and an increase in the Firmicutes phyla (Attaye et al., 2022).

TABLE 1. Chemical composition of milk .

Items	Average
pH	6.61
Acidity%	0.16
Fat%	3.00
Protein%	2.80
Total solids (TS) %	12.10
Solids not fat (SNF)%	9.10
Moisture%	87.9
Specific density	1.0334

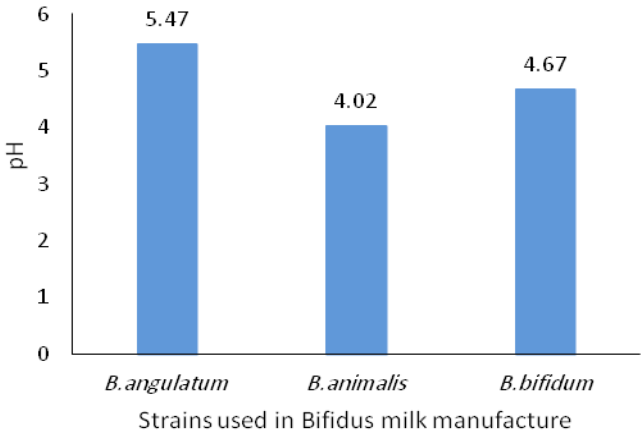

Fig 1. pH values of Bifidus milk after 24 hrs of addition of *Bifidobacteria sp.*

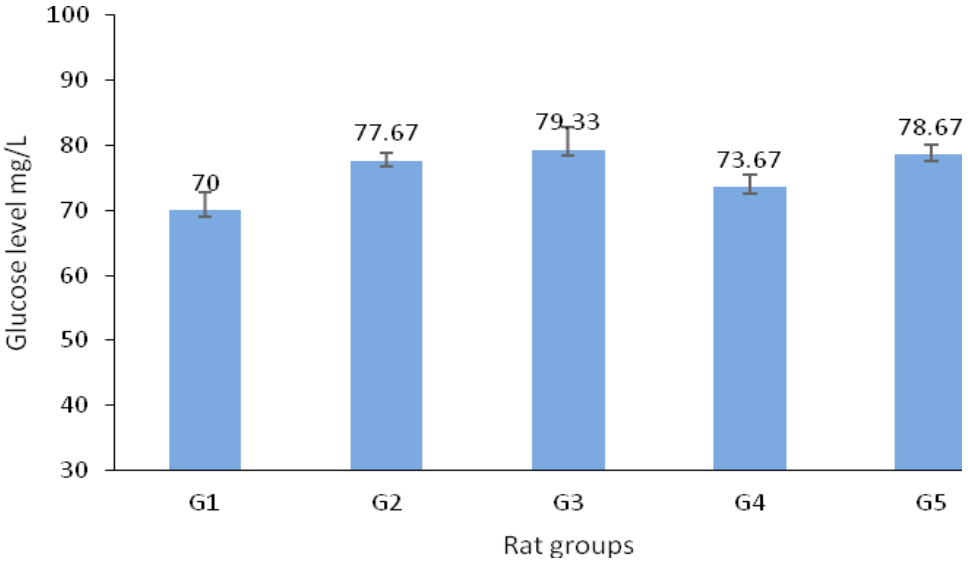

Fig. 2. Glucose level (mg/l) for experimental groups. Results are expressed as mean value and the vertical bar presents the SE, n=7. G1: negative control without keto, G2 positive control with keto, G3: with keto and *Bif. angulatum,* G4: with keto and *Bif. animalis,* G5: with keto and *Bif. bifidum.*

TABLE 2. Changes in weight and liver index for the experimental groups.

Treatments	Body weight			% Liver index
	Initial (g)	Final (g)	Gain (%)	
G1	174.0±2.08	376.3±4.18	1.163±0.005	2.487±0.03
G2	174.7±0.33	311.3±12.84 [a]	0.782±0.071[a]	2.893±0.04[a]
G3	180.0±2.65	344.0±6.083	0.911±0.023	2.503±0.008[b]
G4	180.7±0.88	389.7±14.84 [b]	1.092±0.081[b]	2.393±0.03[b]
G5	180.7±0.67	388.7±10.73 [b]	1.143±0.054[b]	2.550±0.029[b]

Results are expressed as mean value ±SE, p<0.05 a: groups vs G1, b: groups vs G2 (n=7). G1: negative control without keto, G2 positive control with keto, G3: with keto and *B. angulatum*, G4: with keto and *Bif. animalis*, G5: with keto and *Bif. bifidum*. Liver index= (liver weight / final body weight) × 100

However, supplementation with *Bif. breve* M4A and *Bif. longum* subsp. *longum* FA1 may lead to an altered intestinal microbiota that minimizes the amount of energy stored in adipose tissue, thereby, reducing weight gain. The proposed mechanism was that gut microbiota plays an important role in energy homeostasis in the host's intestine (Cani and Delzenne, 2009) or enhanced bile salts signalisation in adipose tissue, thus causing weight loss (Kim et al., 2022). Alsharafani et al. (2016) suggest that bifidobacteria supplementation may help reduce weight gain, reduce serum triglyceride concentrations and reduce hepatic steatosis under high-fat diet. However, the opposite trend was established by Barratt et al. (2022) who studied severe acute malnutrition in infants, supplementation with *Bif. infantis* led to weight gain and reduced signs of inflammation. Yin (2010) noticed three different patterns of *bifidobacteria* towards weight change *Bif. M13-4* resulted in weight gain in rats , *Bif. L66-5* led to weight reduction at the same caloric intake. *Bif. L75-4* and *Bif. FS3-1-1-2* strains did not influence body weight. They concluded that different *Bifidobacteria* strains have various energy responses and metabolism of fat in rats. From previously discussed mechanisms, the behaviour of bifidobacteria on weight is strain-dependent.

Liver index was the highest ($P \leq 0.05$) in the positive control group (G2) compared to the G1 group (Table 2). On the other hand, Giving the Ketogenic diet rat groups Bifidus milk decreased the liver index significantly ($P \leq 0.05$) and the values were close to the value of the negative control group (G1). As shown in Table 2, rats subjected to ketogenic diet have been shown to lowest body weight gain. This weight loss is often attributed to the metabolic shift induced by the ketogenic diet, where the body relies more on fat for energy instead of carbohydrates (Attaye et al., 2022). On the other hand, in a study by Kennedy et al. (2007), the authors found that the weight of KD rats was stable until the end of the study.

The liver enzyme activities

Liver enzymes such as alanine transaminase (ALT) and aspartate transaminase (AST) are commonly measured to assess liver function (Huang et al., 2006). Table 3 indicates the liver function of ALT and AST enzymes as well as some antioxidant biomarkers catalase and superoxidase dismutase (SOD). In the matter of AST levels, there was a decline by the consumption of Bifidus milk (G3 to G5), with more improvement made by G4 in comparison with G2. Moreover, ALT concentrations in G4 did not differ statistically ($P > 0.05$) from G1, all Bifidus milk groups caused statistical enhancement compared to G2 ($p \leq 0.05$).

Some studies suggest that the keto diet may lead to increases in liver enzymes (Kosinski and Jornayvaz, 2017). This suggestion was observed in our study where the AST and ALT levels showed a significant increase in G2 compared to G1. This result is in line with a case study by Anekwe et al. (2020) who reported a marked increase in liver enzymes of AST and ALT during the ketogenic diet. On the other hand, Rezaei et al. (2017) reported that KD did not have any influence on the serum levels of AST and ALP; however, it significantly affected the serum level of ALT. Hizo and Rampelotto (2024) demonstrated that bifidobacteria are safe applicable means for the prevention of liver symptoms including steatosis, steatohepatitis, NAFLD, ALD, fibrosis and cirrhosis via enhancing liver enzymes and other associated biochemical parameters. A metanalysis analyzed 31 studies conducted by Hizo and Rampelotto (2023) proposed that bifidobacteria improved liver pathologies and hepatic disease. About antioxidant activity, catalase activity was inhibited in G2 but reactivated by Bifidus milk, only G5 did not differ significantly with G2. In respect of SOD, treatments were slightly elevated compared to G2, however, all groups have considerably differed from G1. Superoxide dismutase (SOD) and catalase are enzymes that protect cells from radical attack. Catalase disproportionates hydrogen peroxide, and SOD is an oxidoreductase that serves to dismutate the superoxide anion (Matsumoto et al., 1991). Concerning catalase enzyme, our results agree with a previous study by Chen et al. (2021), who found that *Bif. adolescentis* supplementation increased the activity of the catalase enzyme in skeletal muscle and brain tissue of rats. This result agrees with our results in G3 and G4, which presented a significant increase in catalase levels compared to G2.

TABLE 3. Liver function, catalase and SOD enzymes for experimental groups.

Treatments	AST (U/L)	ALT (U/L)	Catalase (U/L)	SOD (U/g)
G1	43.06±4.76	22.36±3.01	1.53±0.02	2.691±0.087
G2	95.76±2.80 [a]	37.71±0.33[a]	0.94±0.11 [a]	1.563±0.150 [a]
G3	69.57±1.01 [ab]	33.12±0.82 [a]	1.44±0.01 [b]	1.736±0.860 [a]
G4	57.50±4.59[b]	23.12±1.85 [b]	1.47±0.05 [b]	2.083±0.150 [a]
G5	65.81±2.02 [a b]	33.31±1.56 [a]	1.11±0.02 [a]	1.736±0.086 [a]

Results are expressed as mean value ±SE, p<0.05 a: groups vs G1, b: groups vs G2 (n=7). G1: negative control without keto, G2: positive control with keto, G3: with keto and *Bif. angulatum*, G4: with keto and *Bif. animalis*, G5: with keto and *Bif. bifidum*.

Lipid profile

Table 4 illustrates the lipid profile for control and treated groups. It is noticed that G2 (ketogenic group) has the highest level in items of TG, TC, HDL, LDL and vLDL, which emphasizes the hepercholestremic role of this diet. On the other hand, Bifidus milk administration involved lower values of TG, TC, HDL, LDL and vLDL compared to G2, especially G4 that was differed statically from G2 and had not any considerable differences with G1 at (p<0.05). G1 the negative control which consumed a healthy balanced diet only hasmoderate rates from each discussed item. G2 composes TG and TC levels about two folds of G1, HDL did not differ statistically in G4 and G5 compared to G1 (p>0.05). vLDL was the highest (P≤0.05) in G2 (41.18±0.77 mg/dl)with a statical difference by G1 (19.05±0.57 mg/dl), about Bifidus milk groups, G5 was higher than G3 followed by G4, 31.45±3.26, 29.04±2.26and 24.52±2.18 mg/dl, respectively. As shown in Table 4, a clear increase in the levels of triglycerides, total cholesterol, HDL, LDL and vLDL can observed in groups of rats followed the keto diet. On the other hand, a clear decrease in these levels was observed in groups of rats fed Bifidus milk after the keto diet. Triglycerides are a type of harmful fat in the blood, and elevated levels can be a risk factor for cardiovascular diseases (Toth, 2016). Also, cholesterol is a fatty substance that is essential for various physiological functions but can contribute to cardiovascular risk when present in excessive amounts, particularly when it comes to low-density lipoprotein (LDL) cholesterol. There is some evidence to suggest that certain strains of bifidobacteria may have a positive impact on triglyceride and cholesterol levels in the blood. This effect could be associated with the ability of probiotics to modulate gut microbiota, influence lipid metabolism, and enhance the body's ability to manage fats (Momin et al., 2023).

The mechanism of lowering cholesterol by bifidobacteria may be due to the ability of *Bifidobacterium* strains to eliminate cholesterol in feces and excrete it by binding with cholesterol. *Bifidobacteria* may also inhibit its reabsorption in the body by interfering with the recycling of bile salt (a metabolic product of cholesterol) which improves the elimination of cholesterol (An et al., 2011). Tahri et al. (1997) reported that *Bif. longum* and *Bif. animalis* could remove cholesterol from the growth media and explained that due to both bacterial assimilation and precipitation of cholesterol. Also, they reported that these bacteria could reduce the serum total cholesterol by inhibiting absorption in the intestine. Concerning the LDL and v-LDL cholesterol, which are commonly referred to as "bad" cholesterol, some studies have indicated that certain strains of bifidobacteria may help lower their levels. The mechanisms through which bifidobacteria may exert this effect that these bacteria could involve the modulation of gut microbiota and the production of short-chain fatty acids (Momin et al., 2023).

As shown in Table 4, G2 led to higher AI values of 0.285 ±0.0135, oppositely G4 showed the lowest AI value of 0.227±0.0125. G1, G3 and G5 seemed to be very close as 0.256±0.0273, 0.253±0.0525 and 0.263±0.0197, respectively. All groups did not appear to have any significant differences (p>0.05).The AI of plasma is a measure that assesses the balance between pro-atherogenic and anti-atherogenic lipoproteins in the blood. It is considered a marker for cardiovascular risk (Zhu et al., 2018). In our study, feeding with Bifidus milk after the ketogenic diet tends to lower triglyceride levels which leads to a favorable decrease in the AI.

TABLE 4. Lipid profile (TG, TC, HDL, LDL, VLDL) for controls and Bifidus milk groups:

Treatments	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	vLDL (mg/dl)	Atherogenic Index
G1	95.23±2.86	91.33±2.40	50.67±2.03	21.62±4.32	19.05±0.57	0.256±0.027
G2	205.9±3.85[a]	215.8±4.50[a]	112.0±2.99[a]	62.65±4.36[a]	41.18±0.77[a]	0.285±0.0136
G3	145.2±11.31[ab]	145.6±5.05[ab]	82.20±9.19[ab]	34.41±12.73	29.04±2.26[ab]	0.253±0.052
G4	122.6±10.88[b]	101.10±3.27[b]	59.80±0.64[b]	16.73±3.40[b]	24.52±2.18[b]	0.227±0.0126
G5	157.2±16.33[ab]	108.8±2.01[ab]	59.6±3.76[b]	17.79±4.23[b]	31.45±3.26[ab]	0.263±0.012

Results are expressed as mean value ±SE, p<0.05 a: groups vs G1, b: groups vs G2 (n=7). G1: negative control without keto, G2: positive control with keto, G3: with keto and *B. angulatum*, G4: with keto and *B. animalis*, G5: with keto and *B. bifidum*. HDL: high-density lipoprotein, LDL: low-density lipoprotein, vLDL: very low-density lipoprotein.

Conclusions

The ketogenic diet showed effectiveness in reducing weight gain, but it possessed unfavourable effects on some metabolic characteristics like liver index, liver function and lipid profile in rats. Feeding on Bifidus milk inoculated by different strains of *Bifidobacterium* during and after the ketogenic diet emerged as a safer means to apply this diet avoiding accompanying metabolic problems.

References

Aebi, H. (1984) Catalase in Vitro. In: Methods In Enzymology, Vol. 105, Academic Press, pp 121-126. https://doi.org/10.1016/S0076-6879(84)05016-3

Alan, W. (2006). Tietz Clinical Guide to Laboratory Tests. In *Transfusion*.

Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W. and Fu, P. C. (1974) Enzymatic determination of total serum cholesterol. *Clinical Chemistry*, **20**(4) , 470–475. https://doi.org/10.1093/clinchem/20.4.470

Alsharafani, M., Roderfeld, M., Roeb, E. and Krawinkel, M. (2016)Bifidobacterium breve M4A and Bifidobacterium longum subsps. longum FA1 Reduced Weight Gain and Hepatic Lipid Droplets in Young Mice Fed High-Fat. *Journal of Probiotics & Health*, **4**(3), 1–8. https://doi.org/10.4172/2329-8901.1000152

An, H. M., Park, S. Y., Lee, D. K., Kim, J. R., Cha, M. K., Lee, S. W., Lim, H. T., Kim, K. J. and Ha, N. J. (2011) Antiobesity and lipid-lowering effects of Bifidobacterium spp. in high fat diet-induced obese rats. *Lipids in Health and Disease*, **10**,116. https://doi.org/10.1186/1476-511X-10-116

Anekwe, C., Chandrasekaran, P. and Stanford, F. C. (2020) Ketogenic Diet-induced Elevated Cholesterol, Elevated Liver Enzymes and Potential Non-alcoholic Fatty Liver Disease. *Cureus*, **12**(1), 1–7. https://doi.org/10.7759/cureus.6605

Ang, Q. Y., Alexander, M., Newman, J. C., Tian, Y., Cai, J., Upadhyay, V., Turnbaugh, J. A., Verdin, E., Hall, K. D., Leibel, R. L., Ravussin, E., Rosenbaum, M., Patterson, A. D. andTurnbaugh, P. J. (2020) Ketogenic Diets Alter the Gut Microbiome Resulting in Decreased Intestinal Th17 Cells. *Cell*, **181**(6), 1263-1275.e16. https://doi.org/10.1016/j.cell.2020.04.027

AOAC International. (2016) Official methods of analysis of AOAC International. *Association of Official Analysis Chemists International*.

Attaye, I., van Oppenraaij, S., Warmbrunn, M. V. and Nieuwdorp, M. (2022) The role of the gut microbiota on the beneficial effects of ketogenic diets. *Nutrients*, **14**(1), 191 https://doi.org/10.3390/NU14010191

Bagheri, S., Heydari, A., Alinaghipour, A. and Salami, M. (2019) Effect of probiotic supplementation on seizure activity and cognitive performance in PTZ-induced chemical kindling. *Epilepsy and Behavior*, **95**, 43–50. https://doi.org/10.1016/j.yebeh.2019.03.038

Barratt, M. J., Nuzhat, S., Ahsan, K., Frese, S. A., Arzamasov, A. A., Sarker, S. A., Islam, M. M., Palit, P., Islam, M. R., Hibberd, M. C., Nakshatri, S., Cowardin, C. A., Guruge, J. L., Byrne, A. E., Venkatesh, S., Sundaresan, V., Henrick, B., Duar, R. M., Mitchell, R. D. and Gordon, J. I. (2022)*Bifidobacterium infantis* treatment promotes weight gain in Bangladeshi infants with severe acute malnutrition. *Science Translational Medicine*, **14**(640), eabk1107. https://doi.org/10.1126/scitranslmed.abk1107

Cani, P. and Delzenne, N. (2009) The Role of the Gut Microbiota in Energy Metabolism and Metabolic Disease. *Current Pharmaceutical*

Design, **15**(13), 1546–1558. https://doi.org/10.2174/138161209788168164

Chen, S., Chen, L., Qi, Y., Xu, J., Ge, Q., Fan, Y., Chen, D., Zhang, Y., Wang, L., Hou, T., Yang, X., Xi, Y., Si, J., Kang, L. and Wang, L. (2021) Bifidobacterium adolescentis regulates catalase activity and host metabolism and improves healthspan and lifespan in multiple species. *Nature Aging*, **1**(11), 991–1001. https://doi.org/10.1038/s43587-021-00129-0

EL-Saleh, A. A., Zahran, A. S. and Abu-Tarboush, H. M. (1998) Growth of Bifidobacteria : environmental conditions and adherence to epithelial cells. *Milchwissenschaft*, **53**, 187–190.

Esposito, E., Iacono, A., Bianco, G., Autore, G., Cuzzocrea, S., Vajro, P., Canani, R. B., Calignano, A., Raso, G. M. and Meli, R. (2009) Probiotics Reduce the Inflammatory Response Induced by a High-Fat Diet in the Liver of Young Rats. *The Journal of Nutrition*, **139**(5), 905–911. https://doi.org/10.3945/JN.108.101808

Ferraris, C., Meroni, E., Casiraghi, M. C., Tagliabue, A., De Giorgis, V. and Erba, D. (2021) One Month of Classic Therapeutic Ketogenic Diet Decreases Short Chain Fatty Acids Production in Epileptic Patients. *Frontiers in Nutrition*, **8**, 613100. https://doi.org/10.3389/FNUT.2021.613100/BIBTEX

Finamore, A., Roselli, M., Donini, L. M., Brasili, D. E., Rami, R., Carnevali, P., Mistura, L., Pinto, A., Giusti, A. M. and Mengheri, E. (2019) Supplementation with Bifidobacterium longum Bar33 and Lactobacillus helveticus Bar13 mixture improves immunity in elderly humans (over 75 years) and aged mice. *Nutrition*, **63**(64),184–192. https://doi.org/10.1016/j.nut.2019.02.005

Fossati, P. and Prencipe, L. (1982) Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry*, **28**(10), 2077-2080. https://doi.org/10.1093/clinchem/28.10.2077

Friedewald, W. T., Levy, R. I. and Fredrickson, D. S. (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, **18**(6),499–502. https://doi.org/10.1093/clinchem/18.6.499

Furth, S. L., Casey, J. C., Pyzik, P. L., Neu, A. M., Docimo, S. G., Vining, E. P. G., Freeman, J. M. and Fivush, B. A. (2000) Risk factors for urolithiasis in children on the ketogenic diet. *Pediatric Nephrology (Berlin, Germany)*, **15**(1–2), 125–128. https://doi.org/10.1007/S004670000443

Gasior, M., Rogawski, M. A. and Hartman, A. L. (2006) Neuroprotective and disease-modifying effects of the ketogenic diet. *Behavioural Pharmacology*, **17**(5–6), 431–439. https://doi.org/10.1097/00008877-200609000-00009

Hamad, M. and Baiomy, A. (2010) Physical Properties and Chemical Composition of Cow's and Buffalo's Milk in Qena Governorate. In *Journal of Food and Dairy Sciences,***1**(7),397–403. https://doi.org/10.21608/jfds.2010.82466

Hizo, G. H. and Rampelotto, P. H. (2023) The Role of Bifidobacterium in Liver Diseases: A Systematic Review of Next-Generation Sequencing Studies. *Microorganisms*, **11**(12), 2999. https://doi.org/10.3390/microorganisms11122999

Hizo, G. H. and Rampelotto, P. H. (2024) The Impact of Probiotic Bifidobacterium on Liver Diseases and the Microbiota. *Life*, **14**(2), 239. https://doi.org/10.3390/life14020239

Huang, X.-J., Choi, Y.-K., Im, H.-S., Yarimaga, O., Yoon, E. and Kim, H.-S. (2006) *Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) Detection Techniques*. http://www.mdpi.org/sensors

Jornayvaz, F. R., Jurczak, M. J., Lee, H. Y., Birkenfeld, A. L., Frederick, D. W., Zhang, D., Zhang, X. M., Samuel, V. T. and Shulman, G. I. (2010) A high-fat, ketogenic diet causes hepatic insulin resistance in mice, despite increasing energy expenditure and preventing weight gain. *American Journal of Physiology - Endocrinology and Metabolism*, **299**(5), 808–815. https://doi.org/10.1152/ajpendo.00361.2010

Kennedy, A. R., Pissios, P., Otu, H., Xue, B., Asakura, K., Furukawa, N., Marino, F. E., Liu, F. F., Kahn, B. B., Libermann, T. A. and Maratos-Flier, E. (2007) A high-fat, ketogenic diet induces a unique metabolic state in mice. *American Journal of Physiology - Endocrinology and Metabolism*, **292**, E1724 –E1739. https://doi.org/10.1152/ajpendo.00717.2006

Kim, G., Yoon, Y., Park, J. H., Park, J. W., Noh, M., Kim, H., Park, C., Kwon, H., Park, J., Kim, Y., Sohn, J., Park, S., Kim, H., Im, S.-K., Kim, Y., Chung, H. Y., Nam, M. H., Kwon, J. Y., Kim, I. Y. and Seong, J. K. (2022) Bifidobacterial carbohydrate/nucleoside metabolism enhances oxidative phosphorylation in white adipose tissue to protect

against diet-induced obesity. *Microbiome*, **10**(1), 188. https://doi.org/10.1186/s40168-022-01374-0

Kosinski, C. and Jornayvaz, F. R. (2017) Effects of Ketogenic Diets on Cardiovascular Risk Factors: Evidence from Animal and Human Studies,**19**(5): 517. https://doi.org/10.3390/nu9050517

Kossoff, E. H., Pyzik, P. L., Furth, S. L., Hladky, H. D., Freeman, J. M. and Vining, E. P. G. (2002) Kidney stones, carbonic anhydrase inhibitors, and the ketogenic diet. *Epilepsia*, **43**(10), 1168–1171. https://doi.org/10.1046/J.1528-1157.2002.11302.X

Le, H. H. and Johnson, E. L. (2020)Going Keto? Say HB-ye Bye to Your Gut Bifidobacteria. *Cell Host and Microbe*, **28**(1), 3–5. https://doi.org/10.1016/j.chom.2020.06.012

Lindefeldt, M., Eng, A., Darban, H., Bjerkner, A., Zetterström, C. K., Allander, T., Andersson, B., Borenstein, E., Dahlin, M. and Prast-Nielsen, S. (2019) The ketogenic diet influences taxonomic and functional composition of the gut microbiota in children with severe epilepsy. *Npj Biofilms and Microbiomes*, **5**(1), 1-13. https://doi.org/10.1038/s41522-018-0073-2

Matsumoto, H., Silverton, S. F., Debolt, K. and Dr. Shapiro, I. M. (1991) Superoxide dismutase and catalase activities in the growth cartilage: Relationship between oxidoreductase activity and chondrocyte maturation. *Journal of Bone and Mineral Research*, **6**(6), 569–574. https://doi.org/10.1002/jbmr.5650060607

Momin, E. S., Khan, A. A., Kashyap, T., Ahad Pervaiz, M., Akram, A., Mannan, V., Sanusi, M. and Elshaikh, A. O. (2023) The Effects of Probiotics on Cholesterol Levels in Patients With Metabolic Syndrome: A Systematic Review. https://doi.org/10.7759/cureus.37567

Motulsky H.J. (1999) Analyzing Data with GraphPad Prism , GraphPad Software Inc., San Diego CA, . *Www.Graphpad.Com.*

Nagawa, M., Nakabayashi, A. and Fujino, S. (1988) Preparation of the Bifidus Milk Powder. *Journal of Dairy Science*, **71**(7), 1777–1782. https://doi.org/10.3168/jds.S0022-0302(88)79745-3

Nishikimi, M., Appaji Rao, N. and Yagi, K. (1972) The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical and Biophysical Research Communications*, **46**(2), 849–854. https://doi.org/10.1016/S0006-291X(72)80218-3

O'Callaghan, A. and van Sinderen, D. (2016) Bifidobacteria and Their Role as Members of the Human Gut Microbiota. *Frontiers in Microbiology*, **7, 295.** https://doi.org/10.3389/FMICB.2016.00925

Paoli, A., Mancin, L., Bianco, A., Thomas, E., Mota, J. F. and Piccini, F. (2019) Ketogenic diet and microbiota: Friends or enemies? *Genes*, **10**(7), 534. https://doi.org/10.3390/genes10070534

Rezaei, S., Kavoosi, M., Shervin Badv, R., Mohammadi, M., Reza Zamani, G., Reza Ashrafi, M., Saeed Yekaninejad, M. and Mahmoudi, M. (2017) *The Influence of Ketogenic Diet on Liver Function in Children and Adolescents with Intractable Epilepsy*. **8**(3), 12609. https://doi.org/10.5812/compreped.12609

Singh, V., Yeoh, B. S., Xiao, X., Kumar, M., Bachman, M., Borregaard, N., Joe, B. and Vijay-Kumar, M. (2015) Interplay between enterobactin, myeloperoxidase and lipocalin 2 regulates E. coli survival in the inflamed gut. *Nature Communications*, **6**. https://doi.org/10.1038/NCOMMS8113

Sinha, S. R., and Kossoff, E. H. (2005) The ketogenic diet. In *Neurologist***11**(3)161–170. https://doi.org/10.1097/01.nrl.0000160818.58821.d2

Slačanac, V., Hardi, J., Čuržik, D., Pavlović, H. and Jukić, M. (2005) Production of antibacterial organic acids during the fermentation of goat and cow milk with *Bifidobacterium longum* BB-46. *Acta Alimentaria*, **34**(3), 277–285. https://doi.org/10.1556/AAlim.34.2005.3.10

Tagliabue, A., Ferraris, C., Uggeri, F., Trentani, C., Bertoli, S., de Giorgis, V., Veggiotti, P. and Elli, M. (2017) Short-term impact of a classical ketogenic diet on gut microbiota in GLUT1 Deficiency Syndrome: A 3-month prospective observational study. *Clinical Nutrition ESPEN*, **17**, 33–37. https://doi.org/10.1016/j.clnesp.2016.11.003

Tahri, K., Grill, J. P. and Schneider, F. (1997) Involvement of Trihydroxyconjugated Bile Salts in Cholesterol Assimilation by Bifidobacteria. *Current Microbiology*, **34**(2),79–84. https://doi.org/10.1007/s002849900148

Toth, P. (2016) Triglyceride-rich lipoproteins as a causal factor for cardiovascular disease. *Vascular Health and Risk Management*, **12,** 171-183. https://doi.org/10.2147/VHRM.S104369

Van Syoc, E. P., Damani, J., DiMattia, Z., Ganda, E. and Rogers, C. J. (2023) The Effects of Bifidobacterium

Probiotic Supplementation on Blood Glucose: A Systematic Review and Meta-Analysis of Animal Models and Clinical Evidence. In *Advances in Nutrition*. Elsevier B.V. https://doi.org/10.1016/j.advnut.2023.10.009

Wieland, H. and Seidel, D. (1983) A simple specific method for precipitation of low density lipoproteins. *Journal of Lipid Research*, **24**(7), 904-909. https://doi.org/10.1016/s0022-2275(20)37936-0

Yin, Y.-N. (2010) Effects of four *Bifidobacteria* on obesity in high-fat diet induced rats. *World Journal of Gastroenterology*, **16**(27), 3394-3401. https://doi.org/10.3748/wjg.v16.i27.3394

Zhu, X., Yu, L., Zhou, H., Ma, Q., Zhou, X., Lei, T., Hu, J., Xu, W., Yi, N. and Lei, S. (2018) Atherogenic index of plasma is a novel and better biomarker associated with obesity: a population-based cross-sectional study in China. *Lipids in Health and Disease*, **17**(1), 37. https://doi.org/10.1186/s12944-018-0686-8