Assessment of dyslipidemia and oxidative stress induced by Ketogenic and Carnivore diets in male rabbits and the potential protective role of the probiotic *Lactobacillus gasseri*

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Abstract

Many societies are witnessing a growing trend towards weight-loss diets, particularly low-carbohydrate diets. Therefore, this study aimed to evaluate the direct health effects of the keto and carnivore diets on several vital indicators. These indicators included body weight, lipid profile, arterial atherosclerosis index (AIP), and oxidative stress markers MDA and GSH. The effect of adding the probiotic bacteria *Lactobacillus gasseri* on the potential disruption of these vital indicators resulting from the two diets was also assessed. It is hoped that the results of this research will contribute to providing a clear scientific perspective on evaluating the potential risks and benefits of these restrictive diets. The study results confirmed the effectiveness of both the keto and carnivore diets in managing body weight. They resulted in a significant weight loss, likely due to the accompanying state of ketosis and decreased appetite. Conversely, both diets caused a significant disruption in blood lipid levels. While they positively affected some values, they had a significant negative impact on others. The results of *L. gasseri* treatment were also mixed. Regarding oxidative stress, both diets led to an increase in the malondialdehyde (MDA) index, but they significantly enhanced the antioxidant glutathione (GSH) level, especially with the addition of the probiotic. Therefore, while the ketogenic and carnivore diets are effective for weight management, they cause significant metabolic and oxidative disturbances. This study strongly recommends the clinical integration of probiotics, such as Lactobacillus gasseri. This could be a necessary preventative strategy to mitigate the dyslipidemia and oxidative stress resulting from such diets.

Keywords: Ketogenic diet, Carnivore diet, *Lactobacillus gasseri*, dyslipidaemia, oxidative stress.

Introduction

The ketogenic diet has gained widespread popularity due to its potential health benefits, most notably in treating obesity. This diet is characterized by a high intake of fats, low carbohydrates, and moderate protein [1]. Numerous studies have explored the potential benefits of the ketogenic diet for various health conditions, including some chronic diseases such as diabetes and liver disease [2]. The liver produces ketone bodies (beta-hydroxybutyrate (βHB), acetoacetate, and acetone) from fatty acids to replace glucose as an energy source during fasting or carbohydrate-restricted diets. The resulting metabolic state is called "auto-ketosis" [3]. One of the major health concerns associated with the ketogenic diet is its potential to cause dyslipidemia. This is primarily attributed to the high proportion of calories obtained from dietary fat, which can reach up to 90% of total daily energy intake, a major risk factor for cardiovascular disease [4].

The carnivore diet is defined as a form of elimination diet, relying entirely on animal-based foods while restricting plant-based foods [5]. Although it is a diet derived from our ancestors, the carnivore diet has only recently gained popularity [6]. The carnivore diet is typically a ketogenic diet. This is due to its lack of carbohydrates and relatively high proportion of fats and protein. Its ability to induce ketosis varies depending on the amount of protein consumed [7]. Despite claims of improved health for some adherents of this diet, some studies have indicated negative health effects in certain areas, such as a significant increase in LDL cholesterol and total cholesterol levels [8].

Probiotics are defined as live microorganisms that, when consumed in sufficient quantities, provide health benefits to the body [9]. They have numerous applications in human, animal, and plant health. They have been used to enhance plant resistance to fungal infections and improve livestock production [10, 11]. Furthermore, they offer significant health benefits to humans through their antimicrobial, anticancer, and other properties [12]. *Lactobacillus gasseri* (LGA) is a naturally occurring human intestinal bacterium and one of the most widely used probiotic strains. Numerous studies have demonstrated LG's ability to inhibit pathogenic microorganisms such as Staphylococcus aureus and Salmonella, among others, as well as its role in boosting immunity and digestive health [13]. LGA has also shown the ability to improve blood lipid levels, attributed to its capacity to metabolize bile acids [14]. Additionally, it increases fecal fat excretion and reduces intestinal fat absorption [15].

The adverse effects, such as dyslipidemia, in followers of ketogenic diets have not been extensively studied. This warrants further research to investigate the potential side effects of these diets [16]. This study examines the effects

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of two diets (ketogenic and carnivore) on dyslipidemia and oxidative stress levels, and the potential role of *Lactobacillus gasseri* in improving blood lipid levels.

Material and Methods

The study was conducted in accordance with the protocol approved by the Scientific Research Ethics Committee at Tikrit University, College of Agriculture, reference number (AgTUA-018-2025).

Experimental Animals:

Twenty albino rabbits were included in this eight-week study. The experiment was conducted from the beginning of July until the end of August 2025. They ranged in age from 14 to 16 months and weighed approximately 1100 grams. This age range was chosen to allow the rabbits sufficient time to grow and reach a suitable weight before the experiment began. The rabbits were randomly divided into five groups of four. They were housed in wire mesh cages and fed twice daily, with a constant supply of fresh water.

The experiment was designed with the following groups: Group 1 (Co): the control group fed a standard diet. Group 2 (KD): the group of animals fed a ketogenic diet. Group 3 (KDL): rabbits fed a ketogenic diet and then administered the probiotic bacteria. Group 4 (CV): laboratory rabbits fed a carnivore diet. Group 5 (CVL): rabbits fed a carnivore diet and administered the probiotic bacterial suspension. The rabbits were given a two-week adjustment period through a gradual introduction of the experimental diets, while the biological experiment lasted for eight weeks.

Preparing diets:

Standard feed

The standard ration provided during the experiment was designed according to the nutritional requirements of laboratory rabbits as defined by Clark *et al.* [17].

Ketogenic diet

The experimental ketogenic diet was prepared according to the method of Augustine *et al.* [18]. With minor modifications, the diet was prepared to consist of a mixture of 15% standard feed, 20% animal fat, 50% shredded coconut, 5% Vitamix, and 10% whey protein. The vegetable fat mentioned in the source used to design the keto diet was replaced with animal fat. Incorporating a mixture of animal and vegetable fats into the keto diet provides a clearer understanding of its health effects. Although plant-based unsaturated fats are typically associated with beneficial health outcomes, animal-based fats have historically been linked to adverse effects. Thus, incorporating animal fats allows for a more realistic assessment of the potential metabolic risks inherent in these restrictive regimens. However, while the use of healthy vegetable fats is recommended for those following the keto diet, there are no strict guidelines on this matter.

Carnivore diet

Since the predominant foods in the Carnivore diet are meat, particularly red meat, eggs, and milk, the average values of fat, protein, and carbohydrates from these foods were calculated. This allowed for the determination of the chemical composition upon which the experimental Carnivore diet could be designed. According to the USDA Food Data Center, the macronutrient ratios for these foods were: 42% fat, 56% protein, and 7.9% carbohydrates [19]. Based on these ratios, we designed the Carnivore diet using the average macronutrient values of the commonly consumed foods. Animal fat was used as the fat source after being melted and mixed with the other diet components.

Table 1. Quantities and proportions included in the design of the experimental carnivore diet.

Ingredients (dry weight)	Protein (%)	Fat (%)	Carbohydrate (%)	Quantity (kg)
ISO whey	90	0	0	4.892
Pure animal fat	0	100	0	2.911
Whole milk	26	26	38	2.097
Whole eggs	48	44	3	0.1

The protein component of the experimental diet was supplemented with whey protein. Whey protein was chosen for its nutritional value being comparable to meat protein. Whole milk was also added to the diet, as were whole eggs, but in very small quantities, due to their poor digestibility by the experimental animals. The ingredients were mixed in the specified proportions, maintaining the above ratios. The quantities of ingredients required to produce 10 kg of the experimental carnivore diet were calculated using a system of simultaneous linear equations. Table 1 summarizes the required quantities of each ingredient and their nutritional ratios.

Probiotic bacteria

Sealed capsules containing the probiotic *Lactobacillus gasseri*, a freeze-dried product supplied by Swanson Health Products, were obtained. The method described by Yaseen et al. (2020) was followed for bacterial activation: the bacteria were transferred twice to MRS broth for 48 hours each time, incubated at 37°C. The third transfer involved

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transferring the bacteria to skimmed milk sterilized at 121°C for 5 minutes. This was followed by a 24-hour incubation at 37°C. The activated colony was then refrigerated until use [20]. Meanwhile, the KDL and CVL groups were fed ketogenic and carnivore diets for two weeks to allow for the development of the metabolic changes associated with these diets. Both groups were then administered the probiotic until the end of the experiment. The rabbits in both groups were given a bacterial suspension at a concentration of 3×10^{8} CFU to achieve maximum effect.

Collection and analysis of blood samples and body weight

The initial weight of all rabbit groups in the experiment was taken on the first day, and the final weight was taken on the last day. The difference in weight was calculated and statistically analyzed using the initial and final weight values.

Blood samples (3 ml) were collected using the cardiac pricking method. For serum isolation and testing, the blood samples were centrifuged (3000 rpm) for 15 minutes. Plasma glucose concentrations, βHB levels, oxidative stress markers (MDA, GSH), blood lipid profile, and atherogenic index were measured.

Statistical analysis

The statistical analysis of the results of the experiment was carried out according to the principles of analysis of variance (ANOVA) at a significance level of (0.05 > P) to test for significant differences between the groups.

Results and Discussion

Body weight

Table 2 shows the results of the experiment, which demonstrate the effect of feeding the keto and carnivore diets on body weight compared to the control group fed the standard diet. The healthy control group showed a significant increase in body weight at the end of the experiment, with an increase of 305 g compared to the starting weight of the animals in the group. In contrast, body weight was affected in all groups fed both diets (keto and carnivore). A weight difference of 13.67 g and -6 g was recorded for groups KD and KDL, respectively. Groups CV and CVL also recorded weight values of 6.67 g and -21.67 g, respectively.

The keto diet has proven highly effective for weight loss, for several reasons. One of the most important is its effect on appetite reduction by curbing hunger and enhancing satiety. Ketone bodies serve as an efficient alternative energy source, particularly in the brain and heart, during periods of low glucose availability. This metabolic transition to a state of nutritional ketosis provides the essential cellular fuel required to maintain

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physiological functions while simultaneously facilitating weight loss. The keto diet reduces appetite by influencing hunger and satiety hormones [21]. Both the carnivore and keto diets are expected to offer similar benefits regarding their impact on body weight. The carnivore diet is considered another type of ketogenic diet due to its metabolic effects. Both diets involve a severe reduction in carbohydrate intake. The difference lies in the fact that the carnivore diet requires a significantly higher protein intake than the keto diet [22]. A low carbohydrate intake promotes lower blood insulin levels, which results in increased ketone production and more efficient fat burning. High insulin levels, on the other hand, would prevent optimal ketosis [23]. Reduced carbohydrate intake stimulates the body to switch to using fatty acids and ketone bodies as its primary energy source instead of glucose [24]. Studies have indicated that elevated levels of β-hydroxybutyrate (βHB), the primary ketone body in the blood, are accompanied by a decrease in ghrelin, leading to reduced hunger. Crucially, appetite does not increase despite weight loss as long as ketosis is maintained [25]. Ketosis and elevated plasma βHB levels are also associated with increased levels of the satiety hormones GLP-1 and CCK [26]. However, the probiotic bacteria had no significant effect on body weight in either treatment group. This suggests that the probiotic bacteria were unable to influence ketosis levels due to the significant carbohydrate reduction in both diets.

Table 2. The effect of the keto and carnivore diets on body weight.

Sample code	Initial weight/g	Final weight/g	Weight difference/g
CO	1175±35 ^{b}	1480.3±58.7 ^{a}	305±80.89 ^{a}
KD	1183±200.28 ^{b}	1169.3±254.4 ^{b}	13.67±200.28 ^{c}
KDL	1100.3±100.8 ^{b}	1094.3±170.9 ^{b}	-6±100.8 ^{c}
CV	1060±134.19 ^{b}	1066.6±185.7 ^{b}	6.67±134.19 ^{c}
CVL	1101.6±158.3 ^{b}	1080±114.39 ^{b}	-21.67±158.3 ^{c}

The numbers represent the mean ± standard error. Letters similar to the control group indicate no significant difference. The numbers shown represent the mean

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of three replicates per sample. Letters different from the control group indicate a significant difference at a significance level of $P < 0.05$.

Lipid profile and Atherogenic index

Tables 3 illustrate the effect of different treatments on blood lipid levels. The tables reveal a wide fluctuation in blood lipids. While some group values showed positive results, others showed negative results. This fluctuation in blood lipid levels confirms the findings of many previous studies. Some studies showed an increase or no significant change in total cholesterol and LDL cholesterol levels after following the ketogenic diet. Conversely, other studies showed a decrease in LDL and total cholesterol and an increase in HDL cholesterol levels, which is consistent with what reported by Di Rosa *et al.* [27].

Our current results in Table 3 show no significant differences in total plasma cholesterol between the two keto diet groups and the healthy control group. Similarly, no significant differences were observed between either group and the control group in LDL and HDL cholesterol levels. Maintaining these levels within the normal range observed in the control group is primarily attributed to the coconut oil content. Some studies have shown that the negative effects of the high fat content of keto diets can be mitigated by replacing saturated fats with coconut oil. Despite the health benefits of various vegetable oils typically that can be included in a ketogenic diet, coconut oil has received significant attention in the design of such regimens due to its high content of medium-chain triglycerides (MCTs) [28]. Adding coconut oil to the diet may improve cholesterol metabolism, as it is effective in lowering lipoprotein A levels [29]. Coconut oil may also reduce cholesterol synthesis due to its ability to decrease the production of ApoB-containing lipoproteins [30]. Furthermore, the presence of *L. gasseri* bacteria in the KDL group may contribute to improved blood lipid levels [31]. *L. gasseri* can reduce the reabsorption of bile salts and increase the excretion of acidic steroids in the feces [32].

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Table 3. The effect of the keto and carnivore diets on blood lipid profiles.

Sample code	TC mg/dl	LDL mg/dl	VLDL mg/dl	HDL mg/dl	TG mg/dl	Atherogenic index
Co	134±1 c	87±12 b	21.33±2.9 c	17.33±7 b	102±14 c	0.43±0.16 b
KD	146±4 c	73±23 b	46±0 b	11±4 b	188±42 b	0.88±0.26 a
KDL	176± 41.2 c	102±25 b	57.33±33 ab	15.33±3 b	218.67±51 b	0.79±0.2 ab
CV	725.33±51 a	539±20.35 a	60.3±32 a	34.33±14 a	243.33±80 b	0.5±0.3 b
CVL	422.33±94 b	368.3±141 a	64.3±26 a	29.33±7 a	413.33±25 a	0.79±0.1 a

The numbers represent the mean ± standard error. Letters similar to the control group indicate no significant difference. The numbers shown represent the mean of three replicates per sample. Letters different from the control group indicate a significant difference at a significance level of $P < 0.05$.

At the same time, the KD and KDL groups showed a significant increase in triglyceride (TG) and VLDL levels. This may be due to the proportion of animal fats used in the diet design. Animal-based fats, which are consumed by many keto dieters, have a detrimental effect on triglyceride levels. This aligns with the findings of Khdher *et al.* [33]. While other studies have indicated positive effects of the keto diet on triglyceride and VLDL levels, as well as other blood lipid markers [34], these conflicting results are primarily attributed to the type of fats used in the keto diet design. Therefore, it is not possible to definitively conclude that the keto diet has a single effect on blood lipid levels. Nevertheless, careful consideration should be given to the type of fats consumed.

The results of our current study indicated very negative effects on blood lipid levels in both groups fed the carnivore diet. Both the CV and CVL groups showed a significant increase in triglyceride (TC), triglyceride (TG), LDL, and VLDL levels. A significant increase in HDL levels was also observed ($p < 0.05$) compared to the control group. This finding is consistent with the study conducted by Klement and Matzat (2025) [35]. This may be due to the extreme reduction in carbohydrate content. A low-carbohydrate diet leads to reliance on fatty acids from adipose tissue, thus increasing both HDL and LDL levels. The increased reliance on fat as an

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energy source leads to increased hepatic secretion of triglycerides (TG) and peripheral absorption, which ultimately results in a significant increase in LDL and HDL levels [36]. This is in addition to the carnivore diet's high content of animal-based fats, which has a significant negative impact on blood lipid levels. It has become known beyond any doubt that animal fats have a negative effect on the lipid profile [37].

The results also showed a significant decrease in TC values in the rabbits fed a keto diet and treated with the probiotic CVL. This is attributed to *L. gasseri*'s TC-lowering effect through increased bile salt excretion. One study indicated an increase in free bile acids, such as 23-norcholic acid, 7-ketolythocholic acid, beta-moricic acid, cholic acid, and deoxycholic acid, induced by *L. gasseri* [38]. Approximately 5% of bile acids are excreted in feces. This excretion is compensated for by cholesterol synthesis in the liver. Increased fecal bile acid excretion leads to increased bile acid synthesis in the liver [39]. Although our current study demonstrated a positive effect of *L. gasseri* dosing on LDL levels, this effect was not statistically significant. The effects on TG, HDL, and VLDL levels were not statistically significant, although slight changes were observed. This may be due to the strict elimination diet of the carnivore regimen and the high content of animal-based fats, which exceeds the regulatory capacity of the probiotic bacteria.

The arterial atherosclerosis index (AIP), which represents the logarithm of the molar ratio of triglycerides (TG) to HDL cholesterol, has been shown in numerous studies to be a significant predictor of cardiovascular disease [40]. The results in Table 2 indicate a contrasting effect of the two diets on the AIP. While the keto diet resulted in an elevated AIP, with a positive effect from the probiotic bacteria, the opposite was true for both carnivore diet groups. Variations in results within a single study or with other studies are highly likely, given that the metabolic effects of these diets depend on their chemical composition. Both diets contain a range of nutrients and compounds with varying effects.

The negative effect of the keto diet on the AIP may be attributed to the proportion of saturated fats used in the diet, despite the inclusion of unsaturated fats. It is well-established that the balance between saturated and unsaturated fat content determines the level of cardiovascular risk factors [41]. In contrast, the CV rabbit group showed no significant differences in AIP levels compared to the control group. This may be due to the high protein content at the expense of carbohydrates. Some studies have indicated that replacing carbohydrates with protein is a dietary approach that can be followed to prevent cardiovascular disease and dyslipidemia [42][43]. While *L. gasseri* bacteria showed a positive effect on AIP levels with the keto diet, they

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showed a negative effect with the carnivore diet. This necessitates further research into the mechanism of action of bacteria on the blood lipid profile and their effectiveness in individuals following high-fat elimination diets such as the keto and carnivore diets.

Oxidative stress

Our current study results indicated a significant increase in Malondialdehyde (MDA) levels in the keto and carnivore diet groups. This may be due to the high fat content. Fat metabolism requires complex processes involving oxidation, reduction, hydroxylation, and conjugation, which increases the production of reactive oxygen species (ROS). When ROS levels exceed antioxidant levels, it leads to the attack of macromolecules in cells and an increase in serum MDA levels [44][45].

Table 4. The effect of the keto and carnivore diets on oxidative stress

Sample code	MDA ng/mL	GSH μg/mL
Co	59.64±17.5 c	7.25±2.5 c
KD	432.8±151 a	17.01±1.8 bc
KDL	186.7±56.6 b	35.28±9.5 a
CV	253.16± b	11.29±4.3 c
CVL	170.9±32 b	32.9±8.5 a

The numbers represent the mean ± standard error. Letters similar to the control group indicate no significant difference. The numbers shown represent the mean of three replicates per sample. Letters different from the control group indicate a significant difference at a significance level of $P < 0.05$.

In contrast, the results in Table 4 showed positive effects on serum glutathione (GSH) levels. All groups fed the ketogenic and carnivore diets showed elevated GSH values compared to the healthy control group. The K and CV groups showed an increase, although not statistically significant ($p < 0.05$). In contrast, the KDL and CVL groups showed a clear and significant increase compared to the control group. Following a ketogenic diet primarily results in an increase in serum beta-hydroxybutyrate (βHB) levels [46]. As previously mentioned, the carnivore diet shares this characteristic with the ketogenic diet. Several studies have indicated that the ketogenic diet is associated with elevated GSH levels [47]. The underlying mechanism for this increase in GSH levels is related to increased upregulation of nuclear factor-associated transcription factor 2 (NRF2) [48]. The role of whey proteins in both diets, and especially in the Carnivore diet, cannot be overlooked. Numerous studies have indicated the ability of whey protein (WP) to raise glutathione (GSH) levels. This is because whey is rich in amino acids necessary for GSH synthesis, particularly cysteine [49][50]. Therefore, the protein content of the carnivore diet used in the experiment primarily enhances glutathione levels.

In addition to the significant increase in GSH levels observed in the two groups treated with *L. gasseri* compared to the control group, a significant increase was also observed compared to the two groups not treated with the probiotic. Beyond the ability of both diets to raise GSH levels, LGA bacteria possess independent activity. Several *L. gasseri* strains have been found to possess enzymes with antioxidant activity within their cells such as NADH Oxidase. They have also been found to produce certain non-enzymatic antioxidant metabolites, particularly thioredoxin and glutathione (GSH) [51].

Conclusion

The study concluded that low-carbohydrate diets (the keto and carnivore diets) are highly effective for weight control and promoting satiety. However, the high fat content of both diets disrupts many metabolic parameters. This was particularly evident with the carnivore diet due to its high animal fat content linked to increased health risks. These disruptions were most pronounced in blood lipid profiles. The results showed that, despite the elevated oxidative stress index (MDA) resulting from increased fat metabolism, both diets contribute to enhanced cellular antioxidants, specifically by raising glutathione (GSH) levels. The study also demonstrated that the probiotic bacterium *L. gasseri* can improve certain blood parameters and increase GSH levels. However, its effectiveness appears insufficient to counteract the severe negative effects of this type of restrictive diet. Therefore, it is advisable against adhering to these restrictive regimens for long-term weight loss, particularly the carnivore diet, due to its exclusion of beneficial plant-based components. A more sustainable approach involves adopting a modified ketogenic diet that incorporates higher fiber or unsaturated fat intake. Furthermore, while *L. gasseri* demonstrated certain protective properties, future research should explore the synergistic effects of multi-strain probiotics or extended supplementation periods to more effectively counteract the lipid imbalances associated with such high-fat dietary patterns

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