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BIOLOGICAL EVALUATION OF PROBIOTIC FERMENTED MILK (RAYEB) ON OBESE RATS

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Key Words: fermented milk (Rayeb), vitamin D, obesity, weight, liver and kidney function.

ABSTRACT

Probiotics have been widely explored in food processing, production, research and disease prevention, therefore, the present study was conducted to evaluate the biological evaluation of probiotic fermented milk (Rayeb) on obese rats for 8 weeks. The rats (n=35) were classified into 5 groups, as follows: Group (1): fed on basal diet and kept as negative control group. While the rats (n= 28) were fed on high fat diet for four weeks to induce obesity then were divided into four groups, group (2) was fed on high fat diet only and served as control positive group. Groups (3 and 4): obese rats were fed on high fat diet and were given orally 1 ml of fermented milk "Rayeb" and Vitamin D at 600 IU/Kg BW respectively. Group (5): was fed on basal diet and was given orally a mixture of (1 ml of Rayeb and Vitamin D at 600 IU/Kg BW).

The results indicated that, the supplementation with fermented milk (Rayeb), vitamin D or their mixture significantly decreased ($P<0.05$) the final body weight compared to the positive control group by 26.27%, 21.84%, 30.03%, respectively due to lowering the feed intake and the feed efficiency ratio of the obese rats. Moreover, the concentration of leptin hormone as well as the percent of peritoneal fat bad were significantly lowered ($P<0.05$) for all treated groups. It was observed that, the supplementation with the tested food materials improved the liver and kidney functions as well as lipid profile of the obese rats. It could be concluded that, fermented milk (Rayeb), vitamin D or their mixture might be useful in weight reduction.

INTRODUCTION:

The prevalence of obesity has reached epidemic proportions over the last few decades. In 2013, 36.9% of adult (age ≥20 years) men and 29.8% of women were considered overweight (body mass index [BMI] 25–29.9 kg m^2) or obese (BMI ≥30 kg m^2) (**Ng et al., 2014)**, and recent trend analyses show that the number of subjects who are overweight or obese is continuing to rise worldwide (**NCDRFC, 2016**). According to the World Health Organization **(WHO, 2000)**, in 2035, 39% of people in today's society will be affected by obesity. That is why there is now an increased need to create new public policies in prevention and in primary health care (**Khayatzadeh-Mahani et al., 2018)**. Because of the multifaceted nature of obesity, there is no single or simple solution to combat this growing epidemic. Novel, and most likely individualized interventions, may thus be necessary to effectively prevent and treat overweight and obesity (**Borgeraas et al., 2018)**.

Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (**Food and Agriculture Organization, 2006)**. Probiotic products are available in many different forms worldwide, including pills, powders, foods, and infant formula **(Sanders, 2008).** In the meantime, emerging evidence reveals a large number of microorganism genera, such as *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Streptococcus*, and *Enterococcus*, whose supplementation in the diet might play a role in prevention or management of obesity (**De Vrese and Schrezenmeir , 2008** and **Morelli, and Capurso, 2012)**.

By-products from the bacterial fermentation process might lower appetite and increase satiety (**Cani et al., 2009**), the microbiota may suppress diet-induced obesity through increased energy expenditure (**Watanabe et al., 2012**). Interestingly, obesity, as compared with normal weight, is associated with a disease-specific dysbiotic shift in the faecal microbiota and also a lower bacterial richness (**Chatelier et al., 2013**). Energy restriction and weight loss, on the other hand, is associated with an increased bacterial richness (**Cotillard et al., 2013**).

Oral administration of viable strains of bacteria (probiotics) has been proposed as a way of manipulating the gut ecosystem to flavor weight reduction or decrease weight gain; however, the mechanisms by which probiotic supplementation may influence the gut microbiota are largely unknown (**Sanders, 2016**)

Pereira-Santos et al., (2015) suggested that there are several possible vehicles for the relationship between 25(OH)Vit D and weight loss. The lack of 25(OH)Vit D status has been thought to be a possible reason behind higher adiposity through the regulation of parathyroid hormone (PTH) and modulation of adipogenesis The meta-analysis lays the foundation for defining the potential clinical efficacy of vitamin D supplementation as a potential therapeutic option for weight loss programs **(Perna, 2019)**.

Therefore, the present study was conducted to evaluate the biological evaluation of probiotic fermented milk “Rayeb" with Vitamin D (VD) on obese rats.

MATERIALS AND METHODS

Materials: Fermented milk (Rayeb) Natural Cow's Milk containing mixture of three strains of probiotics **(*Lactobacillus acidophilus, Bifidobacterium bifidum,* and *Streptococcus thermophilus*)** at the level of 10^7 CFU/ml was purchased from the local market. The viabilities of the administered strains were confirmed.

Rats: A total of 35 adult male albino rats, Sprague Dawley strain, weighing (150 g) were obtained from the Laboratory Animal Colony, Helwan, Cairo.

Chemicals: Casein, cellulose, vitamins and minerals were obtained from Morgan Company for Commerce and Chemicals. Capsules of Cholecalciferol (Vitamin D3) at the concentration of 1000 IU were purchased from local pharmacy. Starch was obtained from the Egyptian Starch and Glucose, Mostorod Factory, Qalyubia, Egypt. Corn oil, Beef tallow and Sucrose were obtained from local market. **Kits** for blood analysis were purchased from Gama Trade Company for Chemicals, Cairo, Egypt.

Microbial media: The microbial media used throughout the study had been purchased from Difco and Oxoid.

Methods: The biological experiment and the chemical analysis were carried out at the Post Graduated Lab, Faculty of Home Economics, Helwan University.

A. Microbiological study: Confirmation of the probiotics bacteria that found in the fermented milk (Rayeb) by identification the bacterial strains was carried out by using specific media and the total counts of probiotic bacteria were determined.

1. MRS broth medium: MRS broth medium was used to cultivation and isolation of all species of ***Lactobacillus*** in Fermented milk (Rayeb) according to **(De Man et al., 1960).** The culture was incubated anaerobically up to 3 days at 37°C.

2. MRS L-cysteine medium (MRSC): MRS broth, Oxoid supplemented with L-cysteine hydrochloride 0.05%. (MRSC) broth medium was used to cultivation and isolation of ***Bifidobacterium*** strains **(Desjardins et al., 1990).** The culture was incubated anaerobically up to 3 days at 37°C.

3. ST agar medium: ST agar medium was suitable for the selective enumeration of ***Streptococcus thermophilus*** from Fermented milk (Rayeb), **(Dave and Shah, 1996).**

B. Biological study: The basal diet (g/kg diet) was consisted of 140 g casein (> 80 % protein), 100 g sucrose, 40 g corn oil, 50 g cellulose, 35 g mineral mixtures, 10 g vitamin mixtures, 1.8 g L-cystine, 2.5 g choline bitartrate and the remainder is corn starch. Diets were formulated according to **(Reeves et al., 1993).**

Induction of obesity : Rats were fed on basal diet for four weeks according to **Reeves et al., (1993)** with some modification included (high fat diet) according to **Min et al., (2004)** containing: casein 14%, cellulose 5%, vitamin mixtures 1%, mineral mixtures 3.5% , sucrose 10%, (beef tallow 19% + corn oil 1%), l-cystine 0.18%, choline bitartrate 0.25 % and the remainder was starch to induce obesity.

After the adaptation period of one week, thirty-five rats were divided into five groups, (7 rats each). **Group (l)** was fed on the basal diet and kept as a control negative (-ve) group. The obese rats (n=28) were classified into 4 groups, as follows: **Group (2)**: obese rats were fed on high fat diet only (Control positive group). **Group (3)**: obese rats were fed on high fat diet and was given orally 1 ml of Rayeb. **Group (4)**: obese rats were fed on high fat diet and was given orally Vitamin D at 600 IU/Kg BW. **Group (5):** was fed on basal diet and was given orally a mixture of (1 ml of Rayeb and Vitamin D at 600 IU/Kg BW).

At the end of the experimental period (8 weeks), rats were fasted overnight before sacrificing and the blood samples were collected from each rat then centrifuged for 15 minutes at 3000 r.p.m.to separate serum. Serum was carefully aspirated and transferred into clean quite fit plastic tubes and kept frozen at -20 °C until chemical analysis. Peritoneal fat pad was dissected from the carcasses, then weighed according to the method of **(Azain et al., 2000).**

Biological Evaluations: All rats were observed each day. Their feed (FI) intake was determined daily and body weights were obtained every week. Body weight gain percent (BWG%) and Feed Efficiency Ratio (FER) was calculated according to **Chapman et al., (1959)** using the following equation:

$$\mathbf{BWG}\% = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

$$\mathbf{FER} = \frac{\text{weight Gain (g)}}{\text{Feed intake (g)}}$$

Biochemical Analysis of Serum: Serum total cholesterol (TC) **(Richmond, 1973),** triglycerides (TG) **(Wahlefeld, 1974),** high density lipoprotein (HDL) **(Albers et al., 1983)** were determined**.** Meanwhile, low density lipoprotein (LDL) and very low-density lipoprotein (LDL) were calculated according to **(Fridewald et al., 1972).** Serum Aspartate amino transferase (AST) and alanine amino transferase (ALT) **(Bergmeyer et al., 1978)** were determined**.** Serum urea **(Kaplan, 1984),** uric acid **(Patton and Crouch, 1977)**, and creatinine were measured according to **(Murray, 1984)**. Leptin hormone was determined using enzyme-linked immunosorbent (ELISA) assay (**Xiong et al., 2005).**

Statistical Analysis: The results were expressed as mean ± standard error (SE). The statistical analysis was carried out by using SPSS, PC statistical software (Version 20.0 SPSS Inc., Chicago, USA) using the Dunk 'test multiple range post-hoc test. Data were analyzed by one way analysis variance (ANOVA). The values were considered significantly different at ($P < 0.05$) **(Armitage and Berry, 1987)**.

RESULTS

Regarding to changes in body weight status, Table (1) illustrated the changes of body weight, feed intake and FER in obese rats fed on high fat diet supplemented with (Rayeb, VD and their mixture). Rats fed on high fat diet had significant increase the initial body weight as compare to the control -ve group, while there were no significant differences in IBW among all obese groups. The high fat diet supplemented with (Rayeb, VD and their mixture) significantly ($P<0.05$) decreased the FBW compared to the positive control group. There were significant differences ($P<0.05$) in FBW among the treated groups. The lowest decrease in body weight was recorded at the group fed a mixture of Rayeb and vitamin D.

Table (1). Effect of high fat diet supplemented with Rayeb, Vit D and their mixture on BW, BWG%, FI and FER of obese rat.

Parameter / Groups	Initial BW (g)	Final BW (g)	BWG (%)	Weight reduction %	Feed Intake (g/day/rat)	FER
Control (-ve)	150.0±1.87 ^{b}	171.50±2.23 ^{cd}	14.40±1.91 ^{b}	-	16.00	0.022±0.028 ^{b}
Control (+ve)	201.0±2.06 ^{a}	239.41±2.67^{a}	19.18±1.89 ^{a}	-	21.60	0.029±0.027 ^{ab}
G1: Rayeb	199.5±2.51 ^{a}	176.50±1.68 ^{c}	-11.46±1.31^{cd}	26.27	16.70	0.023±0.028 ^{b}
G2: Vit D	201.16±1.66 ^{a}	187.16±2.61 ^{b}	-6.93±1.42 ^{c}	21.84	17.50	0.013±0.027 ^{c}
G3: Mixture	195.83±2.34 ^{a}	167.50±1.80 ^{d}	-14.40±1.45 ^{d}	30.03	14.00	0.033±0.037 ^{a}

Data are expressed as mean ± SE.
Means with different superscript letters in the column are significantly differences at ($P < 0.05$).

In regarding to BWG%, obese rats (control +ve) had significant (P<0.05) increased in BWG% as compared to control -ve group. All treated groups significantly decreased BWG% compared to the positive control group. The mixture (VD and Rayeb) caused the lowest BWG% compared to the other treated groups. The percent of weight reduction was recorded by 26.27%, 21.84%, 30.03% for the groups fed on Rayeb, Vitamin D or their mixtures, respectively. It was seen that the FI was lowered for the treated groups compared the control +ve group. FER was statistical lowered at the groups fed either vitamin D or the mixture (Vit D and Rayeb) compared to the positive control group.

Results in table (2) illustrated that positive control group had significant increase (P<0.05) in leptin hormone, compared to the negative control group, while supplementation with (Rayeb, Vit. D and their mixture) significantly reduced (P<0.05) the level of leptin hormone, compared to the positive control group. The highest reduction in leptin hormone concentrations was recorded at the mixture group. In regarding to Peritoneal Fat bad, there was significant difference (P<0.05) in Peritoneal Fat bad concentrations for the treated groups compared to the +ve control. The greatest decrease in Peritoneal Fat bad concentrations was obtained by a mixture of Vit. D and Rayeb.

Table (2): Effect of high fat diet supplemented with Rayeb, Vit. D and their mixture on serum leptin and Peritoneal Fat bad concentrations of obese rats.

Parameter / Groups	Leptin (pg/ml)	Peritoneal Fat bad (%)
Control (-ve)	35.75±0.45 [e]	1.18±0.01 [d]
Control (+ve)	110.30±1.30 [a]	6.94±0.37 [a]
G1: Rayeb	53.00±2.00 [c]	2.55±0.15 [c]
G2: Vit D	69.75±1.85 [b]	3.60±0.10 [b]
G3: mixture	44.80±0.20 [d]	2.03±0.03 [c]

Data are expressed as mean ± SE.
Means with different superscript letters in the column are significantly differences at (P ≤ 0.05).

Obese rats fed on high fat diet had significant increased (P<0.05) the level of serum liver functions (AST, ALT and ALP) compared to the negative control group (Table 3). The treatments with either Rayeb, Vit. D or their mixture significantly decreased (P<0.05) the elevated levels of serum AST, ALT and ALP compared to the positive control group. It was clear that, no significant differences in AST, ALT and ALP levels among the treated groups. Supplementation with mixture of vitamin D and Rayeb caused the highest reduction in liver function.

Table (3): Effect of high fat diet supplemented with Rayeb, Vit D and their mixture on liver function of obese rats.

Parameters / Groups	AST	ALT	ALP
	(μ/L)		
Control (-ve)	50.04±1.19 [c]	26.64±1.88 [c]	1.75±0.09 [b]
Control (+ve)	74.14±1.90 [a]	39.76±1.02 [a]	2.76±0.14 [a]
G1: Rayeb	63.00±1.81 [b]	32.76±1.39 [b]	1.97±0.11 [b]
G2: Vit D	64.48±1.17 [b]	34.14±1.13 [b]	2.08±0.21 [b]
G3: mixture	62.72±2.31 [b]	31.00±1.41 [b]	1.88±0.03 [b]

Data are expressed as mean ± SE.

Means with different superscript letters in the column are significantly differences at ($P \leq 0.05$).

Table (4) shows the effects of high fat diet supplemented with (Rayeb, Vit D and their mixture) in serum kidney functions on obese rats. Feeding with high fat diet significantly increase ($P<0.05$) the level of uric acid, creatinine and urea compared to the control –ve. Feeding obese rats on diet supplemented with (Rayeb, Vit D and their mixture) caused a significant decrease ($P<0.05$) in the mean values of uric acid, creatinine and urea as compared to the positive control group. There were no significant differences in serum uric acid, creatinine and urea between the treated groups.

Table (4): Effect of high fat diet supplemented with Rayeb, Vit D and their mixture on kidney function of obese rats.

Parameters / Groups	Uric acid	Creatinine	Urea
	(mg/dl)		
Control (-ve)	1.76±0.15 [c]	0.66±0.01 [c]	27.44±1.27 [c]
Control (+ve)	3.38±0.11 [a]	1.67±0.09 [a]	43.74±1.09 [a]
G1: Rayeb	2.61±0.15 [b]	1.05±0.08 [b]	34.44±1.20 [b]
G2: Vit D	2.51±0.17 [b]	0.84±0.02 [c]	32.90±1.24 [b]
G3: mixture	2.61±0.17 [b]	0.83±0.02 [c]	33.48±1.28 [b]

Data are expressed as mean ± SE.

Means with different superscript letters in the column are significantly differences at ($P \leq 0.05$).

The effect of high fat diet supplemented with (Rayeb, Vit D and their mixture) on lipids profile of obese rats was illustrated in table (5). Feeding rats with high fat diet caused a significant increase ($P<0.05$) in serum lipid profile, however, serum HDL-C was significantly lowered, compared to the normal rats. Diet supplemented with (Rayeb, Vit D and their mixture) significantly decreased ($P<0.05$) the mean value of serum TC, TG, VLDL-C and LDL-C, however, serum HDL-C level was increased significantly ($P<0.05$), compared to the positive control group.

It was noticed that, no significant difference in TC, TG, HDL, LDL and VLDL levels among the treated groups. It was obvious that, the supplementation with Vit D gave the highest beneficial effect in improving lipid profile in obese rats.

Table (5): Effect of high fat diet supplemented with Rayeb, Vit D and their mixture on lipid profile of obese rats

Parameter / Groups	TC	TG	HDL-C	VLDL-C	LDL-C
	(mg/dl)				
Control (-ve)	76.62 ± 1.85^{c}	64.20 ± 1.73^{c}	57.84 ± 0.77^{a}	12.84 ± 0.34^{c}	5.94 ± 2.04^{c}
Control (+ve)	108.46 ± 1.26^{a}	87.44 ± 1.62^{a}	39.84 ± 0.60^{c}	17.48 ± 0.32^{a}	51.13 ± 1.87^{a}
G1: Rayeb	92.28 ± 1.48^{b}	71.50 ± 1.19^{b}	50.72 ± 2.15^{b}	14.30 ± 0.23^{b}	27.26 ± 2.39^{b}
G2: Vit D	86.18 ± 2.04^{b}	69.62 ± 2.67^{b}	50.98 ± 2.55^{b}	13.92 ± 0.53^{b}	21.27 ± 2.52^{b}
G3: mixture	88.44 ± 3.05^{b}	71.16 ± 1.07^{b}	48.68 ± 1.23^{b}	14.23 ± 0.21^{b}	25.52 ± 3.43^{b}

Data are expressed as mean ± SE.

Means with different superscript letters in the column are significantly differences at ($P \leq 0.05$).

DISCUSSION

The present research was conducted to evaluate the biological evaluation of probiotic fermented milk with vitamin D on obese rats. The probiotics bacteria as bio-starters at the level of "10^7 CFU/ml" in fermented milk "**Rayeb**" including three strains of probiotics ***(Lactobacillus acidophilus, Bifidobacterium bifidum,*** and ***Streptococcus thermophilus)*** were used to examine their beneficial effect on body weight, leptin hormone, peritoneal, liver, kidney functions and lipid profile of obese rats.

The normal intestinal microbiota is important for maintaining host health because it provides energy in the form of short-chain fatty acids (**Cummings and Macfarlane, 1997)**. Functional dairy products, specially fermented milk; recently its availability and popularity increased in the daily-life. Consumers' interest about personal health is reasons in establishing markets for these functional products **(Gasmalla et al., 2017).**

Fermented milk (Rayeb) is a popular dairy product with wide spread worldwide consumption. Studies have reported health benefits of Fermented milk owing to its probiotic content comprising live Lactic acid bacteria (LAB) that prevent pathogen growth, by producing organic acids and bacteriocins in the gastrointestinal tract **(Bourlioux et al., 2003)**. Additionally, yogurt helps prevent colorectal cancer and diabetes and helps prevent and manage hyperlipidemia (**Cho et al., 2006)**. Furthermore, Fermented milk (Rayeb) and yogurt contains various LAB and stimulates the immune system and stabilizes the gut microflora (**Parvez et al., 2006)**.

Commonly used probiotics include *Lactobacillus, Bifidobacterium, Bacillus, Saccharomyces, Streptococcus, Clostridium*

(**Cox et al., 2015).** More than three kinds of probiotics and prebiotics can play a role in fighting obesity by reducing intestinal bacterial lipopolysaccharides, altering bacterial composition, and reducing fat storage (**Swami and Shah**, **2017**). Probiotics have significant effects on oxidative stress, inflammatory factors, and lipids with no adverse events reported. Mechanisms for these obesity related effects include regulation of immune differentiation and insulin sensitivity, inhibition of pathogenic bacteria adhesion to the intestine and translocation to adipose tissue, and improvement of intestinal barrier function. Moreover, treatment of obesity with a probiotic-rich diet is a major advancement in the treatment of obesity **(DiBaise et al., 2008).**

Supplementation with VD to obese rats significantly decreased the higher body weight, this result is in accordance with some studies reported by **Caan et al., (2007)** demonstrated that postmenopausal women who received vitamin D and calcium supplementation had a lower weight gain compared to placebo. **Ortega et al., (2008)** found that women with higher vitamin D responded more positively to low calorie diets and lost more body fat during the experimental period.

In addition, **Zhu et al., (2013)** also supplemented patients with vitamin D and calcium in a low calorie diet and found that the body weight reduction was significantly higher than control group. **Vimaleswaran et al., (2013)** studied the causality and the relationship between BMI and VD in a very large number of individuals (up to 42,000 participants) and concluded that obesity leads to a low status of this vitamin but it is unlikely that the deficiency predisposes to a higher BMI.

Treatment with VD (2.5-10 mcg/kg/day) produced significant dose-dependent decrease ($p<0.05$) in body weight parameters, feed intake, weight and size of fat pads, levels of serum glucose, TGs, TC, and LDL as compared to HFD group. Moreover, the level of serum HDLs was increased as compared to HFD group. So that, VD treatment ameliorated established obesity and associated biochemical consequences **(Verma et al., 2016). Merino et al., (2017)** demonstrate that the nutrition of rats with diet deficient in VD and high fat, promotes overweight by increasing fat deposits, suggestion a cause-effect relationship between VD deficiency and overweight. A significant amount of VD seemed to be stored in the liver and adipose tissue when dietary VD is at a supplementation level; thus excess body adiposity could contribute to relatively low serum 25(OH)D level when vitamin D was supplemented **(Park et al., 2020)**.

Regarding to lipid profile in the current work, obese rats had lowered (P<0.05) lipid profile due to Rayeb and/or VD supplementation. Rayeb helps prevent colorectal cancer and diabetes and helps prevent and manage hyperlipidemia **(Cho et al., 2006).** Probiotics can inhibit cholesterol uptake and increase cholesterol efflux by activating liver X receptors, eventually reducing the overall cholesterol level and potential risk of developing atherosclerosis **(Huang et al., 2015). Parhamfar et al., (2018)** suggests that consumption of milk fermented by *L. fermentum* improves serum lipid trends in rats by lowering serum total cholesterol, triglycerides, LDL-C levels, as well as by increasing HDL-C level. It also plays a role in the prevention of obesity induced by a high-fat diet.

Moreover, the administration of probiotic-fermented milk significantly lowered the levels of TC and TG in rat serum and viscera (P < 0.05) and significantly increased the level of total bile acid in the rat liver and small intestine (P < 0.05) due to the adhesion and absorption of living bacteria cells, the production of short chain fatty acids, the reduction of the reabsorption of bile acids, and the inhibition of lipoprotein lipase activity **(Wa et al., 2019).** Additionally, significant going down of lipid profile, but scale-up of HDL-c were noticed after dietary supplement with probiotic bacteria in hypercholesterolemic rats. So, it is useful of fermented milk for improving the prevention of cardiovascular disease **(Allbban, 2020).** These results are in accordance with the obtained results.

Some studies investigating the association between VD levels and dyslipidemia in obese children have suggested that VD deficiency might be linked to metabolic syndrome **(Rusconi et al., 2015)**. The functions of vitamin D are linked to lipid values. VD regulates calcium metabolism and increases intestinal calcium absorption, thereby reducing intestinal fatty acid absorption **(Wang et al., 2016).** Therefore, a reduction in intestinal fat absorption can lower the cholesterol level. Additionally, increasing the calcium concentration promotes the conversion of cholesterol into bile acids in the liver, resulting in reduced cholesterol level **(Vaskonen et al., 2007).**

VD level seems to affect the lipid profile, even in non-obese children, and a low VD level may progress to dyslipidemia or obesity in non-obese children **(Kim and Jeong, 2019). Pashova-Stoyanova et al., (2019)** confirmed the protective role of vitamin D and its effects in lowering the elevated uric acid levels. Vitamin D decreases glucose and uric acid concentrations and improves the cardiogenic lipid profile (cholesterol/HDL and LDL/HDL). **Cho et al., (2020)** indicate that the consumption of yogurt fermented by *Lactobacillus fermentum* TSI for 8 weeks by obese rats reduces abdominal fat and improves blood lipid metabolism in HD-induced obese rats.

The current results revealed that supplementation with Rayeb and/or VD significantly decreased ($P<0.05$) serum leptin hormone and perirenal fat pads of obese rats. Obesity is associated with an increase in the size and amount of adipose tissue **(Cani et al., 2009)**. Adipose tissue secretes a number of signaling molecules commonly referred to as adipocytokines **(Koerner et al., 2005).** Leptin and adiponectin are the important adipocytokines produced by adipocytes.

In addition, the weights of the mesenteric fat pads, perirenal fat pads, and epididymal fat pads were lower in the *Lactobacillus gasseri* group compared to the control group **(Kang et al., 2010)**

The obtained results are in agreement with **Sundari et al., (2020)** who reported that oral administration of VD (2400 IU) decreased pro-inflammatory substances, such as leptin and mRNA MCP-1 and increased anti-inflammatory substances, such as adiponectin, in visceral adipose tissue of obese female Wistar rats. The result, regarding serum leptin, are similar to the other study that reported by **(Belenchia et al., 2013)**, which state that the administration of high dose VD 2000 IU in twice daily on obese adolescents rats for 3 months has reduced the level of leptin significantly, compared to other obese groups which do not receive the treatment. There is a positive association between leptin level and body fat mass, it means more body fat more leptin **(Erturk et al., 2004)**.

The rats fed the *Lactobacillus gasseri* diet displayed greater numbers of small adipocytes from mesenteric retroperitoneal adipose tissues and leptin concentrations were decreased to 32 % than did those on the control diet. These results indicate a possible role for a fermented milk product in the regulation of adipose tissue growth **(Sato et al., 2008).**

The obtained results showed that the liver functions were significantly improved as results of Rayeb and/or supplementation. Patients with elevated ALT were found to have lower vitamin D. **(Liangpunsakul and Chalasani, 2011).** Low serum levels of vitamin D have been observed in chronic liver diseases, especially with liver cirrhosis **(Kitson and Roberts, 2012).** The number of large-size adipose tissue, the level of triglyceride, leptin and AST were significantly reduced in obese rats fed on yogurt fermented by *Lactobacillus plantarum* **(Park et al., 2016).** In animal models of liver and cardiovascular metabolic damage, the supplementation of 23 IU/day/rat vitamin D_3 shows liver and cardio-protective effects **(Mazzone et al., 2018).**

Finally, it could be concluded that, consumption of Rayeb, vitamin D or their mixture might be useful in weight reduction and alleviate their complications.

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التقييم البيولوجي للَبن المخمر"الرايب" المحتوي علي بادئات حيوية وفيتامين د على الفئران المصابة بالسمنة

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لقد تم استكشاف البروبيوتيك على نطاق واسع في التصنيع والإنتاج الغذائي والبحث العلمي والوقاية من الأمراض ، لذلك أجريت هذه الدراسة للتقييم البيولوجي للَبن المخمر"الرايب" المحتوي علي بادئات حيوية وفيتامين د على الفئران المصابة بالسمنة لمدة ٨ أسابيع. صُنفت الفئران (ن = ٣٥) إلى ٥ مجموعات على النحو التالي: المجموعة (١): تتغذى على النظام الغذائي الأساسي وتبقى كمجموعة ضابطة سالبة. بينما تم تغذية الفئران (ن = ٢٨) على نظام غذائي عالي الدهون لمدة أربعة أسابيع لاحداث السمنة ثم قسمت إلى أربع مجموعات ، المجموعة (٢) تم تغذيتها على نظام غذائي عالي الدهون فقط وكانت بمثابة مجموعة ضابطة موجبة. المجموعتان (٣ و ٤): تم تغذية الفئران البدينة على نظام غذائي عالي الدهون وتم إعطاؤها عن طريق الفم ١ مل من اللبن الرايب وفيتامين د بمعدل ٦٠٠ وحدة دولية / كجم من وزن الجسم على التوالي. المجموعة (٥): تم تغذيتها على الغذاء الاساسى وتم إعطاؤها عن طريق الفم خليط من (١ مل من الحليب الرايب وفيتامين د عند ٦٠٠ وحدة دولية / كجم وزن الجسم) .أشارت النتائج إلى أن تناول اللبن الرايب وفيتامين د أو خليطهما أدى إلى انخفاض معنوي ($P < 0.05$) في وزن الجسم النهائي مقارنة بمجموعة الكنترول الموجبة بنسبة ٢٦.٢٧٪ و ٢١.٨٤٪ و ٣٠.٠٣٪ على التوالي نتيجة انخفاض تناول الغذاء و نسبة كفاءة الغذاء في الفئران البدينة. علاوة على ذلك ، انخفض تركيز هرمون اللبتين وكذلك نسبة الدهون البريتونية بشكل معنوي ($P < 0.05$) لجميع المجموعات المعالجة. لوحظ أن التدعيم بالالبان المخمرة وفيتامين د أدى إلى تحسين وظائف الكبد والكلى بالإضافة إلى صورة الدهون في الفئران البدينة. يمكن القول بان تناول اللبن الرايب وفيتامين د أو خليطهما قد يكون مفيدًا في إنقاص الوزن.