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Feeding Soy with Probiotic Attenuates Obesity-Related Metabolic Syndrome Traits in Obese Zucker Rats

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Abstract

Obesity has reached pandemic levels, being a major concern of health. Overweight and obesity are the precursors for metabolic disturbances in the body. Probiotics and prebiotics administrations have shown to reduce the characteristics of obesity-induced metabolic syndrome in animals. The present study examined the effects of dietary supplementation of soybean meal and *Bifidobacterium longum* (BB536) singly and in combination on obesity-related traits in obese Zucker rats. Control group rats were fed with AIN93-M diet and treatment groups were fed with soybean meal (5 % or 10%) and *Bifidobacterium longum* (0.1%) in single and combinations for 100 days. Weight gain, feed intake, and % of fat in liver were recorded. Serum biochemical parameters such as cholesterol, triglycerides, insulin and glucose were analyzed. Activities of liver enzymatic markers (alanine aminotransferase (ALT), gamma glutamyl-transferase (GGT), and alkaline phosphatase (ALP)) were determined. Dietary supplementation of soymeal at 10% with *B. longum* reduced the weight gain by 30% and liver fat content by 35%. Feeding *Bifidobacterium longum* alone did not have any effect in analyzing serum biochemical parameters and activity of liver enzymatic markers. Serum glucose and insulin levels in rats were lowered by 18% - 24% and 22% - 25% respectively when fed with administration of combinational feeding of soymeal at 5% and 10% with *Bifidobacterium longum*. Hepatic enzyme activity was reduced by 1.3 fold with the combinational diet at higher concentration (soy 10%). The present study provides evidence that supplementation with soymeal with probiotic, *B. longum* attenuates the metabolic disorders induced by obesity in obese Zucker rats.

Keywords

Obesity, Metabolic Syndrome, Soy, *Bifidobacterium longum*

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1. Introduction

Obesity is a major emerging health problem worldwide causing major negative effects. In the US, more than 35.7% people aged 20 years and above are obese and 16.9% children and adolescents between 2 - 19 years are obese [1]. Obesity results from a positive energy balance and accumulation fat cells. Individuals with body mass index (BMI) of ≥25 kg/m^2 are in risk for developing obesity-related complications and ultimately obesity. People with BMI of 25 - 29.9 kg/m^2 are considered overweight and those with BMI of ≥30 kg/m^2 are obese. Diagnosis of obesity will be based on BMI, waist circumference and over health condition and obesity-related complications. Fat accumulation in the adipose tissue triggers a chronic low grade inflammation and proceeds to metabolic syndrome. Metabolic syndrome is a condition consisting of three or more of the following factors: dyslipidemia, insulin resistance, hypertension, overweight and glucose intolerance concurrently [2]. In a long term, these factors may lead to other chronic diseases such as type-2 diabetes, cardiovascular diseases and various types of cancer.

Prevention of obesity is a major public health concern due to associated alarming hikes in health care cost. Increased physical activity and modulation of diet are suggested to reduce the fat deposition and prevention of onset of obesity. In recent years, researchers have focused on potential natural agents to prevent chronic diseases as alternative and complimentary agents. The bioactive constituents in fruits, vegetables and medicinal plants have offered protection against hyperglycemia, hyperlipidemia, insulin resistance, inflammation and oxidative stress to combat the pathogenesis of metabolic syndrome [3].

Probiotics are live bacteria present in supplements and offer potential health benefits to the host when administered in adequate amounts [4]-[6]. *Lactobacilli* and *Bifidobacteria* are the two generally used probiotics as they are the normal inhabitants of the gastrointestinal tract. The consumption of fermented milk and lactic acid producing bacteria successfully prevented the proliferation of pathogenic bacteria in the gut [6] [7]. Consumption of probiotics is influenced by the composition of the food matrix. The liberation of peptides in dairy proteins increases the functionality of probiotics [6]. Potential health benefits of probiotics are strain-specific, and selection of strain is pivotal in the development of probiotic products [6]. Some potential health benefits of probiotics include the treatment and prevention of allergies, inflammatory bowel disease, reduced risk of mutagenicity, and may have a hypocholesterolemic effect [8]. Research suggests a relationship between the groups of bacteria (*Bacteroidetes* and the *Firmicutes*) and obesity [9]-[12]. Results from several studies in obese rat models, mice and humans, indicated a higher proportion of bacteroids in lean counterparts compared to the obese [13]-[15].

Soybean is a member of the pea family, and is a rich source of protein, fiber, isoflavones, phytosterol and lecithin [16] [17]. It has versatility in food application due to low allergic reactions. Consumption of soy has potential benefits in reducing obesity and its related complications such as serum and hepatic total cholesterol, triglycerides. Administrations of soy as a whole and as its constituents have proven to possess anti-obesity properties by several mechanisms in *in-vivo* and *in-vitro* as lowering insulin resistance, improving blood glucose levels, decreasing fat accumulation, improving dyslipedemia, and decreasing inflammatory cytokines by antioxidant properties [18] [19].

Obese Zucker rat is an established experimental animal model for studying the potential anti-obesity agents as well as for studying the traits of metabolic syndrome due to obesity [20]. Leptin receptor is genetically modified in Zucker rats resulting in relentless food consumption. Further, obesity develops by the accumulation of excessive energy consumption and less expenditure, resulting in an increased fat content in the body. Disruption of cellular processes in fat cells due to accumulation of fat leads to the elevated levels glucose, insulin, triacylglycerides, and cholesterol in the blood along with high blood pressure [21]. Zucker rats display the early onset of hyperplastic/hypertrophic obesity, and also trigger the onset of type II diabetes. Fat infiltration or hepatic steatosis is another complication of obesity and can be characterized by increased plasma enzymatic markers (alanine aminotransferase [ALT], gamma glutamyltransferase [GGT], and alkaline phosphatase [ALP]) [22]. Probiotics growth could be enhanced by several ways and one is by the supplementation of prebiotics. Soluble fiber in soy could act as prebiotic to increase the colonization of bacteria. Hence, the present study was conducted to investigate the protective effects of feeding soymeal and *Bifidobacterium longum* singly and in combinations against obesity-induced metabolic syndrome traits in obese Zucker rats.

2. Materials and Methods

2.1. Experimental Design

Three-month-old female Zucker rats (Harlan, IN) were housed in an environmentally controlled animal care fa-

cility with 12 hrs of light and dark cycles. All animal care protocols used in this study were approved by the Alabama A & M Institutional Animal Care and Use Committee. After one week of acclimatization, rats were randomly divided into six groups of 3 rats in each. Rats in control group were fed with AIN 93-diet [23]. The rats in treatment groups received AIN-93M modified diet with soy meal (Bob's Red Mill, ND), at 5% and 10% (w/w) concentrations, singly and in combination with a 0.1% probiotic mixture (*Bifidobacterium longum* (BB36), Morinaga Milk Industries, Japan). All diets were made isocaloric and prepared fresh. Weekly body weight and daily feed intake were recorded throughout the study.

At the end of the feeding experiment (100 days), rats were anesthetized under carbon dioxide and blood was drawn by cardiac puncture. Blood was centrifuged immediately at 4000 rpm for 20 min at 4˚C to separate serum. Aliquots of serum were stored at −80˚C until analysis. Liver was removed, weighed, flash frozen in liquid nitrogen and stored at −80˚C for later biochemical analysis. All reagents used in the study were purchased from Fisher Scientific (Suwanee, GA) and dietary ingredients were purchased from MP Biomedicals (Santa Ana, CA).

2.2. Quantification of Liver Lipids

Gravimetric quantification of liver lipids was performed using Folch's method of lipid extraction. One gram of liver was homogenized in a 20-fold volume of 2:1 chloroform-methanol (v/v) mixture. Following homogenization, 0.9% NaCl solution was added to separate the two phases by keeping at room temperature for 2 hours. The bottom layer was extracted, and evaporated in a water bath for 25 minutes to remove the chloroform and methanol. Later, sample was placed in oven at 120˚C for 30 minutes to remove any excess moisture, cooled in a desiccator for 15 minutes, and weight was recorded.

2.3. Preparation of Liver for Analytical Measurements

One gram of liver was homogenized in mixture of 2:1 chloroform-methanol (v/v). later, sodium chloride (0.58%) solution was added to achieve separation of the phases and centrifuged for 20 min at 500 × g. Precipitate was filtered ((3.2 cm Whatman, Fisher Scientific, Suwannee, GA) and washed with chloroform. The filtered organic phase containing the tissue lipids was transferred to clean vials. Enzymatic activity of alanine-amino transferase (ALT), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) were analyzed using commercial kits (Cayman chemicals, VA) by following manufacturer's instructions.

2.4. Serum Biochemical Profile

Serum levels of glucose, triacylglycerols (TAG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), Low density lipoprotein-cholesterol (LDL-C), were analyzed using commercial kits (Cayman chemicals, VA) by following manufacturer's instructions. Absorbance was measured with Biotek Elx808 microplate reader (Winooski, VT).

2.5. Serum Insulin Level

Serum insulin was quantified using a commercially available enzyme linked immune assay (ELISA, Alpco Immunoassays, Salem NH) kit as per manufacturer's instructions.

2.6. Statistical Analysis

Results are presented as means ± SEM. ANOVA was used to determine any significant differences among the treatment groups. The means were separated using Tukey's Studentized Range Test at $P < 0.05$ (SAS 9.0).

3. Results

3.1. Effect of Feeding Soymeal and *B. longum* in Feed Intake, Weight Gain, Liver Weight and Liver Fat Content

The protective effects of soymeal and *B. longum* singly and in combinations against weight gain and fat content of liver were evaluated (**Table 1**). Feed intake was higher in rats fed with control diet (28.71 ± 2.26 g) compared

Table 1. Feed intake, weight gain, liver weight and liver % fat in rats fed Soybean meal, and *Bifidobacterium longum* (BB536) singly and in combination.

Groups	Feed intake (g/day)	Weight Gain (g/100days)	Liver (g)	Liver %fat
Control	28.71 ± 2.26[a]	240.66 ± 4.56[a]	24.47 ± 2.29[a]	25.02
Soy 5%	23.00 ± 1.74[b]	186.12 ± 3.05[c]	14.35 ± 1.30[c]	17.01
Soy 10%	22.00 ± 1.52[b]	183.15 ± 3.59[c]	14.83 ± 1.26[c]	16.13
***B. longum* (*Bl*) 0.1%**	23.14 ± 1.69[b]	221.50 ± 4.26[b]	19.55 ± 1.65[b]	22.46
Soy 5% + *Bl* 0.1%	22.57 ± 1.69[b]	171.11 ± 3.55[d]	19.40 ± 1.70[b]	16.31
Soy 10% + *Bl* 0.1%	21.57 ± 1.52[b]	168.26 ± 3.02[d]	19.00 ± 1.77[b]	16.22

Values are Mean ± SEM; n = 3. [abcd]Values not sharing common superscript in column are significantly different ($P < 0.05$) using Tukey's studentized range test.

to the treatment groups. There were no significant ($P < 0.05$) differences in feed intake (g/day) among the treatment groups. Feeding soymeal (10%) with *B. longum* reduced weight gain by 30%. Rats in treatment groups fed with soy meal had lower weight gain compared to *B. longum* (221.50 ± 4.26) and control (240.66 ± 4.56) fed rats. Obese rats in control group had highest (25.02%) fat content in liver as well as liver weight (24.47 ± 2.29) compared to the treatment groups. Although there were no significant differences among the treatment groups in liver fat (%), however rats fed with soy meal had lower percentage of liver fat.

3.2. Plasma Glucose and Insulin Levels

Feeding *B. longum* alone did not show any affect in serum insulin level and was not significantly different from the rats in control group (**Table 2**). Rats fed with soy either singly or in combination in their diet had lower ($P < 0.05$) levels of serum insulin and plasma glucose levels compared to rats in control group. Highest insulin (2.99 ± 1.02) and glucose (26.82 ± 2.30) serum levels were found in control rats followed by rats in the *B. longum* treatment group. A 10% - 24% decrease was observed in serum glucose level in rats fed the soy diet. Combinational diet of soy reduced the serum insulin level by 22% - 25% and single administration of soy reduced the level by more than 20% compared to the control diet fed rats.

3.3. Serum Biochemical Markers

Effect of adding soy and *B. logum* to the diet of obese rats in modifying the lipid profile was investigated (**Figure 1** and **Figure 2**). Serum concentrations of total cholesterol, TAG and LDL-C were higher ($P < 0.05$) in rats fed the control diet. Rats fed treatment diets had reduced levels, and pronounced reductions were seen in the rats fed the combinational diet compared to the single treatment diets. Rats fed with combinational diet have significant reductions in TAG. Feeding rats with soy lowered ($P < 0.05$) serum cholesterol level compared to rats fed with control and Bl alone. Serum LDL-C levels were reduced by less than 15% in the treatment groups except the group fed soy (10% + Bl 0.1%), where more than a 35% reduction was observed compared to the control group. A 12% - 19% reduction in total cholesterol was observed following administration of soy in diet. On the contrary, HDL-C was found to be highest in rats fed the combinational diets (1.56 - 1.49), followed by single treatment diets and lowest (1.32) was in control diet fed groups.

3.4. Activity of Hepatic Enzyme Markers

Combinational treatment of soy meal and *B. longum* reduced the elevated levels of liver injury enzyme markers ALT, GGT, and ALP (**Figure 3**). Feeding *B. longum* alone did not affect these enzyme markers, which remained high compared to the control group. Feeding soy alone also resulted differences ($P < 0.05$) in activities of enzymes except GGT. There were no significant differences in enzyme activities of rats fed with soy and combination of soy and BL. However, the combinational diets were more effective in decreasing the activity of hepatic enzymes by 1.1 - 1.3 fold.

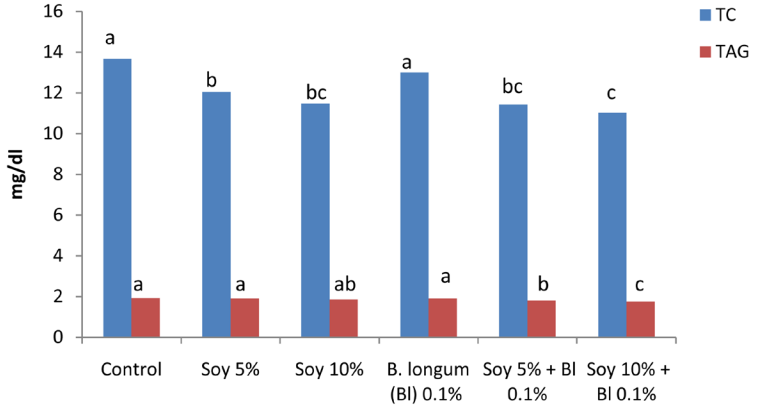

Figure 1. Effect of feeding soybean meal, and *Bifidobacterium longum* (BB536) singly and in combination on total cholesterol and triacylglycerol (TAG) levels in rats. Values are means; n = 3. [abc]Bars not sharing common superscript (among groups) are significantly different ($P < 0.05$) using Tukey's studentized range test.

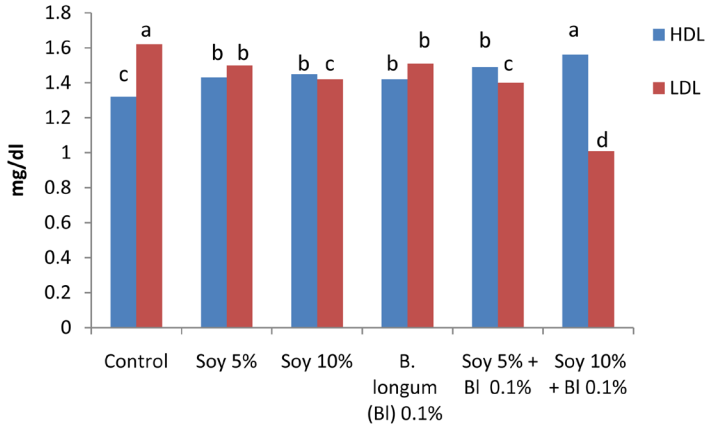

Figure 2. High-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels in obese Zucker rats fed with Soybean meal and *Bifidobacterium longum* (BB536) singly and in combination. Values are means; n = 3. [abcd]Bars not sharing common superscript (among groups) are significantly different ($P < 0.05$) using Tukey's studentized range test.

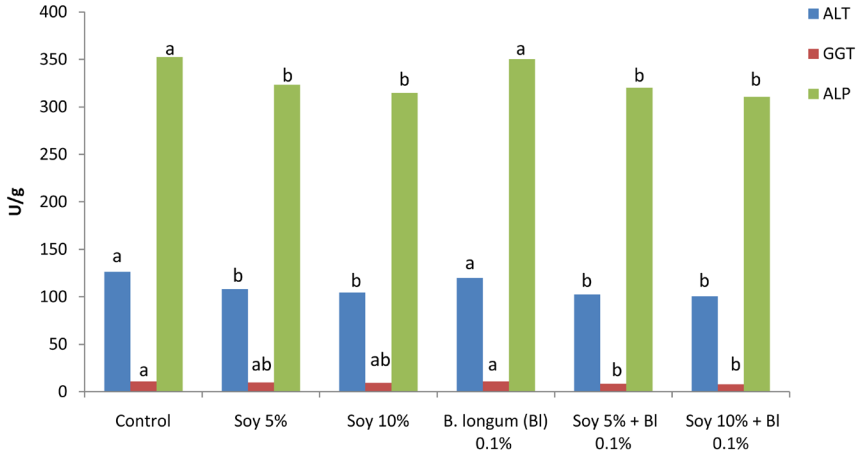

Figure 3. Activity hepatic enzymes in obese Zucker rats fed Soybean meal, and *Bifidobacterium longum* (BB536) singly and in combination (n = 3). ALT—Alanine-amino transferase; GGT—Gama-glutamyl transferase; ALP—Alkaline phosphatase. Values are means; n = 3. [abcd]Bars not sharing common superscript are significantly different ($P < 0.05$) using Tukey's studentized range test.

Table 2. Glucose and insulin levels in rats fed soybean meal and *Bifidobacterium longum* (BB536) singly and in combinations.

Groups	Plasma glucose (mmol/L)	Insulin (ng/ml)
Control	26.82 ± 2.30[a]	2.99 ± 1.02[a]
Soy 5%	24.00 ± 1.36[ab]	2.36 ± 1.12[b]
Soy 10%	21.66 ± 1.30[bc]	2.28 ± 1.01[b]
***B. longum* (Bl) 0.1%**	24.36 ± 1.45[ab]	2.92 ± 1.03[a]
Soy 5% + Bl 0.1%	22.11 ± 1.29[bc]	2.34 ± 0.98[b]
Soy 10% + Bl 0.1%	20.32 ± 1.20[c]	2.22 ± 0.92[b]

Values are Mean ± SEM; n = 3. [abc]Values not sharing common superscript in column are significantly different ($P < 0.05$) using Tukey's studentized range test.

4. Discussion

The present study was conducted to evaluate the single and combinational effect of soybean and *B. longum* against obesity induced traits associated with metabolic syndrome in Zucker rats. A cluster of conditions such as high blood glucose, insulin resistance, dyslipidemia, hypertension and visceral adiposity exists in metabolic syndrome. Obesity and overweight trigger cellular deregulation in adipocytes which proceeds to the development of metabolic syndrome. Change in life style factors such as physical activity and modifications in the diet are the most recommendable changes to manage obesity and metabolic syndrome. A number of studies suggest that feeding dietary components from plant sources such as legumes, beans, and whole grains may play an important role in alleviating metabolic syndrome associated abnormalities [24]-[26]. Feeding soybean meal and a probiotic (*B. longum*-BB536) singly and in combinations at selected concentration offered protection in reducing hyperlipidemia, hypercholesterolemia, and hyperglycemia associated with excess body weight in obese Zucker rats.

Feeding soy meal, and Bl resulted in a significantly ($P < 0.05$) lower feed intake and weight gain compared to the rats fed the control diet. Zucker rats fed Bitter melon (BM) seeds did not have any effect on weight gain or body fat [27]. Another study using BM fruit extract showed a decrease in mean bodyweight and percent body fat. The differences in responses may in part be explained due to differences in supplementation with seeds and fruit extract. Soy meal and Bl may have played a role in decreasing body weight in obese Zucker rats by lowering the energy efficiency. A reduction in weight gain has been observed in animals and humans fed with soybean compared to the casein [28] [29]. The fiber in soy meal may have bound the fat in the diet and resulted in the lower absorption of lipids translating to lower energy absorption. Dietary fiber appears to regulate metabolic disturbance through its modulation of gut micro-flora. There is evidence that supports the hypothesis that obesity and Type 2 Diabetes may be associated with an altered gut micro biota [13] [30]. Dietary fiber rich foods such as soy may also have important prebiotic effects. Several mechanisms have been proposed to explain the positive effect of prebiotics in obesity models. An increase in peptide (GLP-1), which regulates satiety thereby modifies food intake and glucose homeostasis, was observed in prebiotic fed animals [31]. This phenomenon may contribute to the decrease in weight, lower blood glucose levels and significantly improved lipid profile seen in rats fed treatment diets containing soy. A major function attributed to intestinal micro-biota is their capacity to extract energy from non-digestible food components (via short-chain fatty acid (SCFA) production through the fermentation). Increased production of SCFA is assumed to be beneficial by reducing hepatic glucose output and improving lipid homeostasis.

Although the mechanisms by which soy and Bl effect weight gain and feed intake are not fully understood, data suggests that this effect can be attributed not only to lower feed intake by rats fed treatment diets but also to lower energy intake of soy supplemented diets because of their fiber content. Fiber in soy may likely contribute to decrease in weight gain. Fiber may play a role in delaying gastric emptying, increasing meal viscosity and delaying and decreasing absorption of fat and sugar. It also reduces the acceleration of colon transit, and therefore provides satiety.

Rats fed soy and Bl in combination had significantly ($P < 0.05$) lower levels of TAG, and total cholesterol. Prebiotics have also been shown to reduce TAG accumulation and modify inflammatory processes in the liver

[32]. Studies [33] [34] have shown that ingestion of soy proteins improve blood lipid profiles, including lowering TAG, total LDL cholesterol levels, and increasing HDL-C levels. Similar observation was also found in this study. However, in rats fed combination diets (SM + Bl) effects were more pronounced. Soy isoflavones may regulate lipid metabolism by modulating activities of key transcription factors, and thereby altering the expression of several genes (PPARγ) involved in regulation of lipogenesis or lipolysis, and reduce serum insulin and insulin resistance [33] [34]. The hypo-triacylglyceridemia and hypo-cholesterolemia effects of psyllium were attributed to the possible delay in absorption of TAG and sugars from the small intestine [35]. The lower TAG and cholesterol levels in psyllium fed rats are in agreement with results in this study.

The present study demonstrated that feeding soy and Bl significantly ($P < 0.05$) lowered serum cholesterol levels. However, we also observed that feeding treatment diets increased serum HDL-C levels significantly. These results suggest that combinational diet may play a significant role in the reduction of cardiovascular disease risk factors which is one of the long term effects of obesity. The hypolipidemic effects of soy can be explained due to lowered insulin levels. Reduced insulin resistance is known to activate enzyme lipoprotein lipase (LPL), which hydrolyzes LPL-bound TAG [36]. Feeding soy, and Bl in combination also resulted in a hypoglycemic effect. Feeding a new fiber fraction from soybean to Zucker rats for a period of 14 weeks resulted in a decrease in plasma glucose and insulin levels with no changes in TAG and cholesterol levels [37]. The TAG and cholesterol levels in the liver were however lower in fiber fed rats compared to the control. Obese rats developed hypercholesterolemia, hypertriglyceridemia, and hyperinsulinemia. Feeding soy alone to obese rats resulted in significantly lower levels of cholesterol and TAG compared to their obese controls, without changes in insulin serum levels, confirming hypolipidemic effect of soy diet [38]. However feeding combinational diets (Soy, Bl) resulted in lower insulin levels along with other reductions compared to the controls.

The metabolic syndrome is associated with long-term inflammation condition resulting in an increased production of cytokines such as TNF-α [39] [40]. This factor is over-expressed in obesity and may regulate insulin resistance. Soluble fiber has been shown to decrease TNF-α levels when compared to controls. Dietary fiber such as those found in soy meal may play a major role in managing the risk of obesity associated with metabolic syndrome. Some studies [41]-[44] have shown the beneficial effects of soluble fiber on weight management, plasma cholesterol, lipoprotein levels and diabetes.

Zucker Rats fed psyllium husk (3.5%) for 25 weeks had significantly lower body and liver weights compared to the controls. Food intakes of the two groups also significantly differed [42]. This was also seen in the present study where rats fed soy meal had significantly lower weight gain and liver weights compared to the control. Rats fed soy and *B. longum* in combination also had higher reductions in liver weight, weight gain compared to control rats. Epigallocatechin gallate (EGCG), but not related catechins, significantly reduced food intake, body weight, blood levels of insulin, glucose, cholesterol, and triglyceride in obese male Zucker rats [45].

Fat infiltration into liver or hepatic steatosis is the complication of obesity and metabolic syndrome. Elevated levels of hepatic marker enzymes (alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), and alkaline phosphatase (ALP)) are high in the plasma along with increase in liver weight during this condition [46]. Rats fed with control had higher liver weight and higher hepatic enzyme activity indicating that hepatomegaly and liver steatosis. This was improved when diets were supplemented with soy and much pronounced effect was observed in combinational diet. Neyrinck *et al*. (2004) [47] also reported a lower ALT activity following consumption of dietary oligofructose, a prebiotic dietary fiber.

Combinational diet was more effective in offering protective effects in reducing the adverse level of traits related to metabolic syndrome compared to their single counterparts. Although the exact mechanisms were not known, the positive effect might be due to the various cellular and molecular effects of probiotics and soy simultaneously in regulating the cellular processes. The fiber in soy could also increase the colonization of probiotics by providing feed.

5. Conclusion

The results of the study have shown that dietary administration of soy has protective effects against metabolic syndrome traits in Zucker rat model of obesity and metabolic syndrome. These protective effects were accompanied by decreases in body weight, liver weight. Combinational diet has offered more protection by reducing serum lipid profile, glucose level and also by improving insulin resistance. This study has proven the potential benefits of simultaneous supplementation probiotic and soy in reducing the metabolic syndrome characteristics.

Further long-term feeding and mechanistic studies are needed to confirm the additive or synergistic effects of the supplementation.

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