INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–1932/2019/22–4–787–792
DOI: 10.17957/IJAB/15.1131
http://www.fspublishers.org

Full Length Article

Inulin, Fructooligosaccharide and *Lactobacillus acidophilus* Affects Body Weight Gain and Blood Metabolites in Polycystic Ovarian Syndrome Rats

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Abstract

Prebiotics and probiotics have the potential to be an appropriate treatment of polycystic ovarian syndrome (PCOS) as they target the proposed initial pathological consequences in this syndrome. Present study of 16 weeks (involving 6 weeks for adjustment period and 10 weeks for collection period) was carried out to investigate *in vivo* potential effects of prebiotics and probiotic in PCOS induced female Wistar rats. Forty-five female rats were randomly separated in 5 groups (9 rats/group) and alloted names to diets *viz*., **NC** (Negative Control), **PC** (Positive control), **I5** (inulin 5% w/w), **F5** (fructooligosaccharide 5% w/w) and **L1** (1% *Lactobacillus acidophilus* w/w about 1×10^{10} CFU/g). All rats were PCOS induced except rats of NC group. Rats were offered water and feed *ad labitum*. Body weights and blood glucose were recorded weekly. At the end of tenth week of collection period all rats were bled to collect blood samples for analyzing lipid profile. At 8th, 9th and 10th week of trial the dietary treatments significantly ($P < 0.05$) reduced the weight of the PCOS induced rats as compare to PC ($P < 0.05$). Similarly, at 6th, 7th, 8th, 9th, and 10th week dietary treatments decreased blood glucose levels of PCOS induced rats as compare to PC ($P < 0.05$). In case of lipid profile, dietary treatments reduced the overall level of cholesterol, triglycerides and low-density lipoproteins in PCOS rats as compare to PC ($P < 0.05$). In conclusion, prebiotics and probiotics reduce body weight, blood glucose and improve lipid profile. Therefore, addition of prebiotics and probiotics could be novel approach to control PCOS.

Keywords: Syndrome; Infertility; Dietary; Prebiotics; Probiotic; Treatment

Introduction

Nutrient imbalance in women is one of the important cause of polycystic ovarian syndrome (PCOS). Polycystic ovarian syndrome worldwide prevalence is about 4–10% (Asuncion *et al.,* 2000). The PCOS in young girls results in metabolic disorders like hyperinsulinemia, obesity, type II diabetes mellitus (DM2), cholesterol, low high-density lipoprotein, cardiovascular disease (CVD), hypertriglyceridemia and hypertension (Glueck *et al.,* 2006; Norman *et al.,* 2007; Moran *et al.,* 2009).

However, studies showed that PCOS is linked with oxidative stress, insulin resistance and diabetes, which may be caused by advanced glycation end products (AGEs) contents produced by cooking of high fat content diet at high temperature (Altieri *et al.,* 2013). The relationship between risk of occurrence PCOS and irregular diet arrangements is still contrary. In medical treatment of PCOS, medicine like metformin has been used till now (Asuncion *et al.,* 2000; Stamets *et al.,* 2004). The studies regarding dietary treatment of PCOS are limited. That's why prebiotics and probiotics were chosen as utmost modern treatment in controlling PCOS. The literatures have been proved that prebiotics and probiotics either used separately or as a synbiotics, they have anti-hyperlipidemic, anti-hyperinsulinemic and antiobesogenic features (Dikeman *et al.,* 2006). Though, there was limited research work associating the direct impact of prebiotics and probiotics on PCOS. The objective of this investigation was to check individual effects of two types of prebiotics (inulin and fructo-oligosaccharide) and one strain of probiotic (*Lactobacillus acidophilus*) and mode of action by which the prebiotics and probiotics can change blood glucose, body weight and lipid profiles in PCOS induced rats modeling.

Materials and Methods

Study Animals

Forty-five days old, 45 female rats (Wistar Albino) weighing130±10 grams having 2 successive estrus cycles

To cite this paper: Masroor, A., M.U. Nisa, M.K. Khan, U.A. Ashfaq and F. Komal, 2019. Inulin, fructooligosaccharide and lactobacillus acidophilus affects body weight gain and blood metabolites in polycystic ovarian syndrome rats. *Intl. J. Agric. Biol*., 22: 787–792

were procured from National Institute of Health, Islamabad. The rats were kept at 45 to 55% relative humidity and 25 ± 1°C temperature under 12 h light: 12 h dark cycle. All experimental protocols for rats were performed by adopting the procedures for the precaution and usage of laboratory animals accepted by the National Institutes of Health Guide (NIH Publications No. 8023, reviewed 1978). The investigational techniques were permitted by the Animal Ethical Committee of Government College University, Faisalabad, Pakistan. In adjustment period of six weeks PCOS was induced in female rats except NC rats group following the procedure of Ghasemzadeh *et al.* (2013). Initially for acclimatization, a basal diet was given to rats for a week. The composition of experimental and basal diets is given in Table 1. In the I5 and F5 diets the quantity of inulin and fructooligosaccharides (FOS) was replaced with cellulose in basal diets. After seven days of acclimatization, 45 Wistar female rats were separated into 5 groups (each group having 9 rats) entitled according to diets *viz*., NC (Negative Control), PC (Positive control), I5 (inulin 5% w/w), F5 (fructooligosaccharide 5% w/w) and L1 (1% *L. acidophilus* w/w about 1 × 1010 CFU/g). Both control groups (NC and PC) were nourished basal diet. Prebiotics and probiotics both are different domains so their amount of addition in rat's diet is different. The rats fed PC, I5, F5 and L1 diets were PCOS induced. Rats were offered water and diets *ad libitum.* The rats diet was *isonitrogenous* and i*socaloric*. The weekly consumption of feed and water was observed minimum one week earlier from the initiation of treatments in order to examine the quantity of water and feed taken by each rat. Dietary treatments were applied by using Completely Randomized Design (CRD) in such a way that each treatment had three replicates and each replicate had 3 rats.

Body Weight

Body weights of PCOS rats fed different treatment diets were recorded weekly *via* digital weighing scale before morning feeding.

Blood Sampling for Blood Glucose and Lipid Profile Analysis

Blood glucose: Weekly blood glucose was checked by glucometer *ACCU-CHEK*®. The blood sample was obtained from the tail of rat.

Lipid profile analysis: On the completion of trial 5cc blood samples from jugular vein of rats were obtained and arranged for lipid profile analysis (Ghasemzadeh *et al.*, 2013). Lipid Profile was checked by microplate reader URIT 660. Cholesterol was determined with kit method by using Biosystem cholesterol kit REF. 11505 (Barcelona, Spain). Triglyceride was estimated by Triglycerides liquiform mono reagent kit (Paris, France). LDL (low-density lipoproteins) and HDL (High density lipoproteins) were determined by Wiener kit having REF. 1220229 and REF 1220114 respectively (Rosario, Argentina).

Statistical Analysis

The data attained from entire investigational sources was exposed to statistical analysis through CRD, by following the technique defined by Steel *et al.* (1997). Duncan Multiple Range Test was performed to compare means among groups. The significance level was $P < 0.05$.

Results

Result of effects of inulin, FOS and *L. acidophilus* on weekly body weight of rats is presented in Table 2. Results showed that, in first seven weeks, dietary treatments were unable to control the increase of weight in PCOS induced rats as compare to PC diet ($P < 0.05$) (Table 2). However, in last three consecutive weeks (8^{th} to 10^{th} weeks) rats fed I5, F5and L1 diets cope to increase in weight in PCOS induced rats as relate to PC ($P < 0.05$).

Results of effects of inulin, FOS and LA on weekly blood glucose (mg/dL) of PCOS induced rats are presented in Table 3. In case of blood glucose levels, dietary treatments were unable to control the increase in blood glucose for first five weeks of trial in PCOS induced rats as equate to PC (Table 3). However, dietary treatments significantly control the increase in blood glucose level from 6^{th} to 10^{th} week of trials in PCOS induced rats as compared to PC ($P < 0.05$).

The statistical results regarding cholesterol, HDL, triglycerides and LDL levels of rats fed NC, PC, I5, F5 and L1 diets have been shown in Table 4. Results indicated that rats fed I5, F5 and L1 diets exhibited significant decrease in cholesterol and triglycerides levels in PCOS induced rats as compared to PC rats ($P < 0.05$). It is obvious from the results that after giving the treatment diets to PCOS rats, HDL level was increased significantly ($P < 0.05$) in the rats fed I5, F5 and L1 diets but rats fed L1 diet showed significantly ($P < 0.05$) the highest HDL level (than the other rats fed I5, F5 and PC diets). However, the HDL level of rats fed I5 and F5 diet was also significant ($P < 0.05$) as equate to rats fed PC diet, but they revealed non-significant ($P > 0.05$) trend among each other. In case of triglycerides, dietary treatment reduced the level of triglycerides in PCOS induces rats as compare to PC ($P < 0.05$). Similarly, LDL level was decreased by dietary treatments in PCOS induces rats as compare to PC. Interestingly, I5 showed similar result with negative control diet.

Discussion

The current study revealed the beneficial effect of inulin, FOS and LA in reducing weight of PCOS induced rats. The proposed mechanism of weight loss by prebiotics and probiotics effects is unknown. But, specific literature have revealed that fermentation of prebiotics promptly and

Table 1: Ingredient composition of experimental diets

Ingredients (g/1000 g)	NC	PC	I5	F5	L1
Corn-starch	397.5	397.49	397.49	397.49	397.4879
Maltodextrin	132	132	132	132	132
Sucrose	100	100	100	100	100
Casein	200	200	200	200	200
L-Cysteine	3	3	3	3	3
Soybean oil	70	70	70	70	70
Cellulose	50	50	0	0	40
AIN-93-VX vitamin mix	10	10	10	10	10
AIN-93G-MX mineral mix	35	35	35	35	35
TBHQ[b]	0.014	0.014	0.014	0.014	0.014
Choline bitartrate	2.5	2.5	2.5	2.5	2.5
I5	0	0	50	0	0
F5	0	0	0	50	0
L1	0	0	0	0	10
Total energy, kcal	3960	3960	3960	3960	3960

NC (Negative Control), PC (Positive control), I5 (inulin 5% w/w), F5 (fructooligosaccharide 5% w/w) and L1 (1% *L. acidophilus* w/w about 1×10^{10} CFU/g)

Table 2: Effect of inulin, FOS and *L. acidophilus* on weekly body weight (g) of PCOS induced rats

Weeks	Treatments					
	NC	PC	I5	F5	L1	P- Value
1st week	132.7 ± 0.55^{a}	135 ± 0.29^{b}	134.9 ± 0.8^{b}	133.4 ± 0.2^{ab}	133.9 ± 0.51^{ab}	0.042
2nd week	136.2 ± 0.58^{a}	138.2 ± 0.41^{b}	138 ± 0.30^{b}	138.9 ± 0.12^{b}	139.2 ± 0.62^{b}	0.001
3rd week	137 ± 0.62^{a}	142.4 ± 0.49^{b}	144.2 ± 0.98^{b}	141.2 ± 0.39^{b}	144.1 ± 1.21^{b}	0.030
4th week	138.7 ± 0.76^{a}	149.2 ± 0.85^{b}	147.3 ± 0.89^{b}	147.2 ± 0.20^{b}	148.1 ± 0.70^{b}	0.001
5th week	140.1 ± 1.17^{a}	152.6 ± 0.89^{b}	149.0 ± 0.56^{b}	148.5 ± 0.20^{b}	151.2 ± 0.71^{b}	0.005
6th week	142.3 ± 1.19^{a}	155.3 ± 0.99^{b}	155.4 ± 0.89^{b}	154.1 ± 0.30^{b}	153.2 ± 0.40^{b}	0.002
7th week	144.4 ± 0.85^{a}	161.3 ± 0.85^{b}	158.6 ± 0.79^{b}	156 ± 0.78^{b}	157.8 ± 1.10^{b}	0.066
8th week	146.5 ± 0.53^{a}	163.4 ± 0.76^{c}	161.3 ± 0.70^{b}	161.1 ± 0.99^{b}	159.4 ± 0.89^{b}	0.035
9th week	148.1 ± 0.12^{a}	169.5 ± 0.96^{c}	164.5 ± 0.93^{b}	164.5 ± 0.56^{b}	164.6 ± 0.61^{b}	0.002
10th week	150.3 ± 0.42^{a}	174.4 ± 0.76^{c}	167.4 ± 1.23^{b}	167.8 ± 0.18^{b}	168.3 ± 0.85^{b}	0.007

NC (Negative Control), PC (Positive control), I5 (inulin 5% w/w), F5 (fructooligosaccharide 5% w/w) and L1 (1% *L. acidophilus* w/w about 1×10^{10} CFU/g). Mean ± standard error. Values sharing same letters differ non-significantly (P>0.05)

entirely occurs in the colon, and on reaching the liver *via* the portal vein, they cause regulation of cholesterol metabolism, and decrease triglyceride storing capacity of hepatocytes, thus leading to ingestion and metabolism of triglycerides in the body. It is assumed that some probiotics might prevent the consumption of dietary fat *via* elevating the quantity of fat evacuated along with feces (Ogawa *et al.*, 2015). Probiotics belong to family (*Lactobacillus*) have been indicated to purpose in this approach (Hamad *et al.*, 2008). The probiotics could also reduce the incidence of weight gain *via* some mechanisms involving in releasing appetite-reducing) hormone GLP-1 as well as increasing protein ANGPTL4 that further lead to reduce storage of fat (Aronsson *et al.*, 2010). Though, it is significant to understand that these mechanisms are under investigation. The conclusions of recent research work are in line with two researches, which presented that incorporation of inulin/fructose oligosaccharides, for long period (120 and 84 days), could have ability to support obese individuals in lowering weight whose routine diet does not contain any of FOS and inulin (Cani *et al.*, 2006; Delzenne and Cani, 2010). The current results are quite comparable with the work of Sanchez *et al.* (2014), who found that oligosaccharide and inulin significantly reduced weight in female group in the first 12 weeks. Likewise, Dehghan *et al.* (2013) observed that consumption of 10 g oligofructose enriched inulin by PCOS females for 8 weeks considerably reduced weight and body mass index (BMI). The recent results are coherent with the former study of Genta *et al.* (2009) and Parnell and Reimer, (2009) who noticed significant reduction in waist circumference, BMI, body weight and self-reported calorie intake of overweight subjects after a daily supplementation of 0.14g/kg and 21g FOS. The results of study of Shoaei *et al.* (2015) were opposite to present study in which he choose 72 females aged 15–40 (years) old identified with PCOS. These females were casually divided into 2 groups 36 females getting probiotic supplements in form of capsule having multiple strains including LA and 36 females taking placebo in the form of starch and maltodextrins but no bacteria for 8-week. In the end of study, he found no momentous changes in terms of weight and BMI among probiotic and placebo groups at the baseline of the study.

In present investigation the reduction of blood glucose level by rats fed I5, F5 and L5 diets might be due to the individual metabolic effects of inulin, FOS and LA on reducing blood glucose. The prebiotic inulin might regulate serum glucose by decreasing hepatic gluconeogenesis (as the result of propionate production *via* fermentation), delaying stomach emptying and postponing entrance of glucose into blood. Furthermore, variation in gut hormones *e.g.*, GLP-1 (Cani and Delzenne, 2009;

Table 3: Effect of inulin, FOS and *L. acidophilus* on weekly blood glucose (mg/dL) of PCOS induced rats

Weeks	Treatments					
	NC	PC	I5	F5	L1	P Value
1st week	99.22 ± 0.49[a]	164.22 ± 3.39[b]	160.22 ± 2.61[b]	160.66 ± 1.26[b]	161.23 ± 4.78[b]	0.007
2nd week	95.10 ± 1.08[a]	165.20 ± 4.49[b]	161.12 ± 4.25[b]	158.00 ± 5.10[b]	163.00 ± 4.44[b]	0.001
3rd week	92.00 ± 3.75[a]	162.20 ± 3.50[b]	157.29 ± 3.79[b]	151.00 ± 2.49[b]	156.32 ± 4.87[b]	0.015
4th week	92.88 ± 3.85[a]	158.56 ± 4.54[b]	153.56 ± 3.39[b]	152.66 ± 2.59[b]	157.66 ± 4.50[b]	0.001
5th week	90.12 ± 3.45[a]	161.00 ± 4.59[b]	149.26 ± 4.50[b]	151.00 ± 3.55[b]	148.81 ± 5.88[b]	0.002
6th week	88.22 ± 2.51[a]	162.00 ± 3.29[c]	146.00 ± 3.22[b]	146.77 ± 4.29[b]	147.55 ± 2.89[b]	0.001
7th week	87.33 ± 2.53[a]	161.00 ± 3.57[c]	143.19 ± 2.87[b]	144.00 ± 5.19[b]	145.00 ± 3.36[b]	0.007
8th week	91.12 ± 2.39[a]	163.39 ± 3.66[c]	141.55 ± 3.59[b]	142.22 ± 2.86[b]	142.29 ± 4.08[b]	0.04
9th week	94.25 ± 2.89[a]	164.59 ± 3.26[c]	141.19 ± 3.19[b]	141.12 ± 3.35[b]	141.00 ± 3.77[b]	0.005
10th week	96.11 ± 1.30[a]	168.77 ± 3.19[c]	140.20 ± 3.70[b]	140.29 ± 3.89[b]	140.49 ± 3.78[b]	0.002

NC (Negative Control), PC (Positive control), I5 (inulin 5% w/w), F5 (fructooligosaccharide 5% w/w) and L1 (1% *L. acidophilus* w/w about 1×10^{10} CFU/g). Values sharing same letters differ non-significantly ($P > 0.05$)

Table 4: Effect of inulin, FOS and *L. acidophilus* on lipid profile of PCOS induced rats

Parameters	Treatments					
	NC	PC	I5	F5	L1	P-value
Cholesterol	122 ± 4.34[a]	165 ± 3.46[c]	142 ± 2.30[b]	146 ± 2.60[b]	145 ± 3.83[b]	<0.001
HDL	46.49 ± 1.89[a]	28.00 ± 1.51[d]	32.33 ± 1.39[c]	34.25 ± 1.18[bc]	39.01 ± 1.30[b]	0.005
Triglyceride	90.0 ± 2.52[c]	137 ± 2.01[a]	113.0 ± 3.06[b]	117.0 ± 4.17[b]	120.2 ± 2.39[b]	0.002
LDL	133.8 ± 2.76[a]	167 ± 2.85[c]	142.3 ± 1.86[a]	143.5 ± 2.01[b]	145.4 ± 2.01[b]	0.04

NC (negative control), PC (Positive control), I5 (inulin 5% w/w), F5 (FOS 5% w/w), L1 (1% *L. acidophilus* w/w about 1×10^{10} CFU/g), HDL (high density lipoprotein) and LDL (low density lipoprotein). Mean ± standard error. Values sharing same letters differ non-significantly ($P > 0.05$)

Kozmus *et al.*, 2011), reduction in body weight and BMI (Sohaily *et al.*, 2013), *via* colonic prebiotics fermentation short-chain fatty acids formation (Cherbut, 2003; Zhang *et al.*, 2008) might affect metabolism of glucose in the body. In current study FOS also showed optimum effect in decreasing blood glucose level. Oligofructose may regulate the increase in β cell mass, pancreatic insulin, GLP-1 and GLP-2 (Cani *et al.*, 2006) and pancreatic insulin metabolism of glucose regulation. Current study results are in line with Busserolles *et al.* (2003) and presented significant reduction in postprandial glucose by treating rats with FOS. Parallel consequences were also perceived *via* Luo *et al.* (1996) as in diabetic rats postprandial glycemia was reduced later digestion of twenty percent oligofructose comprised diet for the extent of 2 months, regardless of absence of glycemic or insulinemic modification response to a saccharose or maltose load. Oligofructose treated streptozotocin (STZ) induced diabetic rats improved glucose tolerance was informed (Cani *et al.*, 2005). Frequent experimental models in genetically and chemical or diet transformed animals have been discovered that LA has strength for delaying and preventing of diabetes beginning (Yun *et al.*, 2009). The LA might lessen level of blood glucose *via* influence gut bacteria to produced insulinotropic polypeptides and glucagon so increase glucose uptake by muscle. Along with liver confirms the additional blood glucose absorption in the form of glycogen (Al-Salami *et al.*, 2008). The verdicts of current research work were similar as Zhang *et al.* (2015) who studied the probiotics influence in diabetic patients on metabolism of glucose. The outcomes specified the momentous reduction in glycosylated hemoglobin (HbA1c), insulin concentration and fasting plasma glucose. The probiotics antioxidant activity might show a fundamental part in inhibiting diabetic risk factors onset (Willcox *et al.*, 2004). The conclusions of current research are in lined with Yadav *et al.* (2007) who surveyed the low fat (2.5%) yoghurt efficiency (having probiotic and *L. casei*) on rats induced type II diabetes. The diet added with probiotic yoghurt meaningfully overdue onset of hyperinsulinemia, dyslipidemia, glucose intolerance, hyperglycemia, and oxidative anxiety elaborating a reduction in diabetes risk and its related health troubled significances.

In the present investigation the accumulation of FOS, inulin and LA applied valuable influence on lipid profile, as they decreased the triglycerides, LDL, serum cholesterol though enhanced the level of HDL. Prebiotics role in cholesterol lowering is primarily due to SCFAs (short chain fatty acids). Particularly butyrate is familiar to avoid production of cholesterol in liver and convey an energy basis for epithelial cells in colon of human; propionate in liver may delay the creation of fatty acids, consequently reducing the frequency of triacylglycerol excretion (Delzenne *et al.*, 2002). The propionate likewise participates in the control of production of hepatic cholesterol and it brings down production of cholesterol level which finally basis of lowering the plasma cholesterol levels (Delzenne and Kok, 1999). The outcomes of recent research are in line with study of Brighenti *et al.* (1999) in which twelve subjects were taken and subsequently ingestion of inulin, triglycerides and serum cholesterol were lowered. The investigation on 12 healthy young males eaten ready to eat breakfast cereal along with inulin (9 g/day) showed 27% reduction in fasting triglycerides and 5% reduction in total cholesterol (Cani *et al.*, 2006). The investigations of current research work are in agreement with Delzenne *et al.* (2002) who emphasized the

consequence of dietary FOS on lipid decreasing mechanism in mice. According to their research plan, animals were administered dosages of oligofructose (20 gram per 100 gram of food) for the period of thirty days. He observed enormous reduction in the ratio of liver triglycerides and serum in animals as related to controls. Conflicting to current research work two similar research work by Luo *et al.* (1996 and 2000), unsuccessful to designate any momentous cholesterol decreasing properties after providing FOS twenty gram per day for the period of four week to ten adults with noninsulin-dependent diabetes consumption or twelve young healthy males. Certain research work also assured that probiotics might accelerate a decrease in level of blood cholesterol and enhance the resistance of LDL against oxidation hence initiating hypotension (Goel *et al.,* 2006). In current research work LA expressively decreased the LDL, triglycerides and serum cholesterol though enhanced the HDL. Subsequently showing *in vitro* research Liong and Shah (2005) confirmed that LA might eradicate cholesterol not only over the mechanism of absorption throughout development as well as through cholesterol binding at cellular surface level and precipitation of cholesterol with unconjugated bile. The consequences of Kieling *et al.* (2002) were in agreement with current study. He studied the effectiveness of yoghurt supplemented with LA 145 and *B. longum*913 on hypercholesterolemia. After the ingestion of yoghurt (300 g/day) for twenty-one weeks, the attained consequences showed the expressively enhanced HDL cholesterol levels but the ratio of low density lipoprotein and high density lipoprotein, cholesterol was lowered.

Conclusion

Inulin, FOS and *L. acidophilus* had positive influence in lowering blood glucose, body weight, serum cholesterol, triglycerides, LDL while increasing HDL levels. Thus, prebiotics seems to be an appropriate alternative treatment to medications in PCOS females. Though, for definitive and better conclusion, more researches with large sample sizes are needed.

Acknowledgments

The authors would like to acknowledge the Department of Physiology, Government College University, Faisalabad, Pakistan for its support in biochemical analysis.

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[Received 31 Dec 2018; Accepted 11 May 2019; Published (online) 20 Aug 2019]