Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Synbiotic (Lactobacillus Pentosus GSSK2 And Isomalto-Oligosaccharides) Supplementation Modulates Pathophysiology And Gut Dysbiosis In Experimental Metabolic Syndrome

Sakshi Khanna
Panjab University
Kanthi Kiran Kondepudi
National Agri-food Biotechnology Institute (NABI), S.A.S. Nagar
Mahendra Bishnoi
National Agri-food Biotechnology Institute (NABI), S.A.S. Nagar
Geeta Shukla (geeta_shukla@pu.ac.in)
Panjab University

Research Article

Keywords: disease, microbiota, syndrome, histomorphology, prodigious

Posted Date: May 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-529631/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Synbiotic (*Lactobacillus pentosus* GSSK2 and isomalto-oligosaccharides) supplementation modulates pathophysiology and gut dysbiosis in experimental metabolic syndrome

Sakshi Khanna[1], Kanthi Kiran Kondepudi[2*], Mahendra Bishnoi[2], Geeta Shukla[1*]

[1]Department of Microbiology, Panjab University, Chandigarh, India

[2]National Agri-food Biotechnology Institute (NABI), S.A.S. Nagar, Punjab, India

[*]Corresponding author

Professor Geeta Shukla

Department of Microbiology, Basic Medical Sciences Block A, South Campus, Panjab University, Chandigarh, 160014, India. Email:geeta_shukla@pu.ac.in

ORCID: 0000000212122211

Dr. Kanthi Kiran Kondepudi

Healthy Gut Research Group, Food & Nutrition Biotechnology Division, National Agri-Food Biotechnology Institute (NABI), S.A.S. Nagar, Punjab 140306, India. Email: kiran@nabi.res.in

Abstract

Metabolic syndrome a lifestyle disease, where diet and gut microbiota play a prodigious role in its initiation and progression. Prophylactic bio-interventions employing probiotics and prebiotics offer an alternate nutritional approach towards attenuating its progression. The present study aimed to evaluate the protective efficacy of a novel synbiotic (*L. pentosus* GSSK2 + isomalto-oligosaccharides) in comparison to orlistat in an experimental model of metabolic syndrome. It was observed that supplementation of synbiotic for 12 weeks to Sprague Dawley rats fed with high fat diet, ameliorated the anthropometric parameters i.e. weight gain, abdominal circumference, Lee's index, BMI and visceral fat deposition along with significantly increased fecal Bacteroidetes to Firmicutes ratio, elevated population of *Lactobacillus spp., Akkermansia spp., Faecalibacterium spp., Roseburia spp.* and decreased Enterobacteriaceae compared with HFD animals. Additionally, synbiotic administration to HFD animals exhibited improved glucose clearance, lipid biomarkers, alleviated oxidative stress, prevented leaky gut phenotype, reduced serum lipopolysaccharides and modulated the inflammatory, lipid and glucose metabolism genes along with restored histomorphology of adipose tissue, colon and liver compared with HFD animals. Taken together, the study highlights the protective potential of synbiotic in comparison with its individual components in ameliorating HFD-induced metabolic complications.

Introduction

Metabolic syndrome, which encompasses obesity, dyslipidemia, hyperglycemia and chronic inflammation in metabolic tissues has emerged as a public health challenge affecting about one quarter of the world's population and the prevalence is predicted to escalate in developed, developing and under developed countries[1]. Inclination towards high-energy diet and decreased physical activity have led to the increased prevalence of metabolic syndrome globally, and is associated with several pathophysiological alterations such as weight gain, ectopic fat deposition, hyperlipidemia, insulin resistance and alterations in gut microbiota, referred as the "second genome"[2,3]. Moreover, gut dysbiosis associated with high calorie intake, leads to significant loss of microbial diversity, increased energy harvest, disruption of gut barrier integrity, low grade inflammation resulting into metabolic endotoxemia, production of reactive oxygen species and deregulation of genes involved in lipid, glucose metabolism and inflammation which play a

prodigious role in the advancement of metabolic complications[4]. Therefore, development of gut microbiota targeted strategy employing probiotics, prebiotics and synbiotics, that could potentially re-establish the gut homeostasis is budding as the novel prophylactic biointervention for alleviating metabolic disorders.

Probiotics are 'live microorganisms that when administered in adequate amounts, confer a health benefit on the host' and the health promoting potentials include maintenance of gut homeostasis, alienating pathogens, enhancing the bioavailability of nutrients, stimulation and modulation of host immune system[5,6]. Due to their multifarious benefits, probiotics are currently the major focus of attention to be explored as potential biotherapeutics for the management of various gastrointestinal ailments, liver damage, cancers, inflammatory and metabolic disorders[7,8]. Prebiotics, are non-digestible food ingredients that selectively stimulate the growth or activity of beneficial microorganisms in the host[9]. Biofermentation of prebiotic fibres releases short chain fatty acids, which are the messengers of cross talk between gut microbiota and host thereby regulating intestinal inflammatory response and colon health[10].

Synbiotic, combination of probiotic with prebiotic, has been found to have a synergistic effect on host health and various experimental studies have indicated the protective and stimulatory effect of synbiotics[11,12]. Recently, we have observed that oral supplementation of probiotic isolate *L. pentosus* GSSK2 ameliorated the adiposity parameters and various biochemical components of metabolic syndrome and the probiotic isolate was found to metabolize isomalto-oligosaccharides (IMOs), a prebiotic reported to possess protective effects in an array of ailments by modulating immune response, improving gut flora, regulating carbohydrate and lipid metabolism [13-15]. Though some studies have reported that synbiotics can help in alleviating obesity and related complications, but no information is available with reference to the combination of probiotic with IMOs as synbiotic in ameliorating diet induced metabolic syndrome. Therefore, the need of hour is to explore novel biointerventions that are safer yet effective for the management of metabolic syndrome to overcome the adverse effects of commonly prescribed weight loss drugs like orlistat[16]. Thus, the present study aimed to evaluate the protective efficacy of a novel synbiotic (*L. pentosus* GSSK2 + IMOs) in comparison to orlistat in experimental model of metabolic syndrome.

Results

Improved anthropometric parameters and reduced adiposity

It was interesting to observe that animals fed either with synbiotic+HFD (Group VIII) or orlistat+HFD (Group IX) had significantly ($p < 0.05$) reduced body and liver weight, adipose tissue weight, abdominal circumference, BMI and Lee's index followed by *L. pentosus* GSSK2+ HFD (Group IV) and IMOs+HFD (Group VI) compared with HFD (Group II) animals (Fig. 1a, b, c, d & e). However, animals belonging to either probiotic (Group III), prebiotic (Group V) or synbiotic (Group VII) had adiposity parameters comparable to control (Group I) and average feed intake was almost similar in animals belonging to all the groups (Group I-IX, Fig. 1f).

Interestingly, gross macroscopic examination of animals belonging to synbiotic+HFD (Group VIII) showed minimum fat deposits in adipose tissue, followed by orlistat+HFD (Group IX), *L. pentosus* GSSK2+HFD (Group IV) and IMOs+HFD (Group VI) compared with HFD animals (Group II, Fig. S1 a to i).

Improved glucose tolerance

It was observed that administration of either probiotic *L. pentosus* GSSK2 (Group IV), synbiotic (Group VIII) or orlistat (Group IX) to HFD animals significantly ($p < 0.05$) lowered the fasting blood glucose level and enhanced glucose tolerance compared with HFD (Group II) animals that had increased fasting blood glucose level and impaired glucose clearance from circulation as depicted by an increase in the AUC during OGTT (Fig. 2a, b & c). However, animals belonging to IMOs+HFD (Group VI) did not show any significant improvement in glucose tolerance while animals supplemented either with probiotic (Group III), prebiotic (Group V) or synbiotic (Group VII) had blood glucose parameters comparable to control (Group I, Fig. 2a, b & c).

Increased LAB count and lipids in feces

The LAB count in feces, an indicator of healthy gut, increased significantly ($p < 0.05$) in animals belonging to synbiotic, probiotic and prebiotic inspite of HFD feeding compared with control (Group I) and HFD (Group II) animals but maximum increase in LAB was in synbiotic (Group VII) animals (Figure 2d). Further, it was observed that supplementation of synbiotic led to increased fecal lipid excretion inspite of HFD, compared with counter controls (Group II, IV, VI, IX) (Fig. 2e).

Improved gut bacteria composition

Supplementation of synbiotic to HFD animals (Group VIII) led to maximum increase in the Bacteroidetes to Firmicutes ratio followed by *L. pentosus* GSSK2+HFD (Group IV), IMOs+

HFD (Group VI) and orlistat+HFD (Group IX) respectively, compared with HFD (Group II) animals (Fig. 3a, b & c). Further, animals belonging to synbiotic+HFD (Group VIII) had significantly ($p<0.05$) elevated population of *Lactobacillus spp., Akkermansia spp., Faecalibacterium spp.,* and *Roseburia spp.* while *L. pentosus* GSSK2+HFD (Group IV) had significantly ($p<0.05$) high number of *Lactobacillus spp. and Roseburia spp.* while IMOs+HFD animals (Group VI) had increased population of *Lactobacillus spp.* and *Faecalibacterium spp* whereas, orlistat+ HFD (Group IX) animals did not show any significant change in any of the bacterial genera compared with HFD animals (Figure 3d). Moreover, Enterobacteriaceae population decreased significantly ($p<0.05$) in synbiotic + HFD (Group VIII), *L. pentosus* GSSK2+HFD (Group IV), IMOs+ HFD (Group VI) and orlistat+HFD (Group IX) animals compared with HFD animals (Figure 3d). Further, it was found that animals administered with probiotic (Group III) had increased abundance of *Lactobacillus spp.* and *Roseburia spp.,* prebiotic (Group V) animals had increased *Lactobacillus spp., Akkermansia spp, Faecalibacterium spp and Ruminococcus spp.* while synbiotic (Group VII) supplemented animals had increased population of *Lactobacillus spp., Akkermansia spp, Faecalibacterium spp, Roseburia spp.,* and *Ruminococcus spp.* compared with control (Group I) animals (Figure 3d).

Improved serum biomarkers

It was found that though animals belonging to synbiotic+ HFD (Group VIII) and orlistat+HFD (Group IX) had significantly ($p< 0.05$) reduced obesity associated serum biomarkers i.e. total cholesterol, triglycerides and LDL cholesterol with increased HDL cholesterol while *L. pentosus* GSSK2+HFD (Group IV) animals had significantly ($p< 0.05$) reduced levels of triglycerides and LDL-cholesterol and IMOs+HFD (Group VI) had reduced levels of triglycerides compared with HFD animals (Group II, Table 1).

Further, *L. pentosus* GSSK2+HFD (Group IV), synbiotic (Group VIII) and orlistat (Group IX) supplementation to HFD animals significantly ($p<0.05$) reduced bilirubin, AST and ALT levels while IMOs+HFD (Group VI) animals showed significant reduction in bilirubin compared with HFD (Group II) animals (Table 1).

It was also observed that synbiotic+ HFD (Group VIII) and orlistat (Group IX) animals had significantly ($p<0.05$) reduced levels of serum LPS, TNF-α and IL-6 followed by *L. pentosus* GSSK2+ HFD (Group IV) and IMOs+ HFD (Group VI) animals compared with their elevated levels in serum of HFD (Group II) animals (Table 1).

Enhanced antioxidant and suppressed oxidant level

It was interesting to observe that supplementation of synbiotic to HFD animals (Group VIII) led to significant ($p<0.05$) reduction in oxidant MDA level both in adipose tissue and colon whereas orlistat+HFD (Group IX) animals had maximum reduction of MDA in colon followed by *L. pentosus* GSSK2+HFD (Group IV) and IMOs+HFD (Group VI) respectively compared with HFD animals (Table 2). Further, antioxidant GSH and SOD level increased in adipose tissue, colon as well as liver of synbiotic+HFD (Group VIII), followed by *L. pentosus* GSSK2+HFD (Group IV) and orlistat+HFD (Group IX) while IMOs+HFD (Group VI) animals did not show significant ($p<0.05$) change in GSH and SOD level compared with HFD animals (Table 2).

Modulation of gene expression

It was found that daily administration of synbiotic (Group VIII) and orlistat (Group IX) to HFD animals significantly ($p<0.05$) downregulated the expression of genes involved in lipid and glucose metabolism (FASN, HSL, GLUT-4 and glucokinase) and inflammatory markers (TNF-α and IL-6) in liver compared with HFD animals (Group II, Figure 4a). However, animals belonging to *L. pentosus* GSSK2+HFD (Group IV) had significantly ($p<0.05$) decreased expression of FASN, TNF-α and IL-6 while FASN, TNF-α and GLUT-4 were downregulated in IMOs+ HFD (Group VI) animals (Figure 4a).

The expression of adiposity genes, C/EBPα and PPARγ in adipose tissue was significantly ($p<0.05$) downregulated in orlistat+HFD (Group IX) followed by synbiotic+HFD (Group VIII), IMOs+ HFD (Group VI) and *L. pentosus* GSSK2+ HFD (Group IV) respectively compared with HFD (Group II) animals while adiponectin expression was significantly ($p<0.05$) upregulated in synbiotic+HFD (Group VIII) and *L. pentosus* GSSK2+HFD (Group IV) compared with HFD (Group II) animals (Figure 4b). Moreover, the expression of adipokine gene i.e. leptin and inflammatory genes i.e. TNF-α and IL-6 was significantly ($p<0.05$) downregulated in synbiotic+HFD (Group VIII) and orlistat+HFD (Group IX) followed by *L. pentosus* GSSK2+HFD (Group IV) and IMOs+HFD animals (Group VI) compared with HFD animals (Group II, Figure 4b).

The expression of gut integrity gene, claudin in colon was significantly ($p<0.05$) upregulated in synbiotic+HFD (Group VIII) and orlistat+HFD (Group IX) while expression of Muc-2 was upregulated in *L. pentosus* GSSK2+HFD (Group IV) and synbiotic+HFD (Group VIII)

compared with HFD animals (Group II). Further, synbiotic+HFD animals (Group VIII) had maximum downregulation of TLR-4 expression followed by *L. pentosus* GSSK2+HFD (Group IV), orlistat+HFD (Group IX) and IMOs+HFD (Group VI) animals respectively while there was no significant change in CDX-2 expression in animals belonging to various groups compared with HFD (Group II) animals (Figure 4c).

Histological modulation

Histological analysis of adipose tissue of HFD (Group II) animals showed hypertrophied adipocytes indicated by increased mean adipocyte size compared with normal histoarchitecture of adipocytes of control (Group I) animals (Fig. 5a, b & j). Interestingly, supplementation of probiotic, prebiotic, synbiotic and orlistat to HFD animals for 12 weeks led to reduced adipocyte hypertrophy. Maximum reduction in mean adipocyte size was observed in synbiotic+HFD (Group VIII) followed by orlistat+HFD (Group IX), *L. pentosus* GSSK2+HFD (Group VI) and IMOs+HFD (Group VI) animals respectively (Fig. 5d, f, h, i & j). However, adipose tissue of animals belonging to either probiotic (Group III), prebiotic (Group V), or synbiotic (Group VII) had normal histoarchitecture of adipocytes (Fig. 5c, e, g).

The morphological examination of liver of animals belonging to HFD (Group II) showed ballooning degeneration of hepatocytes and hypertrophy compared with normal hepatocyte of probiotic (Group III), prebiotic (Group V), synbiotic (Group VII) and control (Group I) animals (Fig. 6a & b). However, supplementation of either probiotic (Group IV), synbiotic (Group VIII) or orlistat (Group IX) to HFD animals led to reduced hepatic steatosis along with minimal fat deposition in hepatocytes compared with HFD (Group II) animals whereas, IMOs+HFD (Group VI) animals had increased hepatic steatosis with ballooned hepatocytes (Fig. 6c, d, e, f, g, h & i).

The colon segments of HFD (Group II) animals showed disrupted crypts, focal colitis in the form of excess lymphocytes between glands and hyperplasia compared with normal and intact mucosal epithelium of probiotic (Group III), prebiotic (Group V), synbiotic (Group VII) and control (Group I) animals (Fig. 7 a, b, c, e & g). Interestingly, the colon of *L. pentosus* GSSK2+ HFD (Group IV), synbiotic+HFD (Group VIII) and orlistat+HFD (Group IX) too had intact epithelium lining and closely packed mucus glands with minimal infiltration of inflammatory cells compared with focal accumulation of lymphocytes in the colonic mucosa of IMOs+HFD (Group VI) animals (Fig. 7 d, f, h & i).

Discussion

Trialogue between diet, gut microbiota and host immune response has revealed novel prophylactic interventions employing probiotics and prebiotics for metabolic diseases. In our earlier studies, we have observed that indigenous potential probiotic *L. pentosus* GSSK2 showed potent anti-inflammatory activity in LPS induced RAW 264.7 cells, metabolized prebiotic IMOs and ameliorated adiposity parameters and overall body physiology in HFD fed SD rats[14,15]. In present study, an attempt was made to investigate the prophylactic potential of a novel synbiotic biointervention (*Lactobacillus pentosus* GSSK2 + IMOs) in experimental metabolic syndrome compared with orlistat, a commonly used weight loss drug.

Increased body weight is one of the important hallmark of metabolic syndrome and is accompanied by fat mass deposition. Interestingly, synbiotic+HFD animals had improved adiposity parameters with no change in average feed intake which might be attributed to lipoprotein lipase inhibition and release of appetite-reducing hormones glucagon-like peptide-1 and peptide YY either by probiotic or its metabolites, increased abundance of gut bacteria inhibiting fatty acid uptake in adipose tissue resulting into increased energy expenditure, reduced visceral adipose tissue deposits and decreased mean adipocyte size [17,18]. Esposito et al.[19] have also observed that supplementation of VSL#3 to HFD fed rats reduced fat mass with equal food intake, while Liu et al.[20] documented that administration of probiotic *L. plantarum* ZJUFT17 to HFD mice had reduced weight gain due to decreased feed intake.

Hyperglycemia, a key outcome of metabolic syndrome, was ameliorated in synbiotic+HFD animals, suggesting improved glucose metabolism and is in concordance with earlier study[21]. These scientists have demonstrated that probiotic *L. sakei* OK67 reduced the blood glucose levels in HFD-fed mice due to decrease in LPS producing Gram negative gut bacteria and preventing LPS induced inflammation resulting into alleviated β cell dysfunction[22]. The antidiabetic effect of synbiotic may be accounted to increased expression of GLUT-4 transporter and glucagon like peptide-1 mediated by enhanced production of short-chain fatty acids such as butyrate and propionate, resulting into increased glucose uptake by adipose tissue and muscles[23,24].

Accumulating evidences have demonstrated the role of gut dysbiosis in the etiology and progression of diet induced disorders[25]. The decreased ratio of Bacteroidetes to Firmicutes along

with decreased abundance of beneficial bacteria i.e. *Bifidobacteria spp., Akkermansia spp.* along with increased pathobionts i.e. *Enterobacteriaceae* in HFD animals compared with control is consistent with earlier reports and are considered as "dysbiotic signatures"[13,26]. This dysbiotic microbiota contributes to metabolic diseases by increasing energy harvest which could be due to downregulation of angiopoietin-like protein 4, a lipoprotein lipase inhibitor inducing host adiposity[2]. Increased energy harvest by dysbiotic microbiota is also supported by fecal microbiota transplantation studies as transfer of the gut microbiota from obese mice even to germ free mice led to an increase in their body weight[27]. Further, increased abundance of LPS-producing Gram negative bacteria along with increased chylomicron formation on HFD intake leads to elevated serum LPS thereby rupturing the gut barrier integrity, causing TLR-4 induced inflammation and endotoxemia[6,28]. Interestingly, in present study, we found that synbiotic exerted its protective effects even in HFD animals via regulating the gut microbiota. The increased Bacteroidetes to Firmicutes ratio along with increased population of *Lactobacillus spp., Akkermansia spp., Faecalibacterium spp., Roseburia spp.* and decreased abundance of *Enterobacteriaceae* in feces of synbiotic+HFD animals confirmed the shift from obesogenic to non-obesogenic bacteria and is in agreement with previous study where increased Bacteroidetes to Firmicutes ratio and decreased burden of *Enterobacteriaceae* was observed on administration of synbiotic i.e. *Lactobacillus paracasei* HII01 and xylooligosaccharides[29]. Similarly, in another study, Ji et al[30] demonstrated increased abundance of Bacteroidetes and *Lactobacillus spp.* on supplementation of *L. sakei* CJLS03 to HFD-fed animals. Moreover, *Lactobacillus spp., Faecalibacterium spp.* and *Roseburia spp.* are the major contributors of short chain fatty acid production in large intestine, especially butyrate, which has been reported to modulate gut homeostasis by increased hydrolysis of indigestible food, regulating glucose and energy metabolism, promoting satiety and preventing metabolic endotoxemia[31,32]. Furthermore, increased abundance of *Akkermansia spp*., key producer of acetate and propionate in colon, has been documented to protect against metabolic syndrome by reducing systemic LPS levels and gut permeability in HFD mice, which is possibly associated with the ability of *Akkermansia* to preserve the mucus layer thickness[33,34]. Therefore, it can be suggested that, increased short chain fatty acid production due to stabilization of gut microbiota on synbiotic supplementation might have altered the adiposity and inflammatory process, influenced the metabolic capability of liver and adipose tissue, thereby preventing the progression of metabolic complications[6].

Metabolic syndrome has also been associated with dyslipidemia, i.e. elevated levels of total cholesterol, triglycerides and LDL-cholesterol, as well as decreased HDL-cholesterol levels, the major risk factors of atherosclerosis leading to cardiovascular diseases. It was observed that synbiotic supplementation to HFD animals positively modulated the serum lipid profile paralleled with reduced hepatic steatosis in histological analysis and increased fecal lipid excretion thereby suggesting that synbiotic administration actually interfered with dietary lipids, making them indigestible and promoting their excretion instead of redistributing to the liver. Similar observations were reported in previous study where scientists have observed improved lipid profile on administration of *Lactobacillus plantarum* P-8 to hyperlipidemic rats[35]. This may be due to increased bile salt hydrolase activity, cholesterol binding and assimilation by the probiotic cell walls, or physiological actions of the synbiotic metabolites[6]. The improved liver biomarkers (ALT, AST and serum bilirubin) in synbiotic+HFD animals is in concordance with earlier studies where scientists have also observed reduced serum ALT and AST levels upon administration of a probiotic mixture (*Lactobacillus* and *Bifidobacterium*) to HFD-fed rats[36]. The observed improved liver plysiology may be due to alleviation of hepatocellular injury induced by lipid deposition and oxidative stress in hepatocytes[37].

HFD augments oxidative stress due to an imbalance between reactive oxygen species generation and anti-oxidant defense system[38]. Interestingly, in the present study, synbiotic+HFD animals had higher levels of antioxidants (GSH and SOD) and reduced oxidant (MDA) which might be due to quenching of free radicals by probiotic resulting into regulation of host redox status i.e. downregulating enzyme producing reactive oxygen species, chelating metal ions, production of antioxidant metabolites and regulation of gut microbiota[39]. Recently, Li et al.[26] have also demonstrated the amelioration of liver oxidative stress in HFD-fed mice on oral administration of probiotic mixture of *L. plantarum* strains that led to significantly increased SOD, GSH and reduced MDA levels.

Synbiotic supplementation to HFD animals, downregulated the expression of lipid metabolism regulators, FASN and HSL due to suppressed lipid synthesis, increase β-oxidation and improved hepatosteatosis and corroborates with earlier studies where selenium enriched probiotics reduced the expression of lipolytic FASN in HFD mice[40]. Similarly, Singh et al.[41] have also shown decreased HSL expression in cobiotic (IMOs+lycopene) supplemented animals accrediting it to

anti-inflammatory and antioxidant activity of cobiotic. Further, the liver GLUT-4 mRNA level was enhanced while glucokinase was reduced in synbiotic+HFD animals that may be attributed to the improved insulin resistance, enhanced glycemic control and maintained glucose homeostasis[42].

HFD elevated the expression of adiposity genes PPARγ and C/EBPα in adipose tissue, the main transcription factors of adipocyte differentiation, lipid storage and adipokine signalling[43]. Interestingly, reduced expression of PPARγ and C/EBPα in synbiotic+ HFD supplemented animals is suggestive of decreased adipogenesis via limiting the conversion rate of preadipocytes into mature adipocytes. Park et al.[44] have also reported that *L. plantarum* Q180 inhibited 3T3-L1 adipocyte differentiation by downregulation of C/EBPα and PPARγ, fat absorption and reduction of adipocyte size in diet-induced obese mice. Obesity is often accompanied by resistance to leptin, an important hormone secreted by adipocytes, leading to increased hunger and reduced energy expenditure occurring due to hyperleptinemia[45]. Notably, it was observed that synbiotic supplementation to HFD animals led to decreased leptin mRNA expression indicating improved leptin function and regulation of body fat distribution. Soundharrajan et al.[46] have also reported that *L. plantarum* A29 supplementation reduced fat mass and downregulated the expression of leptin gene in adipocytes, resulting in reduced bodyweight of HFD mice.

Adiponectin, an anti-inflammatory adipokine protects against metabolic disorders by regulating glucose levels and promoting fatty acid oxidation, however, its production is inhibited under inflammatory conditions in adipose tissue[47]. It was interesting to observe that animals belonging to synbiotic+ HFD had decreased expression of proinflammatory cytokines, TNF-α and IL-6 and upregulated adiponectin suggesting diminution of inflammation in adipose tissue. Recently, Zeng et al.[48] have observed that administration of *L. pentosus* S-PT84 to lipopolysaccharide and HFD fed mice exerted anti-inflammatory effect on adipose tissue by eliminating metabolic endotoxemia-induced macrophage infiltration, restoring the production of adiponectin, and decreased levels of pro-inflammatory mediators.

Systemic endotoxemia, considered as the ‘core’ of metabolic syndrome is associated with elevated plasma LPS in circulation thereby leading to proinflammatory and oxidant environment[49,50]. Interestingly, reduced serum level of inflammatory markers (LPS, TNF-α and IL-6) in synbiotic+ HFD animals highlighted the anti-inflammatory potential of this intervention

in alleviating metabolic endotoxemia. Lim et al.[21] also observed that *L. sakei* OK67 supplementation antagonized the HFD-induced alterations by inhibiting LPS production, regulating tight junction protein expression and suppressing inflammation. The synbiotic-mediated alleviation of chronic inflammation could be accredited to decreased number of LPS producing Enterobacteriacea in gut microbiota and downregulation of key signaling pathways i.e. NF-κB and MAPK by probiotic or its metabolites as observed in our earlier in vitro study, where *L. pentosus* GSSK2 attenuated LPS-induced inflammation by downregulating MAPK pathway vis-a-vis inhibiting COX-2[14,51].

HFD intake induces an increase in gut permeability by reducing the thickness of mucus layer, impairing the expression of tight junction proteins via LPS-induced activation of TLR-4 pathway[25]. In the present study, mucin gene Muc-2 and tight junction protein claudin were upregulated with reduced expression of TLR-4 in colon suggesting that synbiotic supplementation to HFD animals attenuated mucosal damage and regulated the gut barrier function which is further supported by improved colon morphology with reduced infilteration of immune cells in synbiotic+HFD supplemented animals. Similarly, Mennigen et al.[52] reported that probiotic mixture VSL#3 protected the epithelial barrier function by maintaining tight junction protein expression in a murine model of colitis while Castro-Rodríguez[53] documented reduced systemic inflammation due to decreased TLR-4 expression in *Leuconostoc mesenteroides* subsp. mesenteroides SD23 supplemented HFD mice.

Based on the present study, synbiotic biointervention, a combination of probiotic *L. pentosus* GSSK2 and prebiotic IMOs was found to be the most effective and comparable to antiobesity drug orlistat in terms of improved anthropometric parameters, biochemical markers, gene expression and histoarchitecture of adipose tissue, colon and liver. Therefore, the proposed molecular mechanism of modulation of HFD-induced metabolic alterations by synbiotic may be attributed to remodulation of gut microbiota by probiotic as well as prebiotic, that may have led to altered adiposity by remodeling energy metabolism, activation of nutrient sensing pathways, mobilization of fats by regulating the expression of glucose and lipid metabolism genes, reduced lipid absorption due to increased fatty acid oxidation, cholesterol binding and assimilation by probiotic. Moreover, increased short chain fatty acid production due to biofermentation of prebiotic and reduced circulating LPS levels due to decreased pathobionts may have led to

mitigation of chronic inflammation, alleviation of metabolic endotoxemia, oxidative stress and restoration of intestinal barrier function that in turn regulated the glucose homeostasis. Reduced inflammatory adipokines due to alleviation of systemic inflammation might have prevented fat accumulation in liver and adipose tissue hampering the vicious cycle between metabolic and immune responses thereby preventing the progression of metabolic syndrome.

Taken together, it is proposed that such novel synbiotic intervention may be employed for combating the growing incidence of metabolic syndrome that could be considered as a promising and alternative live bacteriotherapy for maintaining the immune- metabolic homeostasis. However, due to species and strain specific response of probiotics and entirely different gut microbiota of rodent, the present observations need to be validated clinically.

Methods

Animals

Male Sprague-Dawley (SD) rats (150-180g) were procured from inbred population of the Central Animal House, Panjab University, Chandigarh, India. Rats were housed in polypropylene cages with a hygienic bed of husk in room with 12 h light/dark cycle, acclimatized for 7–10 days and given standard pellet diet and water *ad libitum.*

Ethics declaration

All protocols related to the sampling, care and management of animals were approved by Institutional Animals Ethical Committee (IAEC), Panjab University, Chandigarh and the Committee for the Purpose of Control and Supervision on Experiments on Animals **(PU/45/99/CPCSEA/IAEC/2017/27)**. All experiments were performed in accordance with Institutional guidelines and regulations. The study is reported in accordance with ARRIVE guidelines.

Preparation of HFD

Standard pellet diet (SPD) (6% calories from fat) was procured from Ashirwad Industries, Chandigarh, India and HFD (60% calories from fat) was prepared in-house as described previously [15].

Preparation of dose

- **Probiotic:** 18 h old culture of indigenous probiotic *L. pentosus* GSSK2 was centrifuged at 4,000 × *g* for 10 minutes at 4°C, washed, and suspended in phosphate buffer saline (PBS pH 7.4) to contain 1×10^9 lactobacilli/0.1 ml[15].
- **Prebiotic:** IMOs (1g/kg body weight/ 0.1mL PBS[41]) was used as prebiotic.
- **Synbiotic:** Probiotic *L. pentosus* GSSK2 (1×10^9 lactobacilli/0.1 ml) in combination with prebiotic IMOs (1g/kg body weight), was employed as synbiotic.

Experimental design

Animals were divided into nine groups, each comprising of 6 animals and treated as follows.

- **Group I (Control):** Animals were fed with SPD for 12 weeks.
- **Group II (HFD) :** Animals were fed with HFD for 12 weeks.
- **Group III (*L. pentosus* GSSK2):** Animals were fed with a single dose of probiotic (1 x 10^9 lactobacilli/0.1mL) daily via orogastric gavage and were given SPD for 12 weeks.
- **Group IV (*L. pentosus* GSSK2+ HFD):** Animals were fed orally with a single dose of probiotic (1 x 10^9 lactobacilli/0.1mL) daily along with HFD for 12 weeks.
- **Group V (IMOs):** Animals belonging to this group were fed orally with a single dose of IMOs (1g/kg body weight) daily along with SPD for 12 weeks.
- **Group VI (IMOs+ HFD):** Animals were fed orally with a single dose of IMOs (1g/kg body weight/ 0.1mL) daily along with HFD for 12 weeks.
- **Group VII (Synbiotic):** Animals were fed orally with a single dose of both probiotic (1 x 10^9 lactobacilli/0.1mL) and IMOs (1g/kg body weight) daily along with SPD for 12 weeks.
- **Group VIII (Synbiotic+ HFD):** Animals were fed orally with a single dose of both probiotic (1 x 10^9 lactobacilli/0.1mL) and IMOs (1g/kg body weight) daily along with HFD for 12 weeks.
- **Group IX (Orlistat+ HFD):** Animals were fed orally with a single dose of orlistat (10mg/kg body weight/ 0.1mL PBS) daily along with HFD for 12 weeks.

Follow up of animals

Body weight and lactic acid bacteria (LAB) count were monitored once a week, throughout the experiment. A day before sacrificing the animals, fasting blood glucose level was monitored, oral glucose tolerance test (OGTT) was performed and feces of animals were collected, for estimation of fecal lipids and analysis of gut bacterial abundance. Animals were sacrificed after 12 weeks of

418 respective treatments by injecting ketamine hydrochloride (80 mg/kg) intraperitoneally followed
419 by cervical dislocation. Blood was drawn through retro-orbital bleeding for estimation of serum
420 biochemical parameters. Liver, adipose tissue (epididymal and retroperitoneal) and colon were
421 collected for analysis of oxidants and antioxidants, histopathological alterations and molecular
422 markers.

423 **Evaluation of anthropometric parameters and adiposity markers**

424 Body weight of animals was recorded weekly on ordinary balance (SD-300, S.D fine chemicals
425 Ltd, Chandigarh, India) while abdominal circumference was measured at the beginning and end
426 of the study using ordinary measuring tape[15]. Lee's Index was calculated as cube root of body
427 weight (g)/ naso-anal length (cm), Body mass index (BMI) was monitored as body weight
428 (g)/length^2 (cm^2) at the end of experiment[13].

429 Feed intake of animals was recorded twice a month and was calculated by subtracting the
430 amount of residual food in each cage from the weighed amount of food provided on previous day
431 (g/day) and represented as average feed intake (g/day/ rat) by dividing the feed intake by total
432 number of animals per cage[15]. Post sacrifice, liver and adipose tissue were weighed using
433 ordinary balance.

434 **Blood glucose and OGTT**

435 The fasting blood glucose levels of animals were recorded weekly via tail snip method using
436 glucometer (Freestyle Optium Glucometer, Abbott diabetes care Ltd., Oxon, UK). For OGTT,
437 animals were fasted for 6 h and blood glucose level was measured before and after oral
438 administration of D-glucose (2 g/kg) at an interval of 15, 30, 60, 90, and 120 minutes
439 respectively[15]. Area under the concentration-time curve (AUC) was calculated using GraphPad
440 PRISM 5 software.

441 **Fecal LAB count and fecal lipids**

442 To assess the effect of synbiotic supplementation on LAB in the colon, freshly voided fecal
443 material (0.5 g/animal) was collected weekly from each group, homogenized in normal saline,
444 serially diluted and plated on MRS agar. The plates were incubated at 37°C for 48 h and colony
445 forming units (CFU) were recorded [11].

446 Fecal lipids were extracted using phase separation based method by Folch et al.[54] followed by
447 estimation of total lipids by method of Fringes and Dunn[55]. Briefly, 200 mg dry feces was taken
448 in a centrifuge tube and 3 mL of chloroform–methanol mixture (2:1, v/v) was added, vortexed

for 1 minute and centrifuged at 3,000 g for 10 minutes. The chloroform phase containing lipid fraction was collected in a fresh tube and completely dried followed by estimation of total lipids.

Selected gut bacterial abundance

Bacterial DNA was isolated using QIAmp® DNA stool mini kit (Qiagen, Hilden, Germany) from 180 mg of fecal sample, as per manufacturer's instruction. DNA quantification was performed using Infinite® M200 Pro NanoQuant (Tecan). DNA extracted as above was subjected to qPCR to quantify the abundance of *Lactobacillus spp., Bifidobacterium spp., Roseburia spp., Akkermansia spp., Faecalibacterium spp., Ruminococcus spp., Prevotella spp.,* using genus-specific primers and that of Enterobacteriaceae, Bacteroidetes and Firmicutes using phylum-specific primers taking total bacteria as an internal control. q-PCR conditions and primer details are given in supplementary material (Supplementary Table 1). Data was analyzed using the ΔΔCt method and values expressed as fold change relative to the control group[13].

Analysis of serum biochemical parameters

Blood was collected retro-orbitally and serum was prepared to estimate liver function test [Bilirubin, aspartate transaminase (AST) and alanine transaminase (ALT)] and lipid profile [Total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol] using autoanalyser, Sysmex XP-100. Serum lipopolysaccharide (LPS), TNF-α and IL-6 were quantified using commercially available ELISA kits (BT Laboratory, Zhejiang, China) as per manufacturers' instructions.

Assessment of oxidant and antioxidant level

Tissue homogenates of colon, liver and adipose tissue samples were prepared in 0.15 M PBS (pH 7.2) using potter Elvehjem homogenizer. Post mitochondrial supernatant (PMS) was prepared by cold centrifuging tissues homogenates at 16,000 × g for 10 minutes and supernatant was labeled as PMS. Protein concentration in tissue homogenate and PMS was measured using standard method of Lowry et al.[56].

The amount of malondialdehyde (MDA), a measure of lipid peroxidation, was assayed in homogenates as per Wills[57] and results were expressed as nanomoles of MDA per milligram of protein. Superoxide dismutase (SOD) activity was assayed in PMS of tissue homogenates according to the method of Kono[58] and expressed as units of SOD per milligram of protein, where 1 U activity is defined as the amount of SOD required to inhibit the rate of Nitroblue tetrazolium reduction by 50%. Reduced glutathione (GSH) levels were estimated in tissue

homogenates as per Ellman[59], absorbance was measured at 412 nm and results were expressed as µmole of GSH/mg of protein.

Gene expression analysis

q-PCR based gene expression analysis was done for fatty acid synthase (FASN), hormone sensitive lipase (HSL), glucokinase, GLUT-4, TNF-α and IL-6 in liver; CCAAT/ enhancer-binding protein alpha (C/EBPα), peroxisome proliferators-activated receptor gamma (PPAR-γ), leptin, adiponectin, TNF-α and IL-6 in adipose tissue; claudin, CDX-2, Muc2 and TLR-4 in colon. Total RNA was extracted using Trizol (Sigma Aldrich, USA). 1 µg of RNA sample was used for c-DNA synthesis using commercially available kit (Biorad iscript kit 1708891) as per the kit's instructions. Relative expression of different genes was determined by qPCR using SYBR® based dye (Biorad C1000 Touch Real-Time PCR machine). q-PCR conditions and primer details are given in supplementary material (Supplementary Table 2). Data was analyzed using the ΔΔCt method and values were expressed as fold change relative to control group. GAPDH was used as internal reference gene to normalize the expression of target genes.

Histological analysis

A part of distal colon, liver and adipose tissue were fixed immediately in 10% buffered formalin, processed, stained with hematoxylin and eosin, and examined for histological alterations using light microscope. The mean adipocyte sizes in adipose tissue sections (minimum 2 animals per group) were estimated in 10-12 images (40X objective), using Image J software[60].

Statistical analysis

Results were expressed as mean ± standard deviation (SD). The inter group variation was assessed by one-way analysis of variance (ANOVA) followed by Tukey's post Hoc Test using PRISM software (5.0). The statistical significance was defined as p and calculated at $p < 0.05$.

References

1. Saklayen, M. G. The Global Epidemic of the Metabolic Syndrome. *Curr Hypertens Rep* 20, 018-0812 (2018).
2. Arora, T. & Bäckhed, F. The gut microbiota and metabolic disease: current understanding and future perspectives. *J Intern Med* 280, 339-349 (2016).

3. Wang, P.-X., Deng, X.-R., Zhang, C.-H. & Yuan, H.-J. Gut microbiota and metabolic syndrome. *Chin Med J (Engl)* 133, 808-816, doi:10.1097/cm9.0000000000000696 (2020).
4. Dabke, K., Hendrick, G. & Devkota, S. The gut microbiome and metabolic syndrome. *J Clin Invest* 129, 4050-4057 (2019).
5. Garg, A. Acquired and inherited lipodystrophies. *N Engl J Med* 350, 1220-1234 (2004).
6. Xavier-Santos, D., Bedani, R., Lima, E. D. & Saad, S. M. I. Impact of probiotics and prebiotics targeting metabolic syndrome. *J Funct Foods* 64, 103666, doi:https://doi.org/10.1016/j.jff.2019.103666 (2020).
7. George Kerry, R. *et al.* Benefaction of probiotics for human health: A review. *J Food Drug Anal* 26, 927-939, doi:https://doi.org/10.1016/j.jfda.2018.01.002 (2018).
8. Chandel, D., Sharma, M., Chawla, V., Sachdeva, N. & Shukla, G. Isolation, characterization and identification of antigenotoxic and anticancerous indigenous probiotics and their prophylactic potential in experimental colon carcinogenesis. *Sci Rep* 9, 14769, doi:10.1038/s41598-019-51361-z (2019).
9. Gibson, G. R. *et al.* Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol* 14, 491-502, doi:10.1038/nrgastro.2017.75 (2017).
10. Davani-Davari, D. *et al.* Prebiotics: Definition, Types, Sources, Mechanisms, and Clinical Applications. *Foods* 8, 92, doi:10.3390/foods8030092 (2019).
11. Verma, A. & Shukla, G. Synbiotic (*Lactobacillus rhamnosus*+*Lactobacillus acidophilus*+inulin) attenuates oxidative stress and colonic damage in 1,2 dimethylhydrazine dihydrochloride-induced colon carcinogenesis in Sprague-Dawley rats: a long-term study. *Eur J Cancer Prev* 23, 550-559 (2014).
12. Ke, X. *et al.* Synbiotic-driven improvement of metabolic disturbances is associated with changes in the gut microbiome in diet-induced obese mice. *Mol Metab* 22, 96-109 (2019).
13. Singh, D. P. *et al.* Isomalto-oligosaccharides, a prebiotic, functionally augment green tea effects against high fat diet-induced metabolic alterations via preventing gut dysbacteriosis in mice. *Pharmacol Res* 123, 103-113 (2017).

14. Khanna, S., Bishnoi, M., Kondepudi, K. K. & Shukla, G. Isolation, characterization and anti-inflammatory mechanism of probiotics in lipopolysaccharide-stimulated RAW 264.7 macrophages. *World J Microbiol Biotechnol* 36, 74 (2020). https://doi.org/10.1007/s11274-020-02852-z>.

15 Khanna, S., Walia, S., Kondepudi, K. K. & Shukla, G. Administration of indigenous probiotics modulate high-fat diet-induced metabolic syndrome in Sprague Dawley rats. *Antonie Van Leeuwenhoek* 113, 1345-1359 (2020).

16. Kang, J. G. & Park, C.-Y. Anti-Obesity Drugs: A Review about Their Effects and Safety. *Diabetes Metab J* 36, 13-25, doi:10.4093/dmj.2012.36.1.13 (2012).

17. Yadav, H., Lee, J. H., Lloyd, J., Walter, P. & Rane, S. G. Beneficial metabolic effects of a probiotic via butyrate-induced GLP-1 hormone secretion. *J Biol Chem* 288, 25088-25097 (2013).

18. Martinez-Guryn, K. *et al.* Small Intestine Microbiota Regulate Host Digestive and Absorptive Adaptive Responses to Dietary Lipids. *Cell Host Microbe* 23, 458-469 (2018).

19. Esposito, E. *et al.* Probiotics reduce the inflammatory response induced by a high-fat diet in the liver of young rats. *J Nutr* 139, 905-911 (2009).

20. Liu, T., Li, Y., Zhao, M., Mo, Q. & Feng, F. Weight-Reducing Effect of *Lactobacillus plantarum* ZJUFT17 Isolated from Sourdough Ecosystem. *Nutrients* 12, 977 (2020).

21. Lim, S. M., Jeong, J. J., Woo, K. H., Han, M. J. & Kim, D. H. *Lactobacillus sakei* OK67 ameliorates high-fat diet-induced blood glucose intolerance and obesity in mice by inhibiting gut microbiota lipopolysaccharide production and inducing colon tight junction protein expression. *Nutr Res* 36, 337-348 (2016).

22. Miraghajani, M. *et al.* Potential mechanisms linking probiotics to diabetes: a narrative review of the literature. *Sao Paulo Med J* 135, 169-178 (2017).

23. Nakamura, Y. K. & Omaye, S. T. Metabolic diseases and pro- and prebiotics: Mechanistic insights. *Nutr Metab* 9, 1743-7075 (2012).

24. Balakumar, M. *et al.* Improvement in glucose tolerance and insulin sensitivity by probiotic strains of Indian gut origin in high-fat diet-fed C57BL/6J mice. *Eur J Nutr* 57, 279-295 (2018).

25. Li, X., Watanabe, K. & Kimura, I. Gut Microbiota Dysbiosis Drives and Implies Novel Therapeutic Strategies for Diabetes Mellitus and Related Metabolic Diseases. *Front Immunol* 8, doi:10.3389/fimmu.2017.01882 (2017).
26. Li, H. *et al.* Probiotic Mixture of *Lactobacillus plantarum* Strains Improves Lipid Metabolism and Gut Microbiota Structure in High Fat Diet-Fed Mice. *Front Microbiol* 11, doi:10.3389/fmicb.2020.00512 (2020).
27. Turnbaugh, P. J. *et al.* An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444, 1027-1031 (2006).
28. Kim, K. A., Gu, W., Lee, I. A., Joh, E. H. & Kim, D. H. High fat diet-induced gut microbiota exacerbates inflammation and obesity in mice via the TLR4 signaling pathway. *PLoS One* 7, 16 (2012).
29. Thiennimitr, P. *et al.* Lactobacillus paracasei HII01, xylooligosaccharides, and synbiotics reduce gut disturbance in obese rats. *Nutrition* 54, doi:10.1016/j.nut.2018.03.005 (2018).
30. Ji, Y. *et al.* Amelioration of obesity-related biomarkers by *Lactobacillus sakei* CJLS03 in a high-fat diet-induced obese murine model. *Sci Rep* 9, 019-43092 (2019).
31. Tamanai-Shacoori, Z. *et al.* Roseburia spp.: a marker of health? *Future Microbiol* 12, 157-170 (2017).
32. Vallianou, N., Stratigou, T., Christodoulatos, G. S. & Dalamaga, M. Understanding the Role of the Gut Microbiome and Microbial Metabolites in Obesity and Obesity-Associated Metabolic Disorders: Current Evidence and Perspectives. *Curr Obes Rep* 8, 317-332, doi:10.1007/s13679-019-00352-2 (2019).
33. Everard, A. *et al.* Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci U S A* 110, 9066-9071 (2013).
34. Xu, Y. *et al.* Function of *Akkermansia muciniphila* in Obesity: Interactions With Lipid Metabolism, Immune Response and Gut Systems. *Front Microbiol* 11, doi:10.3389/fmicb.2020.00219 (2020).
35. Bao, Y. *et al.* Effect of *Lactobacillus plantarum* P-8 on lipid metabolism in hyperlipidemic rat model. *European J Lipid Sci Technol* 114, 1230-1236, doi:https://doi.org/10.1002/ejlt.201100393 (2012).
36. Liang, Y. *et al.* Probiotic mixture of *Lactobacillus* and *Bifidobacterium* alleviates systemic adiposity and inflammation in non-alcoholic fatty liver disease rats through

Gpr109a and the commensal metabolite butyrate. *Inflammopharmacology* 26, 1051-1055 (2018).

37. Gratz, S. W., Mykkanen, H. & El-Nezami, H. S. Probiotics and gut health: a special focus on liver diseases. *World J Gastroenterol* 16, 403-410, doi:10.3748/wjg.v16.i4.403 (2010).

38. Tan, B. L. & Norhaizan, M. E. Effect of High-Fat Diets on Oxidative Stress, Cellular Inflammatory Response and Cognitive Function. *Nutrients* 11, 2579, doi:10.3390/nu11112579 (2019).

39. Wang, Y. *et al.* Antioxidant Properties of Probiotic Bacteria. *Nutrients* 9, 521, doi:10.3390/nu9050521 (2017).

40. Nido, S. A. *et al.* Effects of Selenium-Enriched Probiotics on Lipid Metabolism, Antioxidative Status, Histopathological Lesions, and Related Gene Expression in Mice Fed a High-Fat Diet. *Biol Trace Elem Res* 171, 399-409 (2016).

41. Singh, D. P. *et al.* A novel cobiotic-based preventive approach against high-fat diet-induced adiposity, nonalcoholic fatty liver and gut derangement in mice. *Int J Obes* 40, 487-496 (2016).

42. Shulman, G. I. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. *N Engl J Med* 371, 1131-1141 (2014).

43. Moseti, D., Regassa, A. & Kim, W. K. Molecular Regulation of Adipogenesis and Potential Anti-Adipogenic Bioactive Molecules. *Int J Mol Sci* 17 (2016).

44. Park, S.-Y., Kim, S. & Lim, S.-D. The Inhibitory Effect of L. plantarum Q180 on Adipocyte Differentiation in 3T3-L1 and Reduction of Adipocyte Size in Mice Fed High-fat Diet. *Korean J Food Sci Anim Resour* 38, 99-109, doi:10.5851/kosfa.2018.38.1.99 (2018).

45. Izquierdo, A. G., Crujeiras, A. B., Casanueva, F. F. & Carreira, M. C. Leptin, Obesity, and Leptin Resistance: Where Are We 25 Years Later? *Nutrients* 11, 2704, doi:10.3390/nu11112704 (2019).

46. Soundharrajan, I. *et al.* Positive metabolic effects of selected probiotic bacteria on diet-induced obesity in mice are associated with improvement of dysbiotic gut microbiota. *The FASEB Journal* 34, 12289-12307, doi:https://doi.org/10.1096/fj.202000971R (2020).

630 47. Achari, A. E. & Jain, S. K. Adiponectin, a Therapeutic Target for Obesity, Diabetes, and
631 Endothelial Dysfunction. *Int J Mol Sci* 18 (2017).
632 48. Zeng, Y., Zhang, H., Tsao, R. & Mine, Y. *Lactobacillus pentosus* S-PT84 Prevents Low-
633 Grade Chronic Inflammation-Associated Metabolic Disorders in a Lipopolysaccharide
634 and High-Fat Diet C57/BL6J Mouse Model. *J Agric Food Chem* 68, 4374-4386 (2020).
635 49. Boutagy, N. E., McMillan, R. P., Frisard, M. I. & Hulver, M. W. Metabolic endotoxemia
636 with obesity: Is it real and is it relevant? *Biochimie* 124, 11-20 (2016).
637 50. Cani, P. D. *et al.* Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes*
638 56, 1761-1772 (2007).
639 51. Dai, C. *et al.* VSL#3 probiotics exerts the anti-inflammatory activity via PI3k/Akt and
640 NF-κB pathway in rat model of DSS-induced colitis. *Mol Cell Biochem* 374, 1-11,
641 doi:10.1007/s11010-012-1488-3 (2013).
642 52. Mennigen, R. *et al.* Probiotic mixture VSL#3 protects the epithelial barrier by
643 maintaining tight junction protein expression and preventing apoptosis in a murine model
644 of colitis. *Am J Physiol Gastrointest Liver Physiol* 296, 12 (2009).
645 53. Castro-Rodríguez, D. C. *et al. Leuconostoc mesenteroides* subsp. mesenteroides SD23
646 Prevents Metabolic Dysfunction Associated with High-Fat Diet-Induced Obesity in Male
647 Mice. *Probiotics Antimicrob Proteins* 12, 505-516 (2020).
648 54. Folch, J., Lees, M. & Sloane Stanley, G. H. A simple method for the isolation and
649 purification of total lipides from animal tissues. *J Biol Chem* 226, 497-509 (1957).
650 55. Frings, C. S. & Dunn, R. T. A colorimetric method for determination of total serum lipids
651 based on the sulfo-phospho-vanillin reaction. *Am J Clin Pathol* 53, 89-91 (1970).
652 56. Lowry, O. H., Rosebrough, N. J., Farr, A. & Randall, R. J. Protein measurement with the
653 folin Phenol Reagent. *J. biol. Chemistry* 193, 256-275, doi:10.1016/s0021-
654 9258(19)52451-6 (1951).
655 57. Wills, E. D. Mechanisms of lipid peroxide formation in animal tissues. *Biochem J* 99,
656 667-676 (1966).
657 58. Kono, Y. Generation of superoxide radical during autoxidation of hydroxylamine and an
658 assay for superoxide dismutase. *Arch Biochem Biophys* 186, 189-195 (1978).
659 59. Ellman, G. L. Tissue sulfhydryl groups. *Arch Biochem Biophys* 82, 70-77 (1959).

60. Sarma, S. M. *et al.* Finger millet arabinoxylan protects mice from high-fat diet induced lipid derangements, inflammation, endotoxemia and gut bacterial dysbiosis. *Int J Biol Macromol* 106, 994-1003 (2018).

Acknowledgements

The financial support provided by the Indian Council of Medical Research, India (3/1/3 JRF-2016/HRD-51) for carrying the present study; Panjab University, Chandigarh and National Agri-food Biotechnology Institute, Mohali for providing research facilities are highly acknowledged.

Author Contributions

S.K: Conducted experiments, compiled and analyzed data, and wrote manuscript; K.K.K: Supervised the study, helped in analyzing and interpreting data M.B.: Helped in designing diet and gene expression experiment; G.S.: Conceived, helped in analyzing, interpreting data and editing the manuscript along with overall supervision of the project.

Conflicts of interest: There is no conflict of interest among the authors.

Figure Legend

Figure 1: Anthropometric parameters and adiposity markers in different groups of animals: (a) Body weight; (b) Weight gain; (c) Liver and adipose tissue weight; (d) BMI (g/cm^2) and Lee's index (e) Change in abdominal circumference; (f) Feed intake; Values are Mean ± SD, $^{\#}p<0.05$ versus control, $*p<0.05$ versus HFD.

Figure 2: Effect of probiotic, prebiotic and synbiotic supplementation in animals belonging to various groups on: (a) Fasting blood glucose; (b) OGTT (c) AUC of OGTT; (d) Fecal lactic acid bacteria count (Log_{10} CFU/ml); (e) Fecal lipid excretion. Values are Mean ± SD, $^{\#}p<0.05$ versus control, $*p<0.05$ versus HFD.

Figure 3: Relative bacterial abundance of different genera in animals belonging to different groups: (a) Bacteroidetes; (b) Firmicutes; (c) Bacteroidetes: Firmicutes; (d) *Lactobacillus, Bifidobacteria, Akkermansia, Faecalibacterium, Roseburia, Ruminococcus, Prevotella, Enterobacteriaceae,* by real-time PCR. Values are Mean ± SD, $^{\#}p<0.05$ versus control, $*p<0.05$ versus HFD.

Figure 4: Relative gene expression of: (a) lipid metabolism genes, inflammatory markers, glucose metabolism genes in liver; (b) adiposity genes in adipose tissue; (c) gut integrity genes in colon of animals belonging to various groups by real-time PCR. Values are Mean ± SD, $^{\#}p<0.05$ versus control, *$p<0.05$ versus HFD.

Figure 5: Photomicrograph of adipose tissue showing: (a) normal histomorphology with uniform, spherical adipocytes in control animals; (b) hypertrophied adipocytes in HFD; (c, e & g) normal histoarchitecture of adipocytes in *L. pentosus*, IMOs and synbiotic animals; (d, f, h & i) reduced adiposity in *L. pentosus* + HFD, IMOs + HFD, synbiotic + HFD and orlistat + HFD animals (H & E staining; scale bar: 50 µm, 400X); (j) mean adipocyte size in animals belonging to different groups.

Figure 6: Photomicrograph of liver of animals belonging to different groups showing: (a) Normal histomorphology with polyhedral hepatocytes having large, rounded vesicular nuclei in control; (b) severe hepatic steatosis and ballooning degeneration of hepatocytes in HFD; (c, e & g) normal histoarchitectureof hepatocytes in *L. pentosus*, IMOs and synbiotic animals; (f) ballooned hepatocytes with vacuolated nuclei in IMOs + HFD animals; (d, h & i) reduced hepatic steatosis in *L. pentosus* + HFD, synbiotic + HFD and orlistat + HFD animals (H & E staining; arrows indicate ballooned hepatocytes; scale bar: 50 µm, 400X).

Figure 7: Photomicrograph of colon of animals belonging to different groups depicting: (a) normal histoarchitecture showing mucosa, submucosa, muscularis propria and serosa in control; (b) severely damaged mucosa with infilteration of lymphocytes and plasma cells in HFD; (c, e & g) normal histomorphology of colon in *L. pentosus*, IMOs and synbiotic animals; (f) inflammation in IMOs + HFD animals; (d, h & i) reduced infilteration of immune cells with intact mucosa in *L. pentosus* + HFD, synbiotic + HFD and orlistat + HFD animals (H & E staining; arrows indicate infilteration of inflammatory cells; scale bar: 100 µm, 100X).

715

716

717 **Table 1:** Serum biochemical parameters (lipid profile, liver function test, LPS, TNF-α and IL-6)
718 of animals belonging to different groups

Parameter	Groups								
	Control	**HFD**	***L. pentosus***	***L. pentosus* +HFD**	**IMOs**	**IMOs+ HFD**	**Synbiotic**	**Synbiotic+ HFD**	**Orlistat+ HFD**
Total cholesterol (mg/dl)	70.2±9.5	110.8$^{\#}$± 9.9	75.6± 7.9	98.4$^{\#}$± 8.3	78 ±9.0	93$^{\#}$ ± 10.4	71.4 ± 9.2	79.8* ± 9.6	82.6* ±7.0
Triglycerides (mg/dl)	82 ± 5.6	118.4$^{\#}$±11.7	76.8 ± 6.2	95.6*± 5.2	79.8±7.9	98.2*± 9.6	85 ± 11.5	91* ± 9.2	89.2*±10.2
HDL cholesterol (mg/dl)	47.7±4.4	32.1$^{\#}$± 4.3	43.2 ±5.3	39.8 ± 3.6	43 ± 4.2	38 ± 5.1	45.2 ± 4.1	45* ± 7.1	45.4*± 2.1
LDL cholesterol (mg/dl)	23.06±3.4	40.72$^{\#}$ ±2.7	28 ± 4.2	26.4*± 6.5	26.8±5.2	41.4$^{\#}$±7.7	22.2 ± 8.2	25.3* ± 4.3	27.2* ± 5.5
SGOT (AST) (IU/L)	95.04±9.8	160.8$^{\#}$ ±9.9	100.6±8.7	132.2*±8.0	104.4±7.1	147$^{\#}$ ±8.7	97.8 ±4.3	106.6*±5.4	103.6*±8.1
SGPT (ALT) (IU/L)	62 ± 9.6	123.6$^{\#}$±11.6	66.4 ± 6.6	80.8$^{\#*}$±7.9	79 ±8.3	115$^{\#}$±10.2	62 ±6.4	73* ±5.4	74.4* ±6.1
Serum bilirubin (mg/dl)	0.48±0.02	1.08$^{\#}$ ±0.03	0.46±0.02	0.66*±0.05	0.57±0.04	0.72*±0.01	0.52±0.03	0.58*±0.06	0.70*±0.04
Serum LPS (EU/L)	24.8±3.02	108.5$^{\#}$±16.3	20.6 ± 1.7	47.6* ± 6.9	27.3 ±8.4	79.1$^{\#}$±9.01	28.2 ± 4.3	38.9*±6.06	42.4*±8.04
Serum TNF-a (pg/ml)	2.3± 0.76	52.5$^{\#}$± 8.23	2.9 ± 0.59	30.3*±3.11	4.2 ± 0.78	41.1$^{\#}$±9.01	3.6 ± 0.93	21.3*±1.42	14.7*±0.94
Serum IL-6 (ng/L)	1.9± 0.12	9.2$^{\#}$ ± 0.73	2.1 ± 0.51	4.3* ± 0.95	2.2 ± 0.14	4.5* ± 0.52	1.9 ± 0.15	3.4*± 0.17	2.8*± 0.24

719 Values are Mean ± SD, $^{\#}$p<0.05 versus control, *p<0.05 versus HFD.

720

721

722

723 **Table 2:** Oxidant and antioxidant levels in animals belonging to different groups

Groups	MDA (nM/mg protein)			GSH (nM/mg protein)			SOD (U/mg protein)		
	Adipose tissue	**Colon**	**Liver**	**Adipose tissue**	**Colon**	**Liver**	**Adipose tissue**	**Colon**	**Liver**
Control	46.6 ± 5.6	52.2 ± 4.3	58.3 ±7.1	3.54 ± 0.2	2.72 ± 0.6	1.44 ± 0.1	3.12 ± 0.2	1.16 ± 0.1	1.25 ± 0.2
HFD	77.1$^{\#}$ ± 7.8	89.5$^{\#}$±8.6	91.2$^{\#}$±4.3	0.84$^{\#}$± 0.3	0.45$^{\#}$± 0.1	0.42$^{\#}$ ± 0.3	0.71$^{\#}$± 0.1	0.49$^{\#}$± 0.1	0.36$^{\#}$± 0.1
L. pentosus	42.2 ± 3.2	57* ± 3.6	52.2±5.5	4.1±0.31	2.51±0.3	1.32± 0.6	3.01±0.1	1.21± 0.3	0.98±0.1
***L. pentosus* +HFD**	65.2* ± 5.4	73.8 ± 4.3	63.8*±4.3	3.09± 0.1	2.03*±0.7	0.99*±0.4	2.6±0.3	1.01*± 0.4	0.66± 0.1
IMOs	50.4 ± 6.5	54.2* ±4.6	61.2±5.9	3.5±0.3	2.10±0.3	1.03±0.5	3.13±0.4	1.23±0.2	1.04±0.3
IMOs+ HFD	71.2 ± 4.4	83.4 ± 5.3	88.3± 3.7	1.23±0.9	0.99±0.4	0.87±0.4	1.45± 0.1	0.33±0.3	0.78±0.2
Synbiotic	48.2 ± 6.3	50.2± 4.4	60.6± 2.9	3.61±0.8	2.54±0.9	1.11±0.2	2.98±0.3	1.03±0.7	1.34±0.8
Synbiotic+ HFD	55.5* ± 7.3	59.9*±6.4	61.2*±6.3	3.2* ± 0.7	1.99*±0.7	0.98*±0.3	2.12*±0.6	1.05*± 0.2	0.99*±0.3
Orlistat+ HFD	60.6* ±4.3	55.5± 4.3	65.5*±4.4	3.01±0.6	1.84*± 0.1	0.52 ± 0.1	2.44*±0.4	1.09*± 0.3	0.54±0.5

724 Values are Mean ± SD, $^{\#}p<0.05$ versus control, *$p<0.05$ versus HFD.

725

726

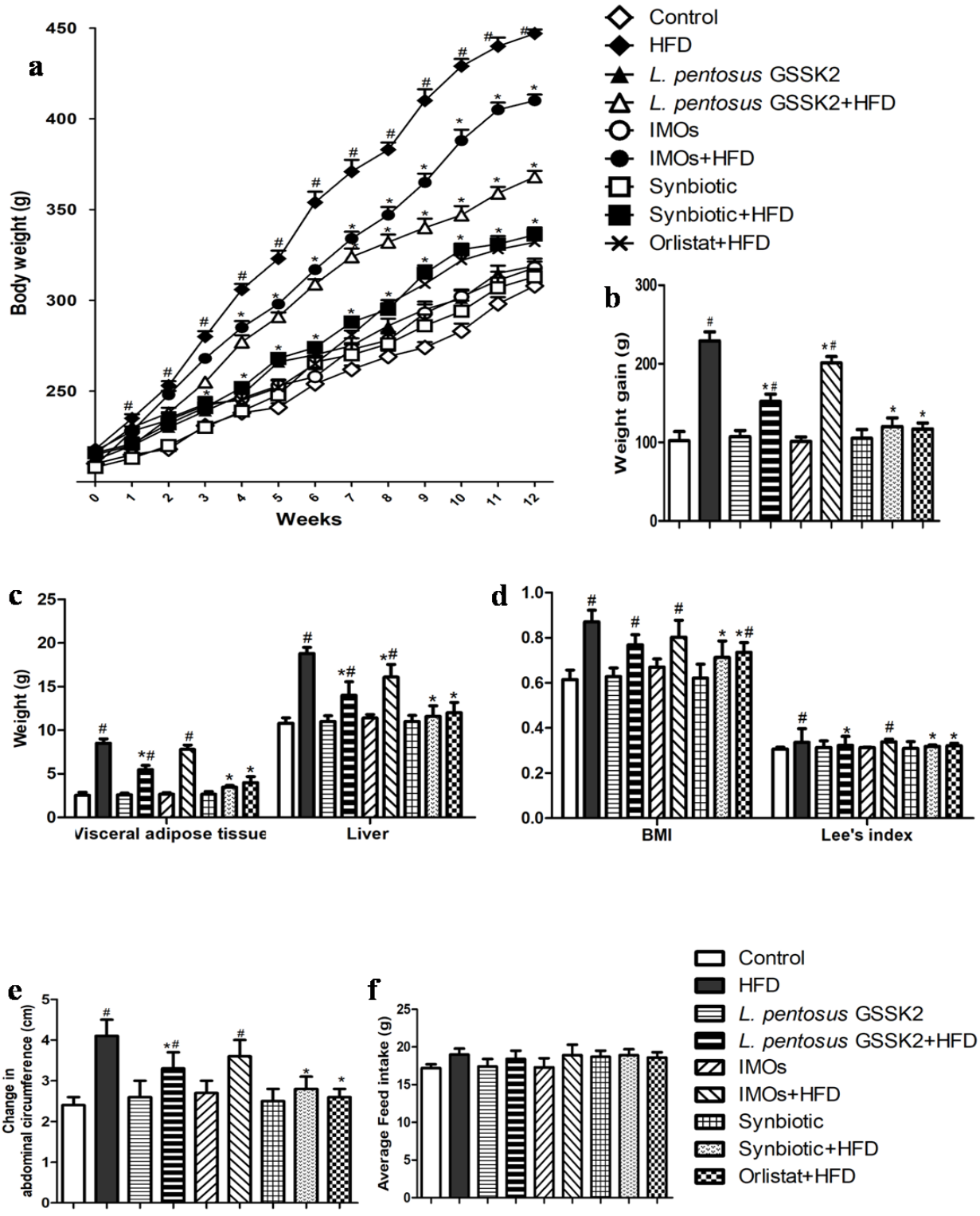
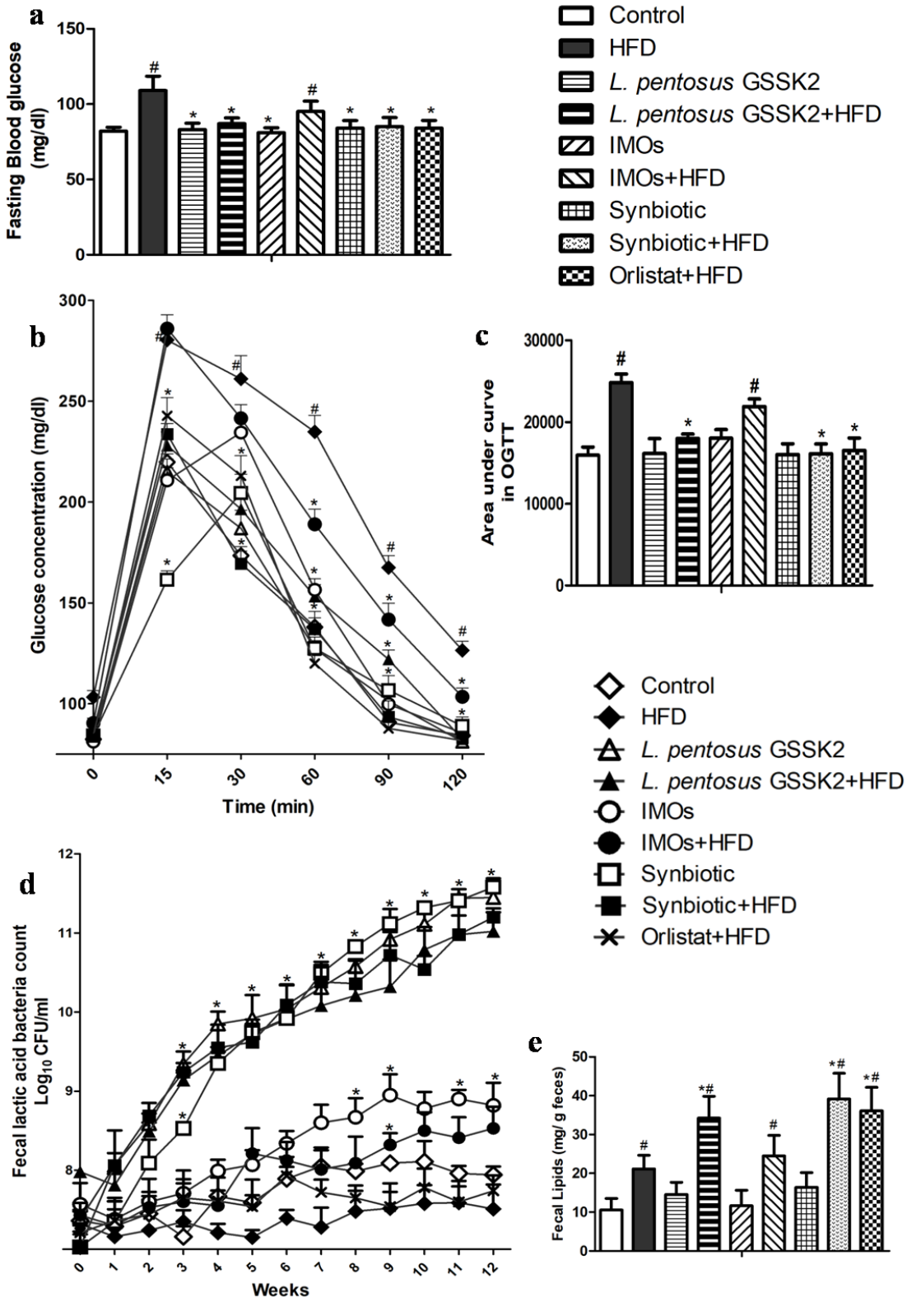
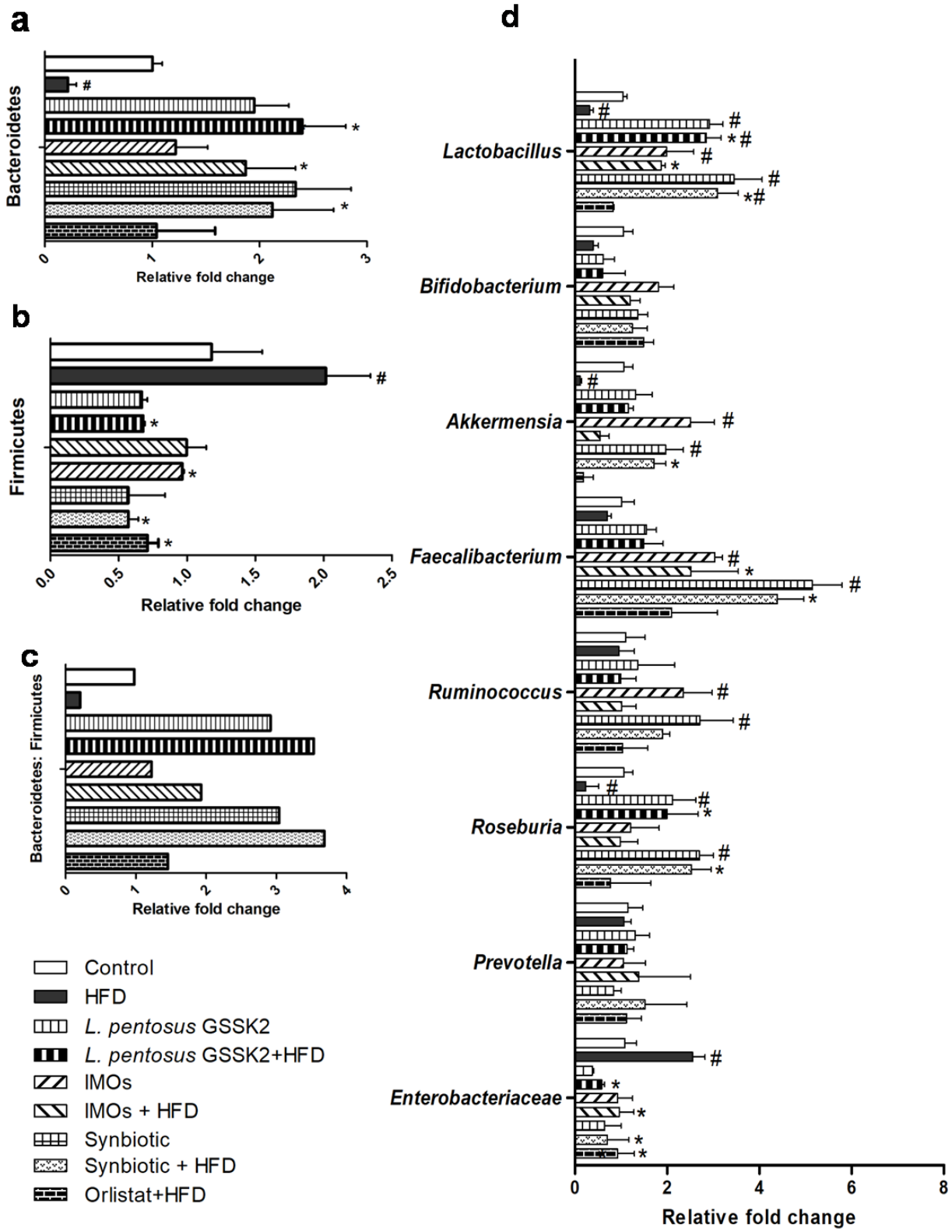

a
Body weight (g)
Weeks
Control
HFD
L. pentosus GSSK2
L. pentosus GSSK2+HFD
IMOs
IMOs+HFD
Synbiotic
Synbiotic+HFD
Orlistat+HFD
b
Weight gain (g)
c
Weight (g)
Visceral adipose tissue
Liver
d
BMI
Lee's index
e
Change in abdominal circumference (cm)
f
Average Feed intake (g)

Figure 1



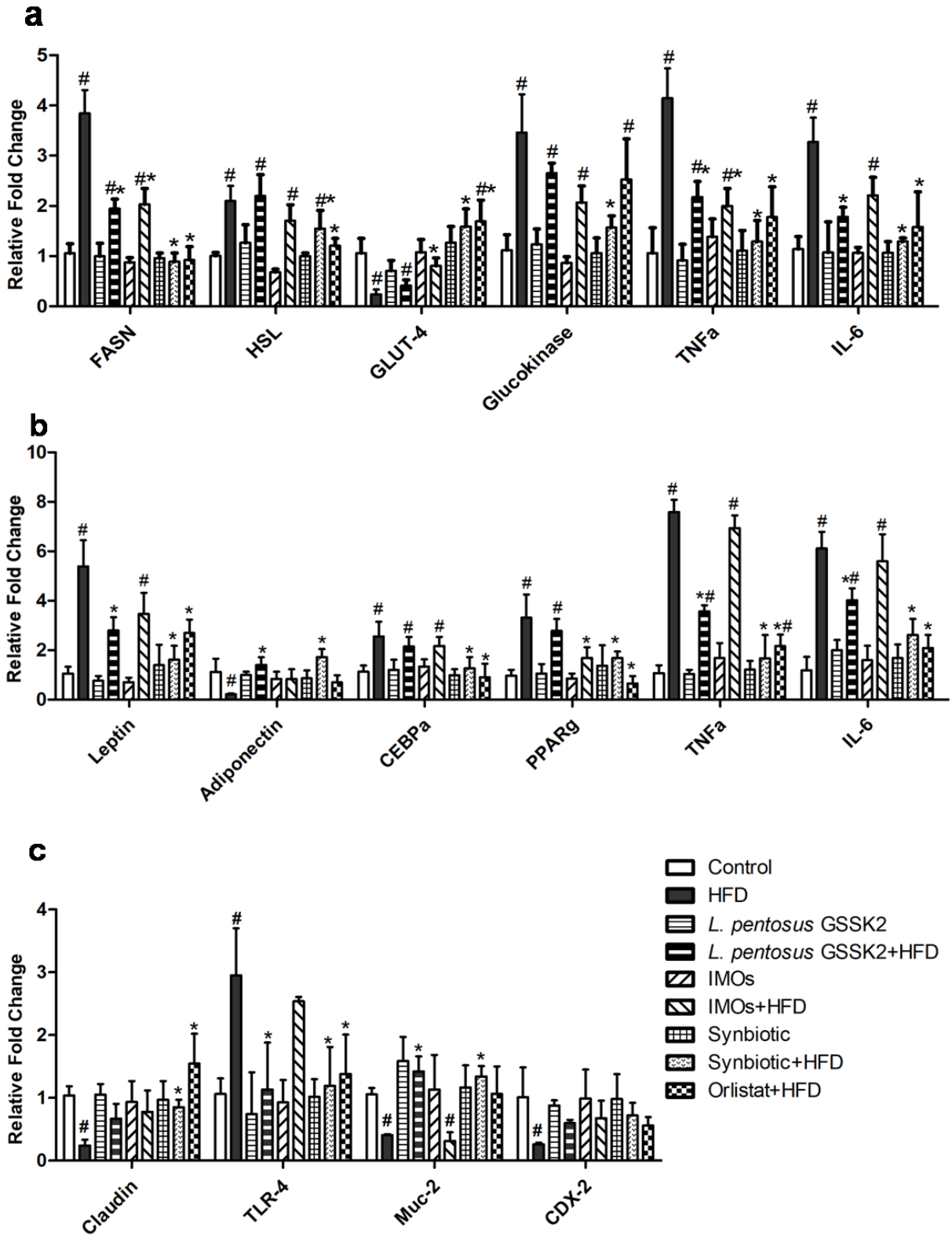
730

731 **Figure 2**



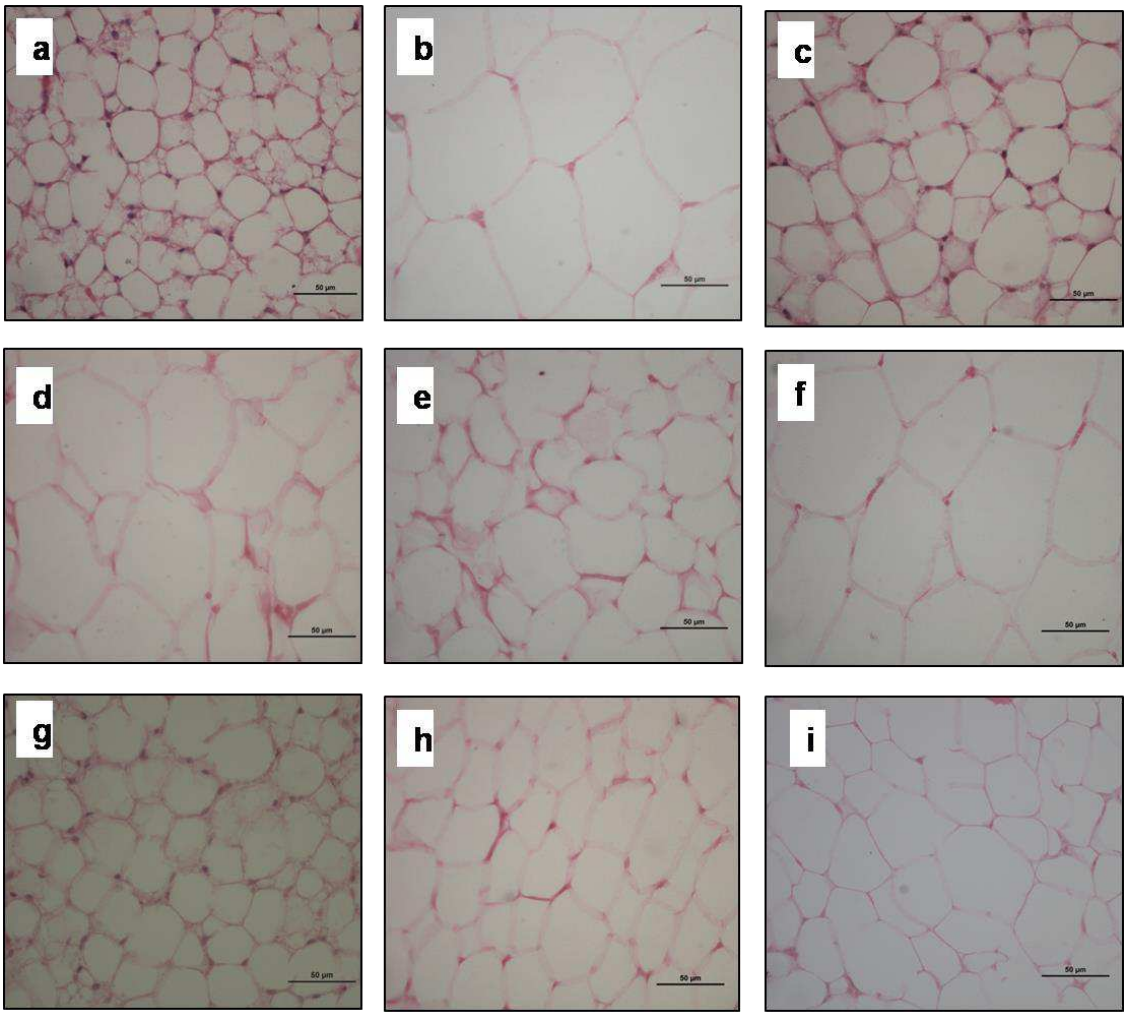
732

733 **Figure 3**



734
735

Figure 4

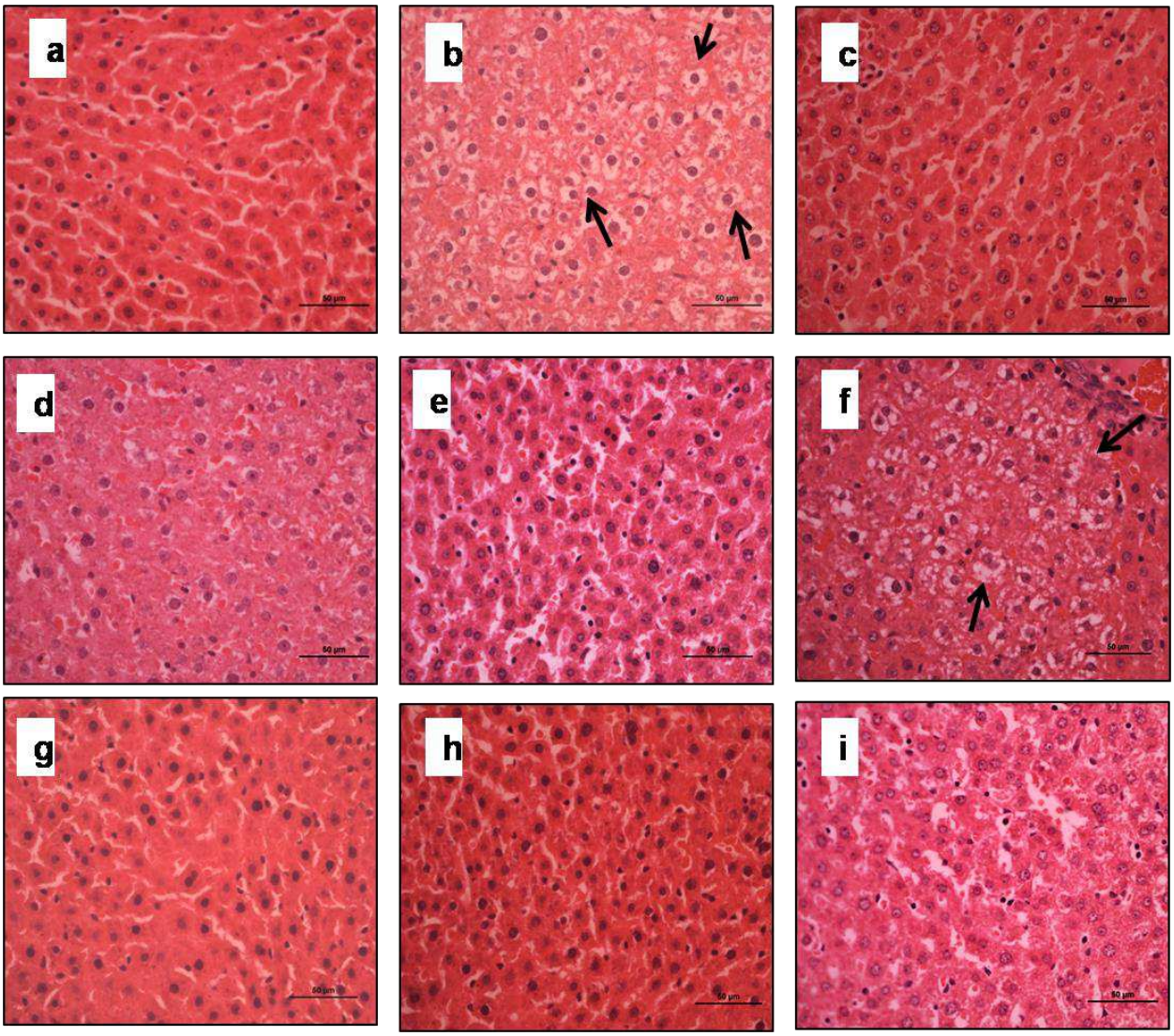


736

737

738 **Figure 5**

739



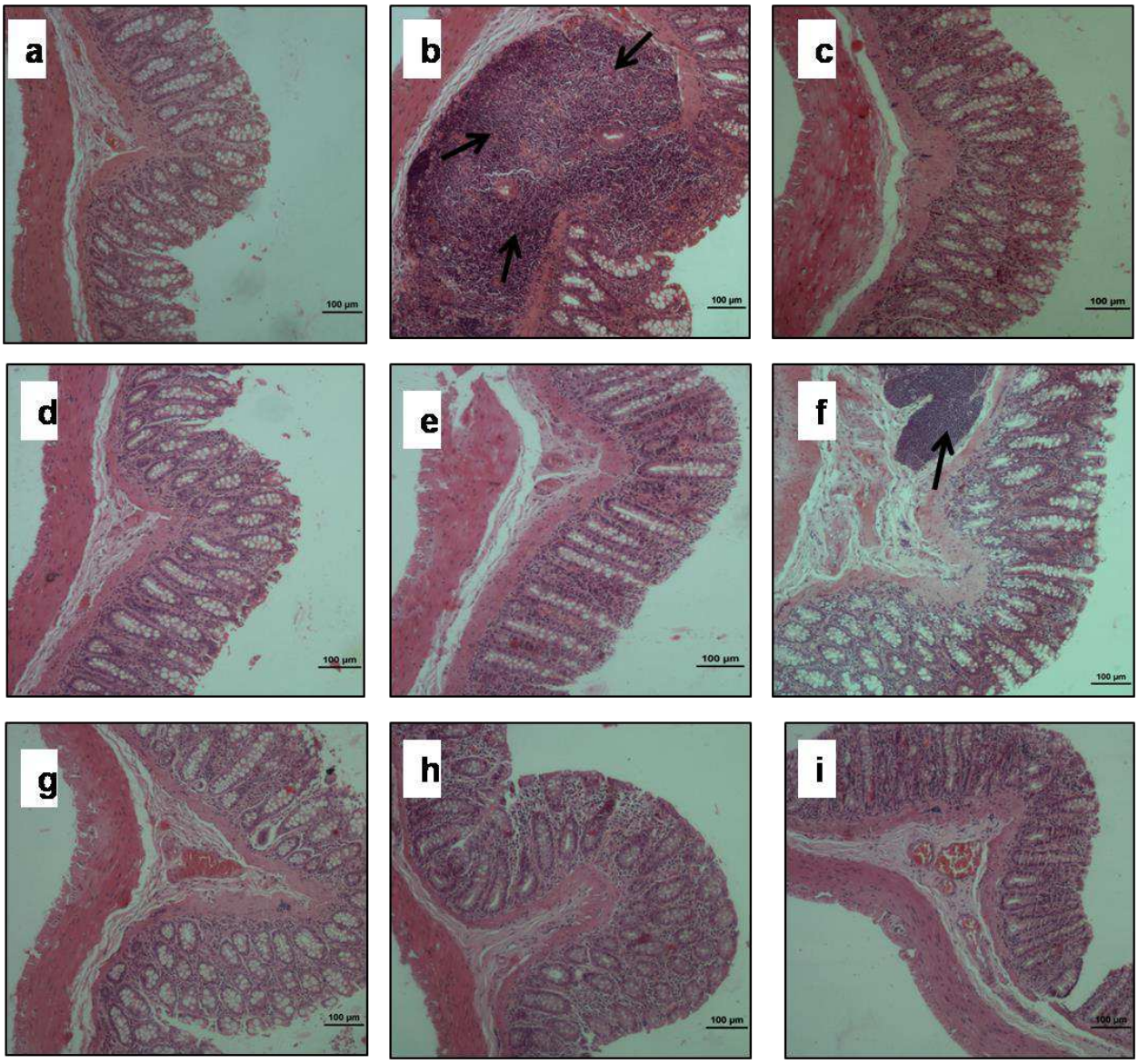
740

741 **Figure 6**

742

743

744



745

746 **Figure 7**

Figures

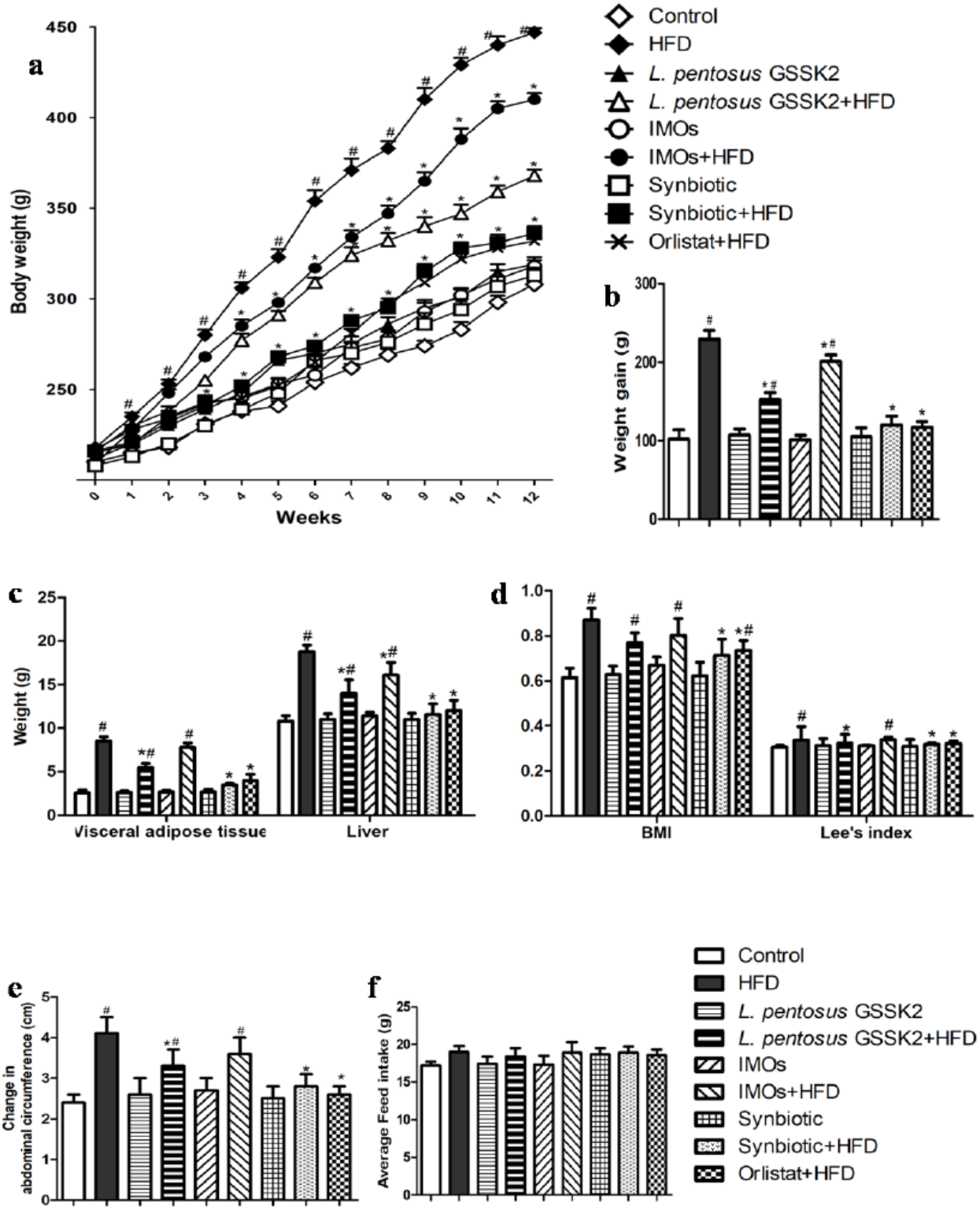


Figure 1

Anthropometric parameters and adiposity markers in different groups of animals: (a) Body weight; (b) Weight gain; (c) Liver and adipose tissue weight; (d) BMI (g/cm2) and Lee's index (e) Change in

abdominal circumference; (f) Feed intake; Values are Mean ± SD, #p<0.05 versus control, *p<0.05 versus HFD.

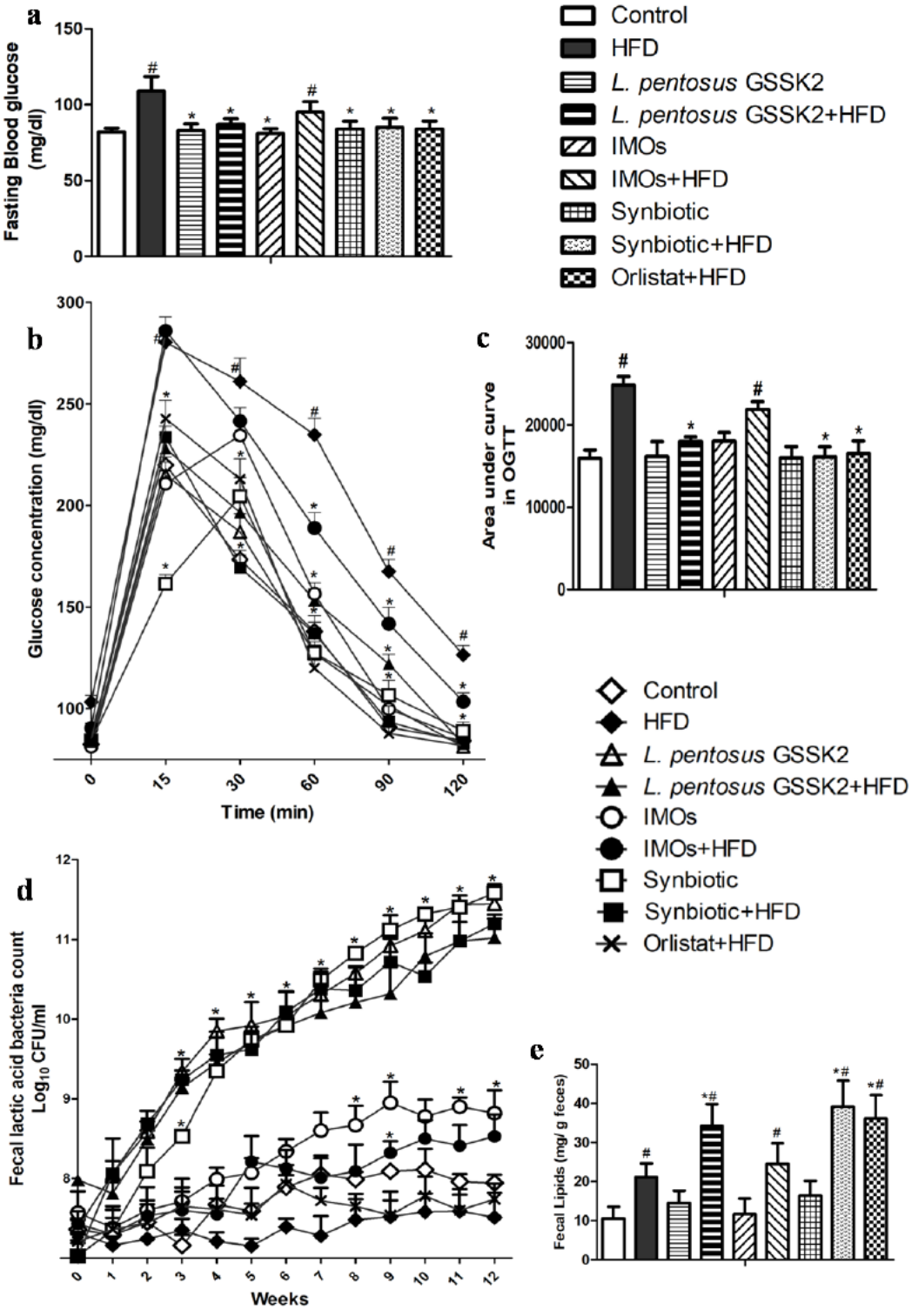


Figure 2

Effect of probiotic, prebiotic and synbiotic supplementation in animals belonging to various groups on: (a) Fasting blood glucose; (b) OGTT (c) AUC of OGTT; (d) Fecal lactic acid bacteria count (Log10 CFU/ml); (e) Fecal lipid excretion. Values are Mean ± SD, #p<0.05 versus control, *p<0.05 versus HFD.

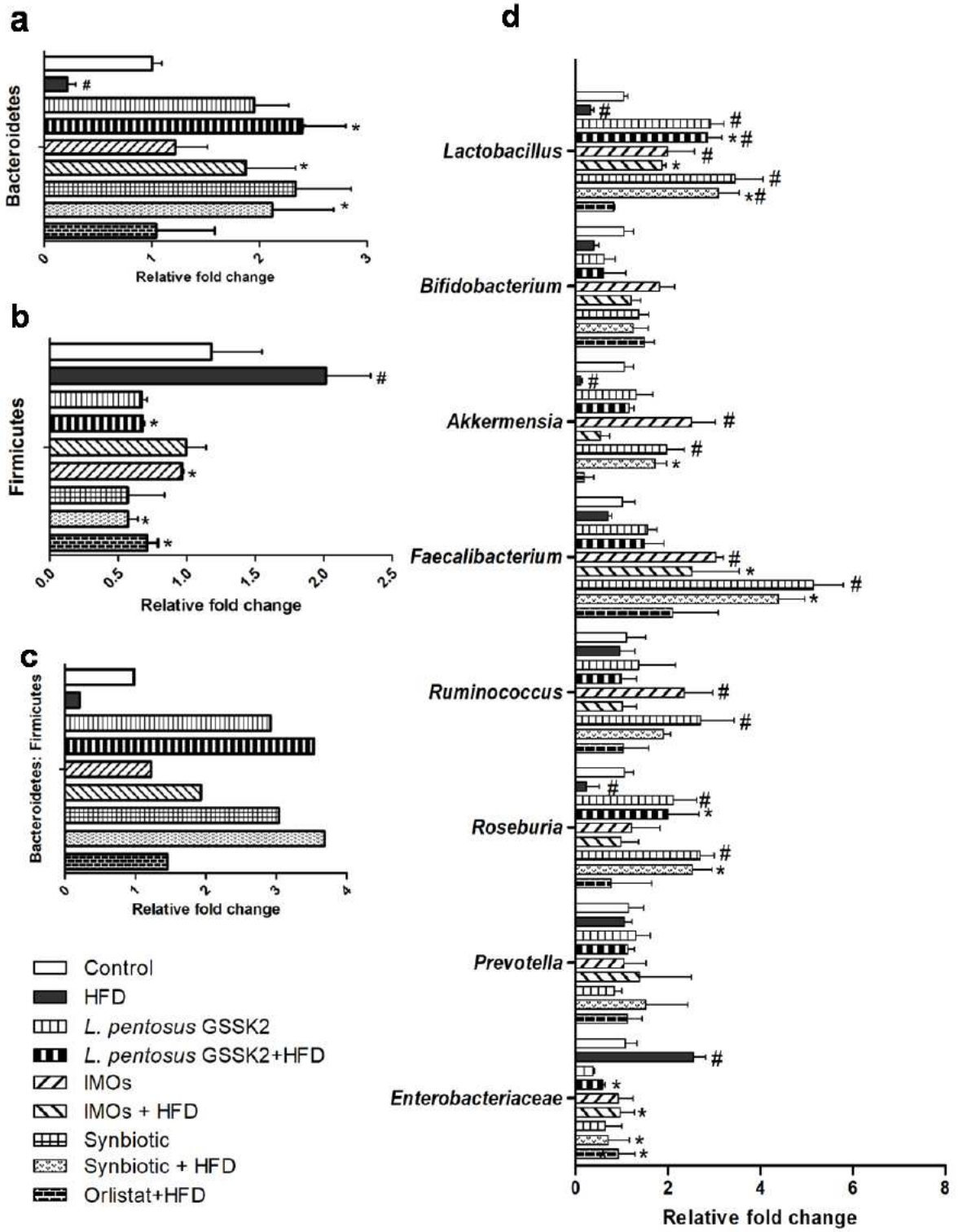


Figure 3

Relative bacterial abundance of different genera in animals belonging to different groups: (a) Bacteroidetes; (b) Firmicutes; (c) Bacteroidetes: Firmicutes; (d) Lactobacillus, Bifidobacteria, Akkermansia, Faecalibacterium, Roseburia, Ruminococcus, Prevotella, Enterobacteriaceae, by real-time PCR. Values are Mean ± SD, #p<0.05 versus control, *p<0.05 versus HFD.

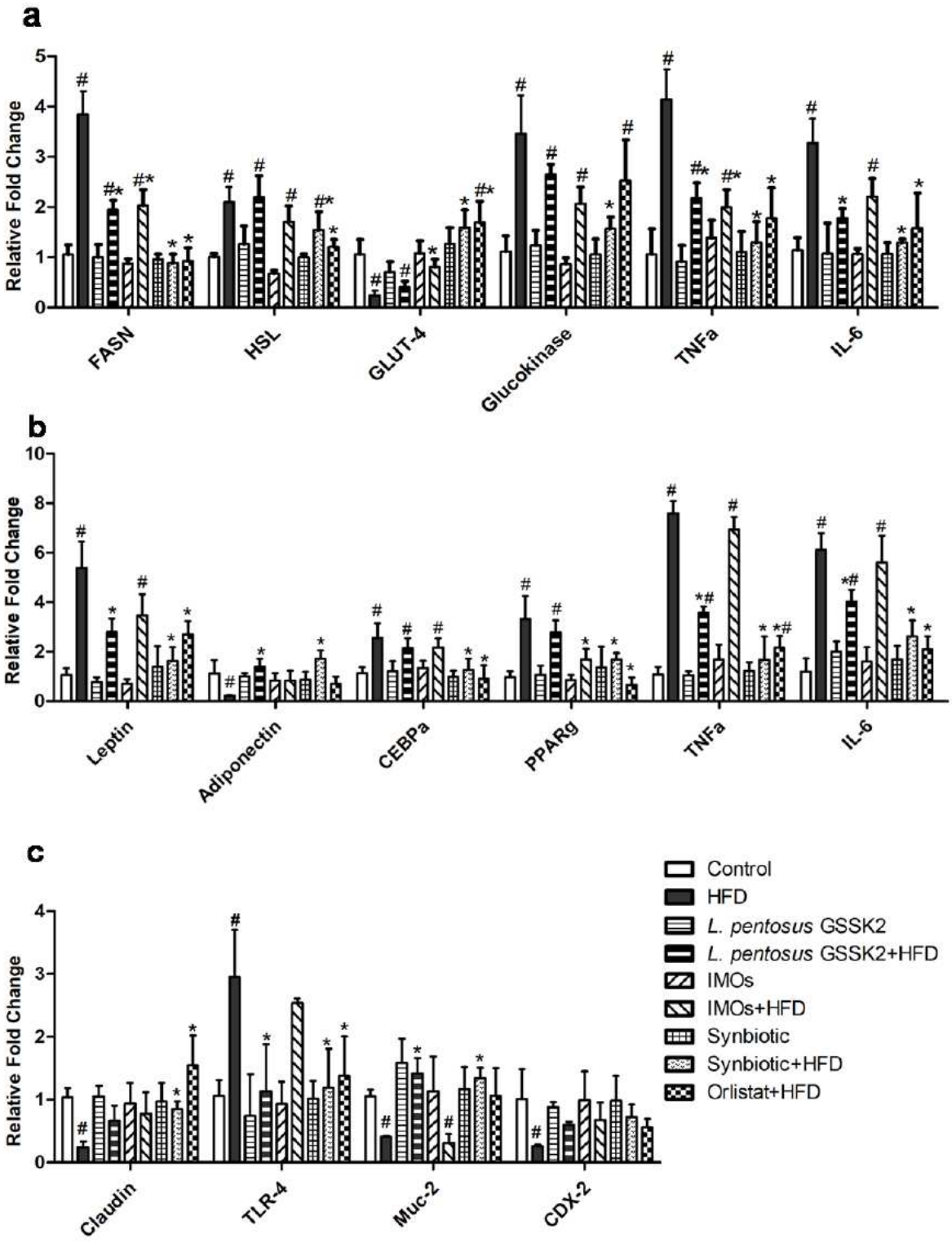


Figure 4

Relative gene expression of: (a) lipid metabolism genes, inflammatory markers, glucose metabolism genes in liver; (b) adiposity genes in adipose tissue; (c) gut integrity genes in colon of animals belonging to various groups by real-time PCR. Values are Mean ± SD, #p<0.05 versus control, *p<0.05 versus HFD.

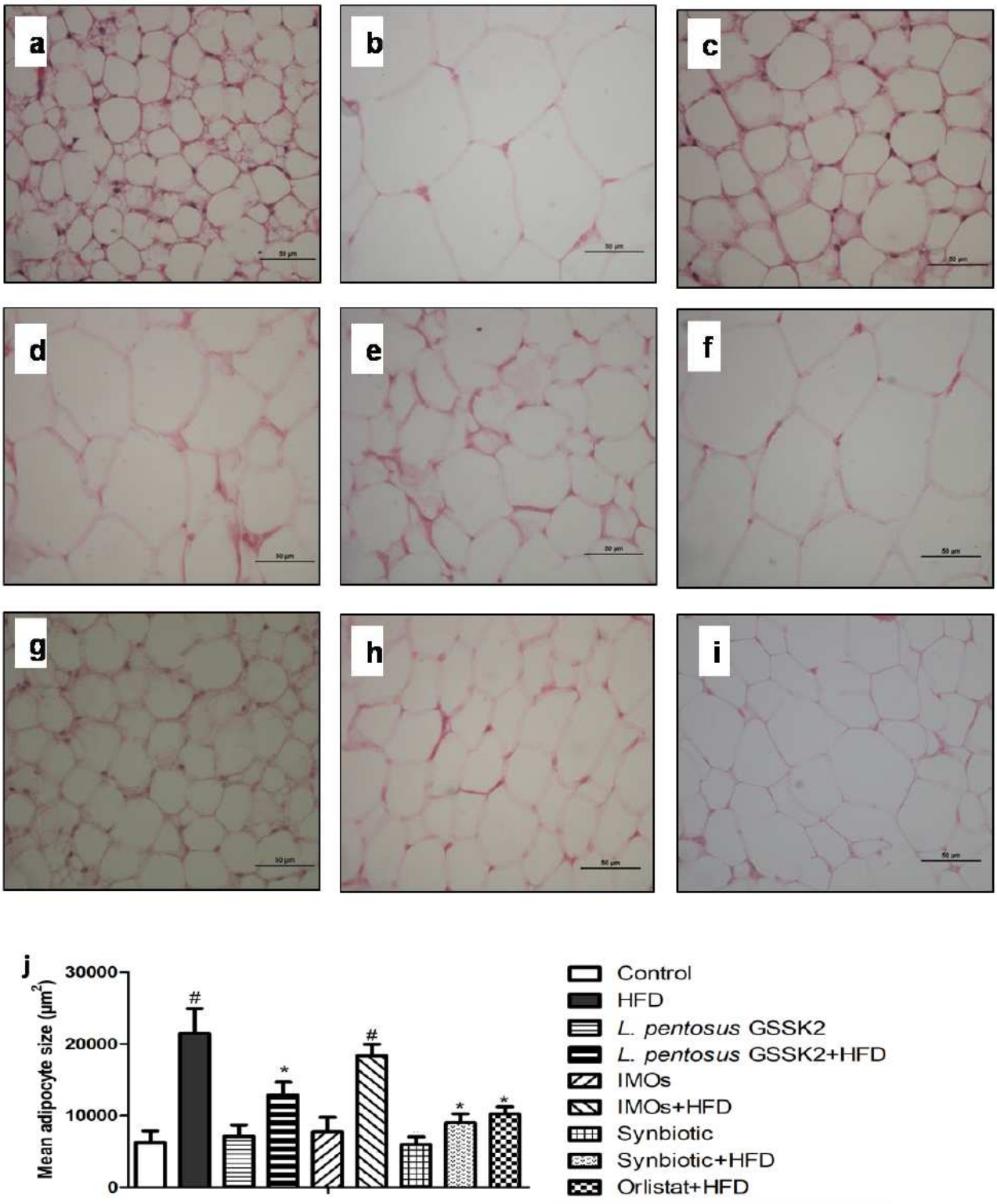


Figure 5

Photomicrograph of adipose tissue showing: (a) normal histomorphology with uniform, spherical adipocytes in control animals; (b) hypertrophied adipocytes in HFD; (c, e & g) normal histoarchitecture of adipocytes in L. pentosus, IMOs and synbiotic animals; (d, f, h & i) reduced adiposity in L. pentosus + HFD, IMOs + HFD, synbiotic + HFD and orlistat + HFD animals (H & E staining; scale bar: 50 μm, 400X); (j) mean adipocyte size in animals belonging 695 to different groups.

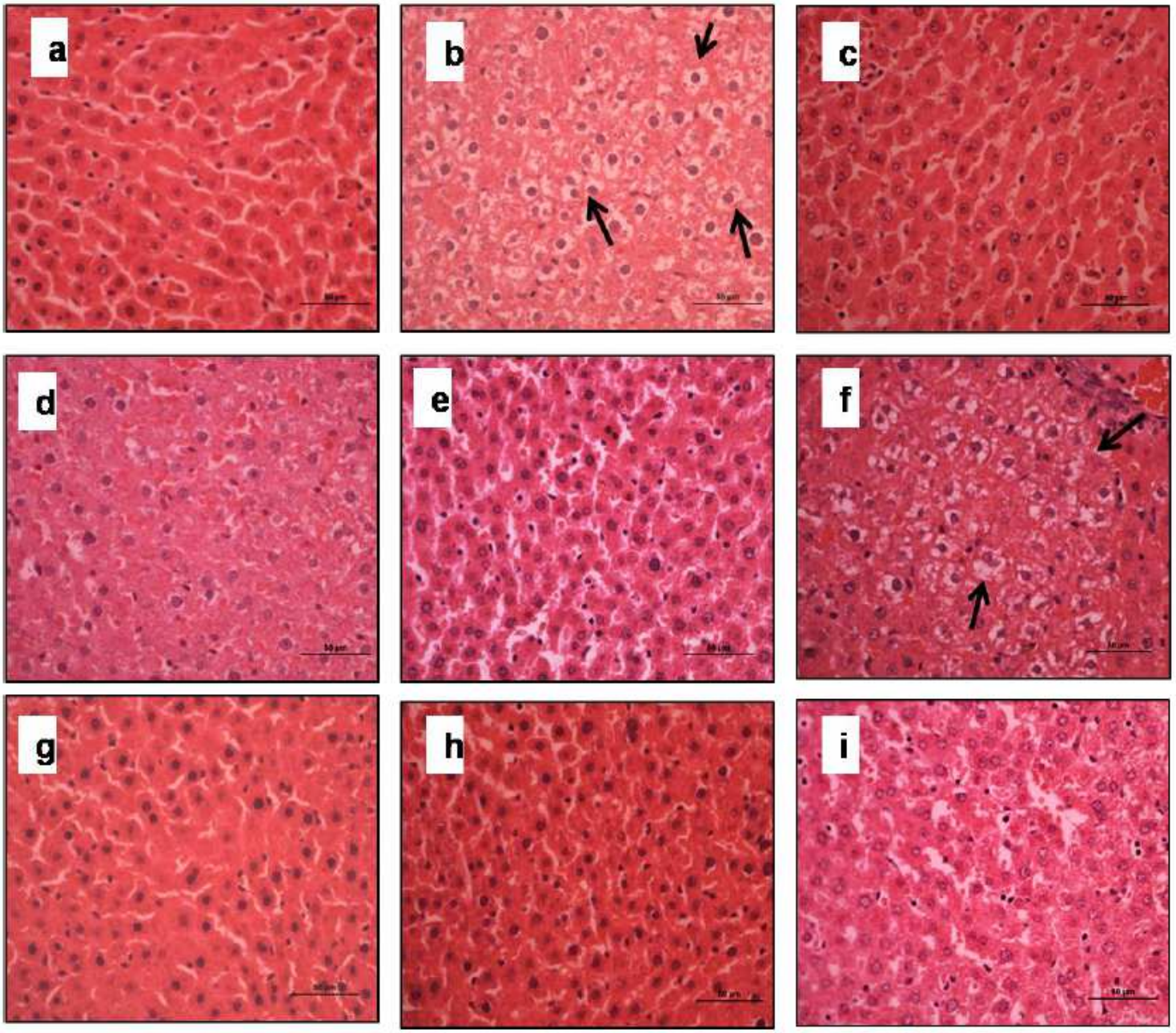


Figure 6

Photomicrograph of liver of animals belonging to different groups showing: (a) Normal histomorphology with polyhedral hepatocytes having large, rounded vesicular nuclei in control; (b) severe hepatic steatosis and ballooning degeneration of hepatocytes in HFD; (c, e & g) normal histoarchitectureof hepatocytes in L. pentosus, IMOs and synbiotic animals; (f) ballooned hepatocytes with vacuolated nuclei in IMOs + HFD animals; (d, h & i) reduced hepatic steatosis in L. pentosus + HFD, synbiotic + HFD and orlistat + HFD animals (H & E staining; arrows indicate ballooned hepatocytes; scale bar: 50 µm, 400X).

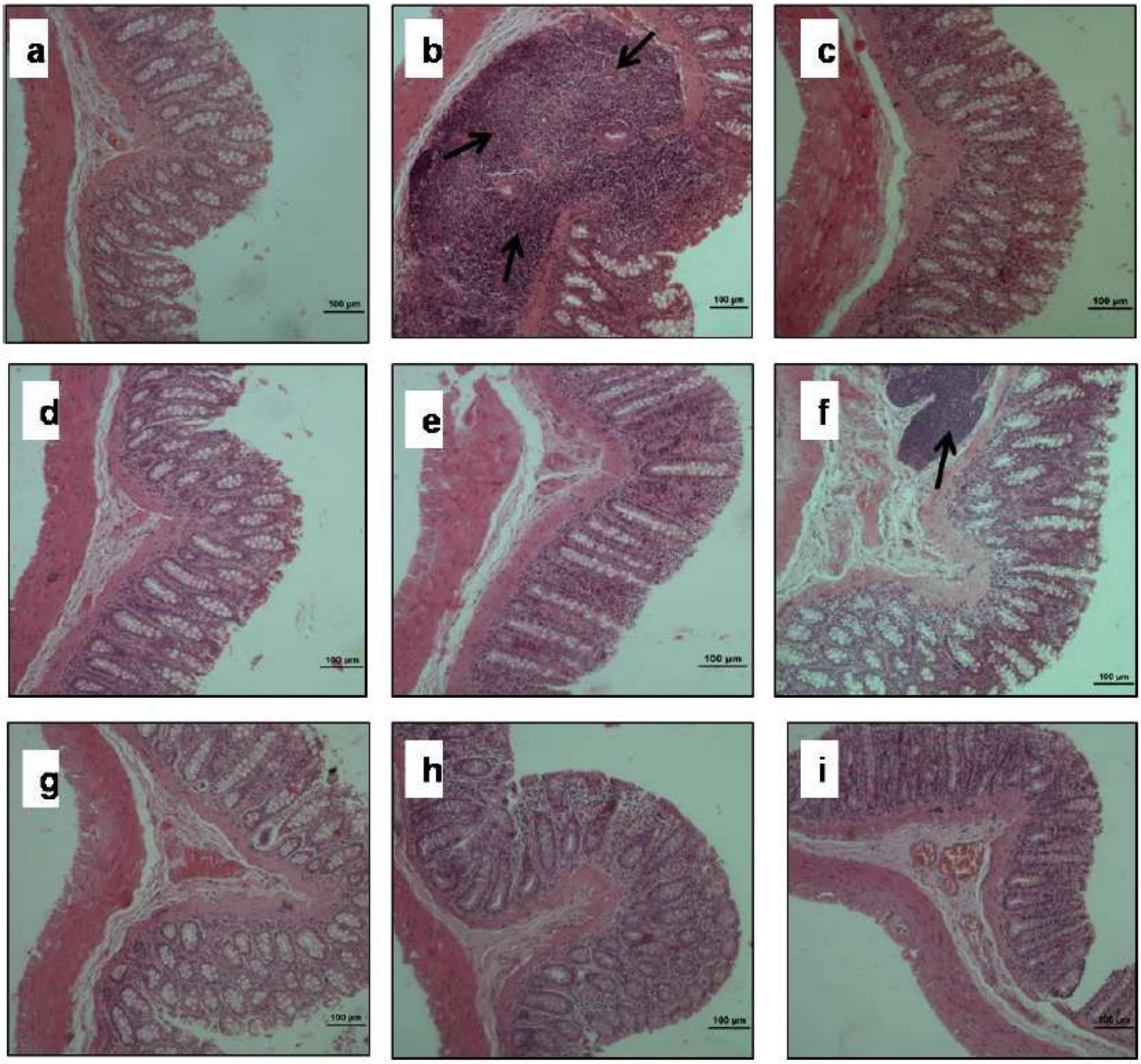


Figure 7

Photomicrograph of colon of animals belonging to different groups depicting: (a) normal histoarchitecture showing mucosa, submucosa, muscularis propria and serosa in control; (b) severely damaged mucosa with infilteration of lymphocytes and plasma cells in HFD; (c, e & g) normal histomorphology of colon in L. pentosus, IMOs and synbiotic animals; (f) inflammation in IMOs + HFD animals; (d, h & i) reduced infilteration of immune cells with intact mucosa in L. pentosus + HFD, synbiotic + HFD and orlistat + HFD animals (H & E staining; arrows indicate infilteration of inflammatory cells; scale bar: 100 µm, 100X).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementarydata.pdf