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Original Article

SELECTIVE CECAL BACTERIAL CHANGES MEDIATE THE ADVERSE EFFECTS ASSOCIATED WITH HIGH PALMOLEIN OR HIGH STARCH DIETS: PROPHYLACTIC ROLE OF FLAX OIL

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ABSTRACT

Objective: Studies on the dynamics of gut bacteria in relation to metabolic adverse effects induced by high palmolein or high starch diets and in relation to health benefits of uncommon foods are lacking. Our aim was to assess under controlled conditions, the impact of vegetable based palmitic acid rich, high fat diet or a high starch diet on various metabolic parameters in relation to selective gut bacterial alterations in rats and also to see the effect of flaxseed oil supplementation on these parameters.

Methods: Wistar Rats were fed for 4 mo either a control diet(CT) or a 30% high fat diet (HF) or HF diet with flax oil supplemented at two different doses (HFF1 and HFF2) or a 78% high starch diet (HC) after which they were sacrificed and analyzed for selective cecal bacteria, hematology, immune function and body composition.

Results: High palmolein diet fed rats showed a decrease in colony forming units of lactobacillus, enterococci, streptococci bacteria and an increase in enterobacteriaceae in the cecum unlike HC fed rats. While high palmolein diet was found to impair immunity and increase inflammation, high starch diet affected body composition and lipid profile. Supplementing the flax seed oil ameliorated most of the adverse effects of high palmolein diet.

Conclusions: Independent of energy intakes both high palmolein and high starch intakes have differential adverse effects. It can be envisaged that the adverse effects of feeding palmolein are mediated through immune impairment and inflammatory response, which in turn are associated with altered gut bacteria profile; and flax oil was found to have a prophylactic role in controlling these adverse effects. This study emphasizes the need to evaluate immunological as well as bacterial profile while assessing the safety of dietary fats in addition to traditional methods.

Keywords: Palmolein, Starch, Flax oil, Calorie intake, Cecal bacteria, Immune function, Body composition, Lipid profile, Omega 3 fatty acids.

INTRODUCTION

Quantity and quality of diet have lots of implications in causing obesity and diabetes. That diet brings about selective gut bacterial alterations which could influence the inflammatory status is a novel theme recently recognized. This has been established beyond doubt using animal fat like lard [1, 2]. Studies on the metabolic complications associated with consumption of high starch diets or vegetable fat, such as palmolein and also the role of uncommon foods with health benefits in relation to gut bacteria are lacking. To the best of our knowledge the effect of flax seed oil (a copious source of omega 3 fatty acids) on high palmolein oil induced gut bacterial changes are not so far documented.

Cereals are the staple diet in many parts of the globe and studies reported in the literature show that besides fat,high carbohydrate consumption also results in an increase in plasma glucose, insulin, triglycerides and non-esterified fatty acids leading to insulin resistance [3, 4].

However, in general, much of the work linking food and metabolic disorders (type 2 diabetes and insulin resistance) has been focused on studying food fat content and less have been concentrated on the importance of carbohydrate composition. In almost all studies reported thus far on gut bacteria in relation to high fat diets,the fat used is lard which is of animal origin [5]. Another fat similar to Lard in its fatty acid profile is palmolein [6] which is of plant origin and is widely used all over the globe in the fast foods and bakery products and also in households. It is not surprising to see that childhood obesity is on the rise as the fast foods and bakery products are the preferred food choices of children nowadays. However, there is a long standing controversy on whether palmolein is really beneficial and more so on higher doses than the recommended intakes of dietary fat. Likewise, studies reported on the effects of palmolein on lipids and other biochemical parameters are controversial and not many surveys are there on the long term usage of palmolein more so in relation to gut bacteria. Omega-3 fatty acids from fish oil have been shown to reduce inflammation and help prevent certain chronic diseases, such as heart disease and arthritis [7, 8]. However, flax seed oil, which is rich in omega 3 fatty acids, has limited scientific evidence. Keeping in view all the above facts, the present study was designed with the following objectives: To assess under controlled conditions, the impact of a vegetable based high fat (HF) diet or a high carbohydrate (HC) diet on various metabolic parameters in relation to selective gut bacterial alterations in Wistar/NIN rats. Another objective of the study was to see if flax seed oil could influence these gut bacterial alterations in these rats fed high fat diet.

The present study was designed to study the effects of HF/HC diet, with palmolein as the source of high fat and corn starch as the source of carbohydrate compared with a control group fed groundnut oil at 10% (w/w) in the diet on selective cecal bacteria, plasma biochemical parameters, body composition, immune function and inflammation in rats. Also flax seed oil was supplemented at two different concentrations to test its influence on the HF diet fed rats.

MATERIALS AND METHODS

This study has been conducted after taking the approval of the Institute's (National Institute of Nutrition, Hyderabad, India) ethical committee on animal experiments which strictly adhered to the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health[9]. Permit number: (P21/8-2009/RH). The current study, which is a part of a project was initiated during 2012.

Treatment of rats

Forty Wistar/NIN male rats of weanling age were obtained from the National centre for Laboratory Animal Sciences (NCLAS), National Institute of Nutrition, (ICMR), Hyderabad, India. The rats were housed in a standard poly carbonated open cages with top grill having facilities for feed and drinking water in polycarbonate bottles with stainless steel sipper tubes. The animal rooms were maintained at 20±2 0C and relative humidity was kept between 50-55% with a 12 h light/dark cycle. Purified water collected through activated charcoal filters and exposed to UV rays (Aqua guard on-line water filter-cum-purifier). The rats were randomly divided equally into 5 groups and were fed diets ad libitum according to Table1 for a period of 16 w.

Table 1: High palmolein/High starch diet composition (g/100g Diet)*

Groups	Starch	Sucrose	Total calories from carbohydrate	Casein	Fat
CT (408)	60 (58.8%)	0	(58.8%)	19.5(19%)	10Groundnut (22%)
HF (508)	19 (15%)	19(15%)	(30%)	21.5(16.9%)	30Palmolein(53.1%)
HFF1 (508)	19 (15%)	19(15%)	(30%)	21.5(16.9%)	30Palmolein(53.1%) +Flax oil 1
HFF2 (508)	19 (15%)	19(15%)	(30%)	21.5(16.9%)	30Palmolein(53.1%) +Flax oil 2
HC (383)	55.9(58.4%)	18.6(19.4%)	(77.8%)	10(10.44%)	5Palmolein(11.7%)

*Cellulose-5%, Salt Mix–4%, Vitamin Mix—1%, L-Cysteine–0.3%, Choline Chloride-0.2%, Values in parentheses are calories. CT = Control; HF = High Fat (palmolein) diet; HFF1 = High Fat (palmolein) diet+Flax seed oil 7.5 ml/kg body weight; HFF2 = High fat (palmolein)+Flax seed oil 15 ml/kg body weight; HC=High carbohydrate/High starch.

The group I rats served as the Control group (CT). Group II (HF) received a high fat diet with 30% palmolein (obtained from local market), group III (HFF1) and group IV (HFF2) rats also were fed the same high fat diet, but in addition received flax oil (obtained from the Shiv sales corporation, New Delhi) by gavage daily at 7.5 ml or 15 ml per kg body weight respectively. Group V (HC) rats were fed a high carbohydrate diet (78%).

Microbial parameters

Selective culture media like Rogosa agar for Lactobacilli, Bifidobacterium agar for Bifidobacterium spp, Mac Conkeymedium for Enterobacteriaceae, KF Streptococcus agar for Streptococcus, Bile Esculinazide agar for Enterococci [10] were obtained from Merck AG, Darmstadt, Germany, and their selectivity was tested with the pure bacterial strains namely Lactobacillus acidophilus, Bifidobacterium brevis, Escherichia coli, Streptococcus Agalactiae and Enterococcus faecalis respectively which were revived from the bacterial swabs obtained from American Type Culture Collection (ATCC).

Bacterial enumeration

The concentrations of various bacteria in the ceacum were analysed using conventional culture method [11].

Plasma biochemistry

Plasma was analysed for glucose, creatinine, urea, total protein, albumin, total bilirubin, total cholesterol, HDL cholesterol, Triglycerides and enzymes namely alanine amino transferase (ALT), aspartate amino transferase (AST), gamma glutamyltranspeptidase (GGT) and alkaline phosphatise (ALP) by using auto analyser as per the instructions given in the manufacturers manual (Schiapparelli Biosystems Inc. Fairfield, USA).

Body composition

Body composition was analysed by using Total body electrical conductivity (TOBEC) which is a most advanced non-invasive technique to measure the total body composition in live animals [12].

Haematology parameters

Haematology parameters were measured by using ADIVA 120 analyzer as per manual instructions.

Splenocyte proliferation assay

The Splenocyte proliferation was assessed by measuring the stimulation in DNA synthesis in terms of incorporation of radioactive thymidine into the cultured spleen cells in the presence or absence of a mitogen Concanavalin A [13]. The stimulation index is expressed as the ratio of Counts per min in Concanavalin A stimulated culture to Counts per min in un stimulated culture.

Bone marrow cellularity

Bone marrow was collected from femur and the number of bone marrow cells, was determined using a haemo cytometer and expressed as total live cells per femur [14].

Statistical analysis

All results are expressed as mean±SEM. The inter group variation between various groups was measured by one way analysis of variance (ANOVA) using the SPSS version 19.0 software. Log transformation of data and data analysis using non parametric tests was carried out wherever necessary. Probability values (P Value) for the various parameters are given in the tables provided. Results were considered statistically significant when $p<0.05$.

RESULTS

The average energy intakes and body weights monitored for the first 12 w period by the various groups studied are given in table 2.

In the HF fed group while the total food intake, total calorie intakes were significantly lower with respect to the CT group, there were no differences in the body weights at any time point in the study. While the total food intake by HFF1, HFF2 was significantly lower with respect to the CT group only a non-significant decrease in total calorie intakes was seen in HFF1and no change was seen in HFF2 group. The body weights were significantly higher in HFF1 and HFF2 groups in the first 4 w, which by the end of the study became comparable with that of CT group. Amongst the high fat fed groups, while there was a decrease in total food intake by both HFF1 and HFF2 groups (P=0. 190 and P=0. 049 respectively), in terms of total energy intake, there was an increase by both these groups (P= 0.226 and P= 0.027 respectively) with no differences seen in the body weights (P=0. 864 and P=0. 602 respectively) compared to HF group.

The food intake, total energy intake and body weights during the study period between the HFF1 and HFF2 groups were not significantly different (P=0.484, P=0.279 and P=0.486 respectively) (table 2).

The food intake and total energy intakes by the HC group were comparable with that of the CT group. On the other hand, a significant steep increase in body weights was observed in the first 5w after which there was a gradual decrease in body weights which by the end of 16 w became comparable to that of the CT group (table 2).

Hematological examination of rats showed that the numbers of total red blood cells, monocytes and eosinophils, blood platelets, hemoglobin and haematocrit between the HF fed group and the CT were comparable. Nevertheless, a statistically significant increase in neutrophil percent and a decrease in total WBC and lymphocyte percent were observed in the HF fed rats compared to CT group (fig. 1).

Table 2: Effect of high palmolein/high starch feeding on energy intakes and body weights–influence of flax seed oil

Parameters	Groups				
	CT	HF	HFF1	HFF2	HC
Initial body weight (g)	60.6 ±6.70(7)	60.3 ±6.57(7)	60.4 ±6.51(7)	60.0 ±6.35(7)	59.7 ±5.86(7)
Final body weight (g)	325.20±10.2 (5)	316.67 ±19.1(6)	320.33 ±12.9(6)	305.86 ±14.6(7)	303.00 ±14.8(5)
Mean body weight at the end of 12w (g)	173.29 ±24.3(8)	174.1 ±21.6(8)	176.65 ±21.2(8)	174.94 ±18.2(8)	189.06 ±16.5(7)
Food intake (g/w)	91.1 ±5.24(8)	61.9 ±3.25**(8)	54.7 ±1.22**(8)	50.9 ±1.58**(8)	93.3 ±6.26(7)
Total Energy Intake (Calories/w	365.08 ±23.5(7)	300.33 ±18.1*(6)	336.80 ±13.5(6)	369.31 ±24.0(6)	339.56 ±20.9 (5)
Gain in body weight (g/w)	27.2 ±1.92(7)	25.9 ±1.58(6)	25.2 ±1.01(6)	24.2 ±1.48(6)	20.3 ±2.10**(5)
Feed Conversion ratio	13.6 ±0.93(7)	11.8 ±0.99(6)	13.5 ±0.91(6)	15.7 ±1.75(6)	17.1 ±1.18(5)
Feed efficiency	0.075 ±0.005 (7)	0.087 ±0.006(6)	0.076 ±0.005(6)	0.068 ±0.008(6)	0.060 ±0.005(5)
Carbohydrate Intake (Calories/W)	218.51 ±12.6 (8)	57.0 ±2.99**(8)	50.3 ±1.13**(8)	46.8 ±1.45**(8)	277.87 ±18.7** (7)
Fat intake (Calories/W)	81.9 ±4.71(8)	167.30 ±8.77**(8)	205.78 ±9.08**(8)	253.47 ±16.0**(8)	42.0 ±2.82**(7)
Protein intake (Calories/W)	71.0 ±4.08(8)	53.3 ±2.79**(8)	47.1 ±1.05**(8)	43.8 ±1.36**(8)	37.3 ±2.50**(7)

*P<0.05, **P<0.01 Values are mean±SE. Fig. in parentheses indicate sample size.

CT = Control; HF = High Fat (palmolein) diet; HFF1 = High Fat (palmolein) diet+Flax seed oil 7.5 ml/kg body weight; HFF2 = High fat (palmolein)+Flax seed oil 15 ml/kg body weight; HC=High starch. P Value is with respect to Control group.

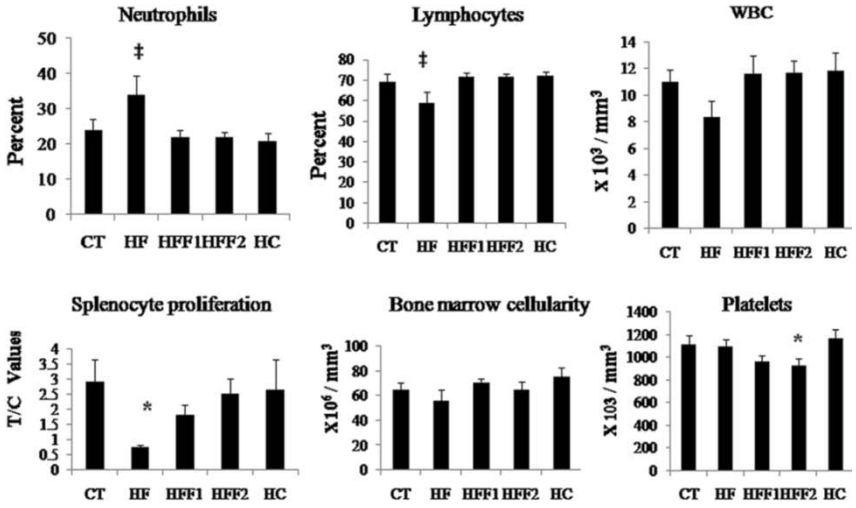


Fig. 1: Effect of feeding high palmolein/high starch diet on immune and inflammatory parameters–influence of flaxseed oil

The splenocyte proliferation in response to mitogen that indicates splenocyte function also showed a substantial diminution in the HF fed group compared to CT rats (fig. 1). When flax seed oil was fed along with HF at two different concentrations, all the hematological parameters were comparable to the CT group. The platelet count in these groups was lower than CT and HF groups; however, it was in the physiological range. The neutrophil, total WBC and the lymphocyte counts and the mitogenic response to concanavalin-A in both the HFF1 and HFF2 groups were comparable to the CT group (fig. 1). All the hematological parameters studied in the HC diet fed rats were comparable with the CT group. Similarly, the splenocyte proliferation activity was not different in the HC fed rats when compared to that of the CT group (fig. 1).

Coming to the plasma biochemistry there were no changes in the concentration of plasma glucose, triglyceride and total cholesterol, but a significant decrease was seen in HDL cholesterol concentration and thereby the HDL/Cholesterol ratio in the HF fed rats compared to CT group. The plasma concentration of various metabolites and enzymes tested in the HF fed rats did not differ from the CT group (table 3). The total cholesterol concentration in both the HFF1 and HFF2 groups was significantly lower when compared to the HF and the CT groups. Moreover the HDL cholesterol/total cholesterol ratio in the HFF1 group was comparable with that of control (fig. 2).

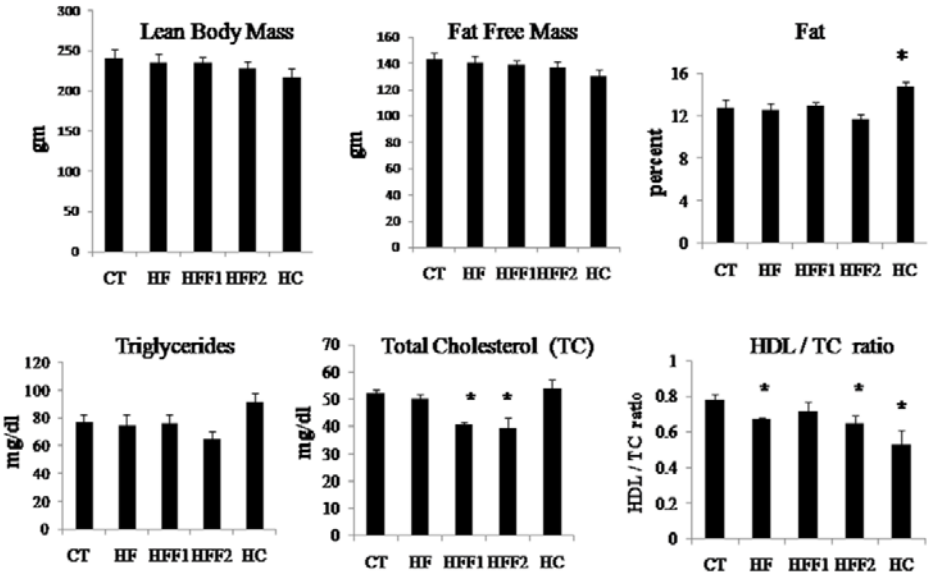
Plasma total bilirubin and ALT concentrations were significantly higher and the plasma calcium concentration was lowered below the CT values in HFF2 group (table 3), however these values are in the physiological range. Plasma glucose, cholesterol concentration in the HC fed group was not different from that of CT rats, whereas HDL cholesterol and HDL/Cholesterol ratio were lower compared to CT rats. In addition, there was an increase in the triglyceride concentration of plasma in the HC compared to CT rats, which however were not statistically significant(fig. 2). Cecal bacterial profile showed a decrease in colony forming units of Lactobacillus, Enterococcus and streptococci bacteria(fig. 3) in HF diet fed rats.

Table 3:The effect of high palmolein/high starch feeding on some plasma biochemical parameters influence of flax seed oil

Parameters	Groups				
	CT	HF	HFF1	HFF2	HC
Glucose (mg/dl)	95.0 ±3.62 (6)	95.5 ±5.17(8)	99.6 ±5.95 (8)	84.5 ±4.66(8)	97.4 ±9.83(8)
Urea (mg/dl)	28.40 ±0.99 (5)	23.3 ±1.66 (7)	21.0 ±2.81*(8)	18.86 ±1.26**(7)	24.86 ±2.34(7)
Creatinine (mg/dl)	0.64 ±0.081(6)	0.70 ±0.042 (8)	0.60 ±0.072(8)	0.64 ±0.088(8)	0.77 ±0.086(8)
Total Protein (g/dl)	7.84 ±0.469 (6)	6.9 ±0.357 (4)	6.71 ±0.328(7)	7.08 ±0.520(7)	7.37± 0.119(8)
Albumin (g/dl)	4.17 ±0.117 (6)	4.09 ±0.068 (8)	4.15 ±0.133(7)	3.85 ±0.58(7)	4.45 ±0.128(8)
ALP (IU/l)	548 ±77(6)	476 ±59(7)	492 ±56(7)	641 ±70(6)	446 ±48(8)
ALT (IU/l)	24.6 ±1.75 (5)	21.4 ±3.61(7)	40.3 ±4.13 (7)	51.8 ±9.01**(8)	32.5 ±6.41 (6)
AST (IU/l)	84 ±8.16(6)	79.5 ±5.12 (6)	90.8 ±8.78 (8)	104.30 ±15.0(8)	94.1 ±5.67 (8)
Total Bilirubin (mg/dl)	0.32 ±0.032 (6)	0.36 ±0.069 (8)	0.41 ±0.050(8)	0.48 ±0.056(8)	0.42 ±0.048(8)
Calcium (mg/dl)	10.4 ±0.26(6)	9.95 ±0.3(8)	9.76 ±0.31(8)	8.13 ±0.50**(8)	8.99 ±0.740(8)

*P<0.05, **P<0.01, Values are mean±SE. Fig. in parentheses indicate sample size.

CT = Control; HF = High Fat (palmolein) diet; HFF1 = High Fat (palmolein) diet+Flax seed oil 7.5 ml/kg body weight; HFF2 = High fat (palmolein)+Flax seed oil 15 ml/kg body weight; HC=High starch. P Value is with respect to the Control group.

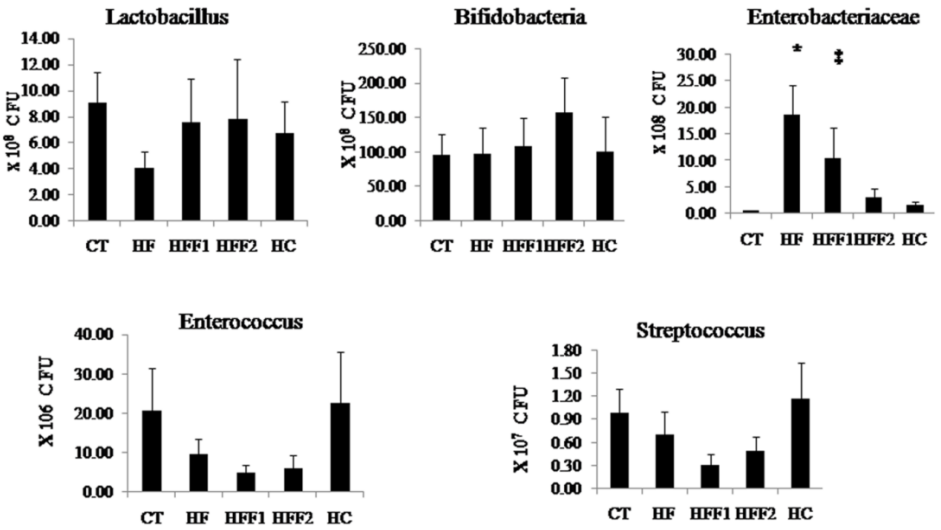


***P<0.05**

Fig. 2: Effect of feeding high palmolein/high starch diet on body composition and lipid profile–influence of flaxseed oil

On the other hand, a significant increase in colony forming units of Enterobacteriaceae was seen in the cecum of HF diet fed rats when compared with the control group. There were no changes in the concentration of bacteria belonging to bifidobacteria. In both HFF1 and HFF2 groups, the concentration of lactobacilli was comparable with the CT group.

With respect to bifidobacteria concentration, there was no change in the HFF1 group, however a statistically non-significant increase was seen in the HFF2 group compared to the CT group. Compared to CT group, the concentration of Enterobacteriaceae in HFF1 treated rats were higher while in HFF2 group they were not different. Enterococcus and streptococcus concentrations were lower than the control group, however these were statistically not significant (fig. 3). When compared to HF group a statistically non-significant growth in the lactobacilli concentration was examined both with HFF1 and HFF2. While there was no change in bifidobacterial concentration with HFF1, a statistically non-significant increase was seen with HFF2 when compared to HF group. The concentration of Enterobacteriaceae in HFF1 and HFF2 groups was lower compared to HF (P<0.140 and P<0.011 respectively). Both Enterococcus and streptococcus concentration in HFF1 and HFF2 were comparable to HF group. In the HC diet fed rats, there were no changes seen in the concentration of all bacteria studied when compared with the control(fig. 3). All the indices of body composition like lean body mass, fat free mass and body fat % in rats fed an HF diet with or without flax oil supplementation were comparable with that of CT group. However, in the HC group there was a significant increase in body fat % when compared to CT rats. There was a decrease in lean body mass and fat free mass in HC fed rats, however, this was statistically not significant from that of CT rats (fig. 2).



***P<0.05,++P<0.01**

Fig. 3: Effect of feeding high palmolein/high starch diet on selective gut bacteria–influence of flaxseed oil

DISCUSSION

The effects of dietary fats on the risk of coronary artery disease have traditionally been estimated from their effects on serum total cholesterol. However, their effects on gut bacteria which have implications in producing metabolic syndrome a relatively new concept was not known earlier. Emerging from the most recent studies is a key mammalian host–gut-microbial relationships wherein an increase in gram negative bacteria with a concurrent decrease in Bifidobacterium has been shown in mice fed high fat of animal origin [1]. Thus Gram negative bacteria have been implicated in the increased lipopolysaccharide release in circulation, contributing to toll like receptor stimulation and associated increase in inflammation [1]. Similar alterations in the ceacal bacterial profile, such as decrease in gram positive bacteria (Lactobacillus) and an increase in gram negative bacteria (Enterobacteriaceae), with no change in bifidobacteria concentrations was observed with palmolein oil, which is a vegetable oil. A decrease in enterococci and streptococci bacteria was also observed, though we cannot conclude if these effects have any beneficial effects on the host. Hitherto there is one paper published on the effects of fish oil, canola oil and safflowerоil on gut microbiota [10]. To the best of our knowledge, there are no studies reported on the effects of palmolein, which is commonly consumed, on gut microbiota,barring one recent report [15]. Interestingly, administration of flax seed oil, although further increased the total fat intake (in HFF1 and HFF2), it prevented the adverse effects of palmolein oil (HF) on gut bacteria. Flax seed oil has been shown to regulate lipid profile, but as far as our knowledge goes, this is the first study showing the effects of flax seed oil on body composition, immune function, inflammation and cecal bacteria in high fat fed rats, more so using vegetable derived palmitic acid rich fat. The increase in the concentration of bifidobacteria in the HFF2 group and the improvement in the lactobacilli concentrations seen both in HFF1 and HFF2 groups suggests that flax oil may have a prebiotic potential.

In contrast to that seen with HF diet, none of the bacteria studied were affected with HC diet. Studies on high carbohydrate diets, especially using high concentrations of corn starch on gut microbiota are scanty. Hitherto there is one report on feeding horses with different diets on gut flora, wherein they found increased counts of Lactobacilli and Streptococciin horses maintained on a high starch diet compared to a high fiber diet. These workers also reported changes in some other bacteria which we have not analyzed [16]. Therefore, from our study, we cannot rule out the possibility of HC diet affecting other bacterial populations. In spite of several years of research, predicamentstill exists as to whether an HF or HC diet is beneficial towards body weight management in order to prevent the other metabolic complications associated with obesity. On one hand the American Diabetes Association, the American Heart Association and other national agencies recommend that a large portion of energy be obtained from carbohydrates and on the other hand, we have the most debatable Aitkin's diet that is claimed to reduce body weight and thereby the associated metabolic complications [17, 18].

In the present study, although the HF diet was designed to give high energy and high fat, due to low food intake in the rats, it turned out to be a low calorie, high fat diet and a relatively a low protein diet compared to the control. In spite of a higher palmolein intake (53.1 energy percent), there was no increase either in the body fat or in the body weight compared to control group, although earlier studies showed increase in body fat on using lard, beef tallow or hydrogenated fat at 40 to 50% energy source in the diet [19–21].

Inclusion of additional fat in the form of flax oil did not bring about any changes in the body composition parameters (LBM, FFMand Fat %) in both the HFF1 and HFF2. Moreover, the flax oil has offset the adverse effects that were observed in the HF group again strengthening the fact that the overall effects of oils are determined by both the quality and quantity used.

Coming to the HC group, though these rats received a high carbohydrate diet, the total energy intakes matched with that of the control group due to comparable food intakes, low protein and low fat intakes. It is interesting to note that high starch diet contributed to increased body fat % unlike the high palmolein diet. Moreover, the HC diet increased fasting plasma triglyceride (TG) concentrations in line with other reports [22].

M. Waddell and H. J. Fallon [23] in their studies on the effect of feeding diets containing 75% glucose or fructose showed an increase in liver triglyceride formation in the rat. In the current study wherein we used a polysaccharide namely starch we did find the same effect, suggesting that both simple and complex sugars when taken in excess lead to hypertriglyceridemia. The dietary intakes of HC group mimic the dietary intakes of people of lower socioeconomic classes in developing countries where fat, protein and total energy intakes are low together with high carbohydrateconsumption. This suggests that high carbohydrate consumption might be contributing to hypertriglyceridemia in this population.

The differential effects of high palmolein or high carbohydrate diets were also reflected in immune and inflammatory markers. Thus, while HF diet was found to impair immunity and increase inflammation, HC diet did not have any effect. Although it is well

known that dietary fat or carbohydrates affects metabolism in general [24], very few studies have reported their effects on the immune system in particular. Beatriz E Martínez-Carrillo *et al.* in 2010 [25] have reported differential effects of feeding high fat or high carbohydrate on the immune system. Unlike in our study, in their studies the source of high fat was lard while the source of carbohydrates was simple sugars like Maltodextrin and Sucrose.

Supplementation of flax seed oil to HF fed rats improved or normalized the total WBC, lymphocyte and neutrophil count besides improving the splenocytefunction, thus suggesting that flax oil prevented the adverse effects seen with HF diet on the immune system.

In the present study HF diet did not bring about any changes in any of the biochemical parameters tested except for a decrease in HDL cholesterol and thereby the HDL/Cholesterol ratio. Saturated fatty acids (SFA) such as palmitic acid are thought to be cholesterol-raising. Not many studies are there on the long term usage of palmolein and the vast number of existing studies reported on the effects of palmolein on lipids and other biochemical parameters are not equivocal [26–29].

The decrease in HDL cholesterol/total cholesterol ratio brought about by the HF diet was found to be normalized with HFF1 which evidently is due to lowering of total cholesterol concentration, a well known effect of flax oil. However, we did not see this effect with higher doses of flax oil. Flax oil at higher dose as used in the present study decreased serum calcium concentration, indicating that there could be some channelling of serum calcium towards bone mineralization, an effect well known of ω-3 fatty acids [30]. Though the plasma total bilirubin and ALT concentrations were significantly higher and the plasma calcium concentration and blood platelets were lowered below the CT values in HFF2 group, they were all in the physiological range. No adverse effects were seen on histopathological examination of the vital tissues. Perhaps it is good to work with slightly lower doses of flax seed oil to match these with that of the CT group.

The observed changes seen in HC diet given rats, such as increased body fat%, increased triglycerides, decreased HDL are effects associated with an increased risk of cardiovascular diseases by a variety of mechanisms [31]. From the results seen with palmolein we can conclude that a high palmolein diet has a more deleterious effect on the immune system than HC diet in rats. Since probiotics like lactobacilli are known to positively influence the immune system and that gram negative bacteria impair immune function, a correlation may be drawn between impairment in the immune system and drop in the cecal lactobacilli count and increase in gram negative enterobacteriaceae seen in the HF fed rats in the present study. As we were preparing this manuscript we came across a very recent report, which also showed an increase in E. coli and inflammatory responses with palm oil [15].

In general, majority of the studies with palmolein at 10-20% in the diet have only looked at serum lipids, especially considering HDL: LDL ratio and also have reported unequivocal results [26-29]. Our data (unpublished) on the use of palm oil at 10% in an isocaloric diet fed rats showed no adverse effects in terms of lipid profile, body composition and there was no increase in the concentration of enterobacteriaceae unlike that observed in the present study. However a drop in lactobacillus concentration was noted at 10% palmolein.

We report that both high palmolein and high starch diets have different adverse effects on the host; while high palmolein diet is found to impair immunity and increase inflammation, high starch diet was found to affect body composition and lipid profile. The current study also goes to prove that independent of energy intakes both high fat or high carbohydrate intakes have adverse effects compared to a standard diet. The current study also emphasizes the need to look into immunological as well as bacterial profile to assess the safety of dietary fats besides considering biochemical profile and body composition parameters. Also for the first time we are showing the beneficial effects of flax seed oil on HF induced adverse effects which are probably mediated through gut bacteria.

Limitations of our study

Due to financial constraints and time problem, assessment of parameters like plasma endotoxin, plasma insulin and plasma cytokines, which we initially intended to carry out could not be carried out. However, the results in hand, are sufficient enough to draw the following conclusions.

CONCLUSION

From the results obtained in the present study it can be envisaged that the adverse effects of feeding palmolein at 53 energy % in the diet (HF) are mediated through immune impairment and inflammatory response mediated through alteration in selective gut bacteria and that flax oil has a prophylactic role and may serve as an alternative dietary strategy in the management of diseases caused by high fat induced bacterial changes probably by serving as a prebiotic. Incorporation of flax oil into the preparation of high fat diets and also the use of symbiotic formulations with flax oil may also have potential clinical utility in normalizing many of the high fat induced adverse effects.

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CONFLICT OF INTERESTS

Declared None

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