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Evaluation of Effects of Milk Lipid (Ghee) Supplementation and Thermal Environment on the Metabolic Profile of Alloxan-diabetic Rabbits (*Oryctolagus cuniculus*)

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Abstract: The type of dietary fat and thermal environment may influence the metabolic profile and insulin sensitivity in normal and diabetic conditions. The objective of this study was to evaluate the effects of supplementation with anhydrous milk fat, ghee (FS) and season (summer vs winter) on blood metabolites and Body Weight (BW) in alloxan-diabetic and non-diabetic rabbits. In each season, twenty rabbits were assigned to 4 groups of 5 animals each. Two groups were rendered diabetic using alloxan monohydrate injection. Two groups, a normal and a diabetic received orally 2.5 g kg⁻¹ b.wt. of ghee daily for 8 weeks. Blood samples collected weekly were used for determination of insulin and blood metabolite concentrations. During summer, insulin (INS) level for the diabetic and non-diabetic-fat supplemented (FS) rabbits was higher than the non-diabetic rabbits fed Lucerne. Diabetic FS rabbits had slightly higher INS level than diabetic group fed Lucerne. Glucose (GL) level was significantly higher in diabetic groups and GL level of FS diabetic rabbits was significantly higher than respective values of diabetic group fed Lucerne only. GL level of diabetic groups was significantly higher in summer. In both seasons, serum total lipids (TL) was significantly higher in diabetic than non-diabetic groups. TL was significantly higher in FS diabetic group in summer. FS increased triglyceride (TG) levels in diabetic and non-diabetic animals, TG level was significantly lower in winter in non-diabetic groups of rabbits. In both seasons, the FS diabetic significantly higher serum cholesterol (CHOL) level than the diabetic control and non-diabetic groups of rabbits. The CHOL level in FS non-diabetic rabbits was significantly higher during winter. In both seasons, serum urea (UR) level was significantly higher in diabetic groups. Diabetic groups showed significantly higher UR during winter compared to summer values. Creatinine (CR) level of FS nondiabetic group was higher during summer. During summer, diabetic groups showed significantly lower mean BW than non-diabetic groups, while with FS, non-diabetic group showed significantly greater mean BW than the other groups. Diabetic FS group had significantly lower mean BW during winter. The findings have implications in pathophysiology and nutritional management of DM.

Key words: Rabbits, alloxan diabetes, fat supplementation, season, blood metabolites

INTRODUCTION

Diabetes Mellitus (DM) is a common metabolic disease with alterations in carbohydrate, protein and lipid metabolism as a consequence of defects in insulin secretion, insulin action or both (American Diabetes Association, 2010). Recently, the incidence of DM has increased drastically in both developed and underdeveloped countries. In Sudan, DM is currently emerging as an important public health problem, especially in urban areas. Previous survey indicated higher prevalence of diabetes in adult population with marked spatial distribution (Elbagir *et al.*, 1996). The diet composition may have significant effect on insulin

sensitivity as well as risk and complications of diabetes (Mann, 2006). Medical nutrition therapy is usually adopted as an effective measure to control hyperglycaemia and dyslipidaemia in diabetic patients (Franz, 2004). The dietary recommendations for diabetic patients seems to be similar for insulin dependent DM (IDDM) and non-insulin dependent DM (NIDDM).

The composition and quantity of dietary fat induce metabolic disorders in normal and diabetic subjects. Chronic consumption of high saturated fat diets deteriorates the metabolic profile and impairs glucose tolerance and increases the risk of type 2 diabetes (Feskens *et al.*, 1995). Also prolonged feeding of high-fat diet in rats, induced hyperinsulinaemia and insulin

resistance (Ghatta and Ramarao, 2004). Prolonged feeding of high-fat diet in rats (Woods *et al.*, 2003; Winzell and Ahren, 2004) was associated with increased body weight, hyperinsulinaemia and insulin resistance. Jen *et al.* (1991) reported that alloxan-diabetic rabbits developed hypercholesterolaemia and hypertriglyceridaemia in response to cholesterol feeding. Yin *et al.* (2002) noted that a diet high in saturated fat and sucrose impaired glucose regulation in rabbits and resulted in high serum levels of total cholesterol and triglyceride.

The NIDDM which is associated with tissue resistant insulin, is strongly linked with obesity; however, the mechanism by which increased adiposity causes insulin resistance has not been clarified. Yin *et al.* (2004) reported that in miniature pigs, feeding a high-fat and high-sucrose diet resulted in increase in the size of adipocytes and plasma GL levels and insulin resistance. Dysfunctions of lipoprotein lipase (LPL) were associated with dyslipidaemia, obesity and insulin resistance. An increase in LPL activity in transgenic rabbits induced marked lowering of plasma triglycerides (TG) and non-esterified fatty acids, however, the basal levels of glucose and insulin (INS) were not altered (Kitajima *et al.*, 2004). In Golden Syrian hamsters, increased saturated fat intake was associated with disturbance of homeostasis, severe hypertriglyceridaemia and increase in creatinine (CR) concentrations related mainly to nephropathy (Popov *et al.*, 2003).

The anhydrous milk fat (ghee), is an important dietary fat used for culinary purposes including dressing of foods and cooking. Ghee also has medicinal uses including treatment of allergy, skin and respiratory diseases (Sharma *et al.*, 2010). In Sudan, ghee is used for topping of sauce and it is also drunk for therapeutic purposes (Dirar, 1993). The conjugated linoleic acid in milk fat proved to have anticarcinogenic (Pariza, 1991) and antiatherogenic properties (Lee *et al.*, 1994). Ghee constitutes a source of lipid nutrients, fat-soluble vitamins and essential fatty acids (Chand *et al.*, 1986). In milk fat, the short chain and medium chain fatty acids of milk fat are readily digested, absorbed and oxidized by carnitine pathway (Bansal and Kansal, 1996). Kumar *et al.* (1999) reported lower concentrations of serum triglycerides, total cholesterol and Low Density Lipoprotein (LDL) cholesterol in rats fed anhydrous milk fat.

Epidemiological studies revealed seasonal pattern in the incidence of DM; the highest numbers of cases were diagnosed during the cool months in winter (Nystorm *et al.*, 1990; Elbagir *et al.*, 1996). However, gestational DM risk diagnosis was significantly higher in summer (Vasileiou *et al.*, 2012). Seasonal variation was also reported in glycosylated haemoglobin (HbA1c) (Asplund, 1997; Carney *et al.*,

2000), insulin sensitivity (Berglund *et al.*, 2012) and lipid profile (Kamezaki *et al.*, 2010; Bardini *et al.*, 2012).

Chemical induction of DM in laboratory animals proved to be useful in elucidating metabolic and pathophysiological changes. The rabbit model is widely adopted in experimental and clinical physiology. The present study was designed to evaluate the effects of dietary ghee supplementation and season on blood metabolites in normal and alloxan-diabetic rabbits.

MATERIALS AND METHODS

Animals: Clinically healthy adult male rabbits (*Oryctolagus cuniculus*) were used. Animals were procured from a private farm and were aged 8-10 months at the commencement of the experiment.

Housing and management: The rabbits were individually caged in a well ventilated animal facility with natural photoperiod at the Department of Physiology. Animals were allowed to adapt to the housing conditions and experimental procedures for two weeks and had free access to fresh Lucerne and clean tap water. Clinical examination was performed before and during the course of the experiment. Animals were given prophylactic anthelmintic injection (Ivomec: 0.02 mL kg⁻¹ b.wt., Alpha Laboratories Ltd., India) and antibacterial injection (Oxytetracycline: 7.5 mg kg⁻¹ b.wt., Alpha Laboratories Ltd, India).

Feeding: The animals were given fresh Lucerne (*Medicago sativa*) (Dry matter basis: CP 17.5%; ME 7.8 MJ kg⁻¹). Commercial anhydrous milk fat (ghee) purchased from the local market was used for treated groups of animals. The proximate composition of ghee is triglyceride 98.6%, cholesterol 161.7 mg/100 g, phospholipids 10.4 mg/100 g (Kumar *et al.*, 1999).

Climatic conditions: The ambient temperature (Ta) and Relative Humidity (RH) measurements were obtained from the Meteorological Unit located about 500 m from the experimental site. The data for the experimental periods under summer and winter conditions are depicted in Table 1.

Induction of diabetes mellitus (DM): The treated groups of rabbits were made diabetic by a single intravenous

Table 1: The ambient temperature (Ta) and relative humidity (RH) prevailing during the experimental period

Season	Ta (°C)			RH (%) (Mean)
	Max.	Min.	Mean	
Summer	39.27±1.07	26.14±0.90	32.71±0.83	39.12±5.64
Winter	37.04±2.46	17.97±1.99	27.50±1.86	20.72±4.04

injection of 150 mg kg⁻¹ alloxan monohydrate (Sigma, St. Louis, MO) dissolved in 0.9% NaCl. Therapeutic measures were adopted to secure the survival of animals by administration of glucose to tide over initial hypoglycaemic phase and injection of insulin (INS) during the acute phase of hyperglycaemia. The Glucose Tolerance Test (GTT) was performed to ensure the diabetic status. Animals with plasma GL levels higher than 200 mg dL⁻¹ were regarded as diabetic and used in the study. Animals developed diabetes mostly on the third day following injection of alloxan.

Collection of blood samples: Blood samples (4 mL) were withdrawn from the jugular vein by plastic syringes. One milliliter of blood was kept in a test tube containing sodium fluoride and after centrifugation, the plasma was used for glucose determination. The rest of the blood sample was left at room temperature for 3 h and then centrifuged (Hettich-Germany) at 3000 rpm for 15 min. Haemolysis-free serum samples were stored at -20°C for subsequent analysis.

Analysis of blood metabolites: The plasma glucose (GL) concentration was determined by colorimetric method (Trinder, 1969) using a commercial kit (Spinreact, S.A. Spain). Serum urea (UR) concentration was determined by enzymatic colorimetric test (Berthot) using a kit (Spinreact, S.A., Spain). Serum creatinine (CR) was determined by colorimetric extraction method (Owen *et al.*, 1954). Serum Total Lipids (TL) were estimated according to the method of Frings *et al.* (1972) measuring the coloured complex with a phosphate ester of vanillin. Serum triglyceride (TG) concentration was determined using the enzymatic method (Liner Chemical, Spain). Serum cholesterol (CHOL) concentration was determined by enzymatic colorimetric method (Spinreact S.A., Spain).

Serum insulin: Serum insulin (INS) concentration was determined by radioimmunoassay kit (Beijing Institute of Atomic Research, Beijing, China).

Experimental procedure: Twenty rabbits were randomly assigned to 4 groups of 5 animals each. Two groups were redered diabetic using alloxan monohydrate as previously explained. Animals in two groups, a normal and a diabetic group were supplemented with milk fat, ghee (FS). Each animal received orally 3 g (2.5 g kg⁻¹ b.wt.) daily. All animals were given free access to fresh Lucerne and tap water. The rabbits were subjected to the dietary experimental protocol for 8 weeks under typical summer and winter climatic conditions. During the experimental period, Body Weights (BW) of rabbits were recorded

weekly. Blood samples were collected weekly at 7:00 a.m. The samples were used for the determination of the concentrations of plasma glucose, serum metabolites and insulin.

Data analysis: For each group of animals, the mean values were computed during the course of experimental period. The data are presented as Mean±Standard Deviation (SD). The Analysis of Variance (ANOVA) (SAS, 1998) and Duncans Multiple Range Test (DMRT) were used to evaluate the effects of diabetes, supplementation with fat (FS) and season on the parameters investigated. Differences were considered to be statistically significant at p<0.05.

RESULTS

The results of the effects of milk fat supplementation (FS) on serum insulin, plasma glucose, serum metabolites and Body Weight (BW) in summer and winter are shown in Table 2 and 3, respectively. The effects of season on parameters investigated are depicted in histograms.

Serum insulin (INS): During summer (Table 2), the diabetic groups of rabbits had significantly (p<0.001) lower values of serum INS concentration (7.56-7.79 Mu L⁻¹) compared to the values measured for non-diabetic groups (15.47-16.46 Mu L⁻¹). However, the INS concentration for FS groups was slightly higher than values of groups fed Lucerne only. Winter data (Table 3) indicate that diabetic groups of rabbits maintained significantly (p<0.001) lower insulin levels compared to the respective values obtained for the non-diabetic groups. However, FS did not influence the INS level. Figure 1 shows that seasonal change in thermal environment had no significant effect on serum INS concentration in diabetic and non-diabetic groups of rabbits.

Plasma glucose (GL): During summer (Table 2), the diabetic FS group of rabbits exhibited significantly (p<0.001) higher GL concentration than the respective value of diabetic group fed Lucerne only. The non-diabetic groups of rabbits maintained steady GL level during the experimental period. During winter (Table 3), GL level was highly significantly (p<0.001) higher in the diabetic groups of rabbits compared to non-diabetic rabbits. Figure 2 shows that in both diabetic groups of rabbits, GL level was significantly (p<0.001) higher in summer compared to respective winter value. There was no change related to season in non-diabetic groups of rabbits.

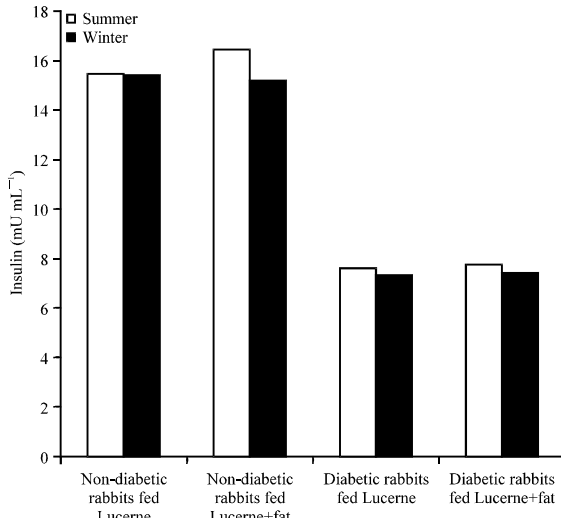


Fig. 1: Effect of supplementation with milk fat (Ghee) and season on serum insulin concentration in diabetic and non-diabetic rabbits

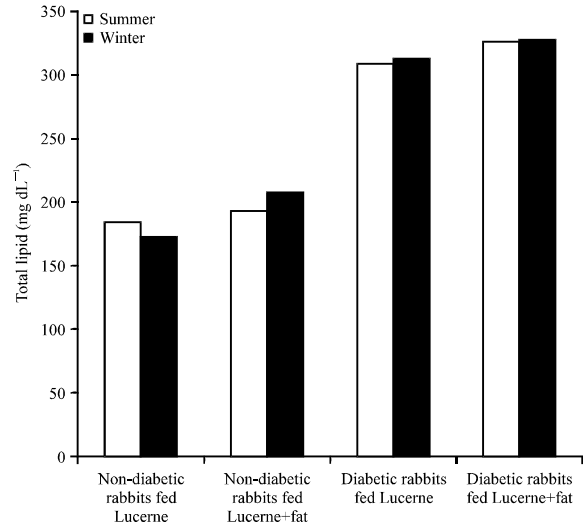


Fig. 3: Effect of supplementation with milk fat (Ghee) and season on serum total lipid concentration in diabetic and non-diabetic rabbits

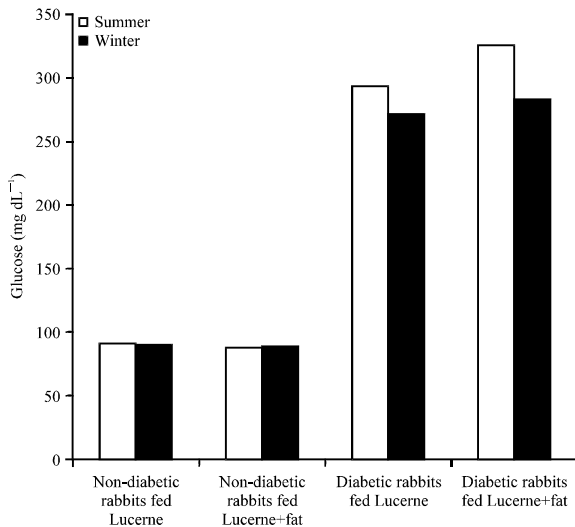


Fig. 2: Effect of supplementation with milk fat (Ghee) and season on plasma glucose concentration in diabetic and non-diabetic rabbits

Serum total lipids (TL): During summer (Table 2), diabetic groups of rabbits had significantly ($p < 0.01$) higher serum TL concentration compared to the non-diabetic groups of rabbits. However, the diabetic FS group of rabbits had significantly ($p < 0.05$) higher TL concentration compared to the diabetic group fed lucerne. In non-diabetic groups, the FS group had slightly higher value. During winter (Table 3), diabetic groups of rabbits had significantly ($p < 0.01$) higher serum TL than the respective values of non-diabetic groups. However, the

diabetic FS group of rabbits had significantly ($p < 0.05$) higher serum TL concentration compared to the diabetic group fed Lucerne. Figure 3 shows that seasonal change in thermal environment had no significant effect on TL in diabetic and non-diabetic groups of rabbits.

Serum triglycerides (TG): During summer (Table 2), diabetic FS rabbits had significantly ($p < 0.05$) higher triglyceride concentration. During winter (Table 3), FS had significant ($p < 0.05$) effect on TG concentration in diabetic groups of rabbits, it was higher in the diabetic group of rabbits supplemented with fat. For non-diabetic groups, the FS group had a higher value. Figure 4 shows that TG concentration was significantly ($p < 0.05$) higher during summer compared to the respective winter value in non-diabetic groups of rabbits.

Serum cholesterol (CHOL): During summer (Table 2), the diabetic FS group had significantly ($p < 0.001$) higher CHOL than the diabetic control and non-diabetic groups of rabbits. Diabetic group of rabbits fed Lucerne only had significantly higher CHOL level than the corresponding values of the non-diabetic groups of rabbits. However, serum CHOL concentration was significantly ($p < 0.05$) higher in non-diabetic group supplemented with fat than non-diabetic group fed Lucerne only. During winter (Table 3), the diabetic FS group of rabbits had significantly ($p < 0.05$) higher serum CHOL than the diabetic group fed Lucerne and non-diabetic groups of rabbits. However, there was no significant difference in CHOL concentration of the diabetic group fed Lucerne

Table 2: Effect of supplementation with milk fat (Ghee) on serum insulin, plasma glucose, serum metabolites and body weight (BW) in diabetic and non-diabetic rabbits during summer

Parameter	Non-diabetic rabbits fed Lucerne	Non-diabetic rabbits fed Lucerne+fat	Diabetic rabbits fed Lucerne	Diabetic rabbits fed Lucerne+fat	LS
LS insulin (Mu L^{-1})	15.47±3.75 ^a	16.46±3.65 ^a	7.56±1.74 ^b	7.79±2.23 ^b	***
Glucose (mg dL^{-1})	90.67±7.95 ^c	87.60±7.62 ^c	293.52±56.23 ^b	325.97±41.09 ^a	***
Total lipids (mg dL^{-1})	184.46±49.39 ^c	193.65±37.16 ^c	309.02±47.89 ^b	325.76±78.43 ^a	*
Triglyceride (mg dL^{-1})	70.45±17.72 ^b	74.05±31.16 ^{ab}	76.70±26.56 ^b	81.15±30.43 ^a	*
Cholesterol (mg dL^{-1})	40.89±9.62 ^c	47.22±12.94 ^b	52.97±14.97 ^b	60.48±15.25 ^a	***
Creatinine (mg dL^{-1})	1.53±1.02 ^a	1.38±0.26 ^a	1.32±0.21 ^a	1.35±0.21 ^a	NS
Urea (mg dL^{-1})	50.22±6.17 ^b	48.54±6.18 ^a	63.94±7.41 ^a	65.43±7.66 ^a	***
BW (g)	1185.00±61 ^b	1233.00±110 ^a	1173.00±145 ^b	1204.00±194 ^{ab}	*

Mean values within the same row bearing different superscripts are significantly different, LS: Level of significance, * $p < 0.05$, *** $p < 0.001$, NS: Not significant

Table 3: Effect of supplementation with milk fat (Ghee) on serum insulin, plasma glucose, serum metabolites and body weight (BW) in diabetic and non-diabetic rabbits during winter

Parameter	Non-diabetic rabbits fed Lucerne	Non-diabetic rabbits fed Lucerne+fat	Diabetic rabbits fed Lucerne	Diabetic rabbits fed Lucerne+fat	LS
Insulin (Mu L^{-1})	15.39±3.66 ^a	15.22±3.89 ^a	7.37±1.62 ^b	7.43±2.05 ^b	***
Glucose (mg dL^{-1})	90.26±9.50 ^b	88.72±7.82 ^b	271.38±45.90 ^a	283.24±48.05 ^a	***
Total lipid (mg dL^{-1})	172.52±44.30 ^c	207.745±58.04 ^c	313.50±47.89 ^b	327.36±71.25 ^a	*
Triglyceride (mg dL^{-1})	52.85±21.01 ^b	63.95±20.48 ^{ab}	78.25±29.30 ^a	85.15±47.69 ^a	*
Cholesterol (mg dL^{-1})	45.72±4.65 ^c	57.30±12.45 ^b	56.00±17.00 ^b	65.20±15.28 ^a	*
Creatinine (mg dL^{-1})	1.30±1.02 ^a	1.36±0.24 ^a	1.32±0.21 ^a	1.18±0.20 ^a	NS
Urea (mg dL^{-1})	53.80±13.17 ^b	48.98±7.52 ^b	77.07±10.07 ^a	76.47±11.60 ^a	***
BW (g)	1255.00±231 ^a	1243.00±149 ^a	1232.00±105 ^a	1147.00±132 ^b	*

Mean values within the same row bearing different superscripts are significantly different, LS: Level of significance, * $p < 0.05$, *** $p < 0.001$, NS: Not significant

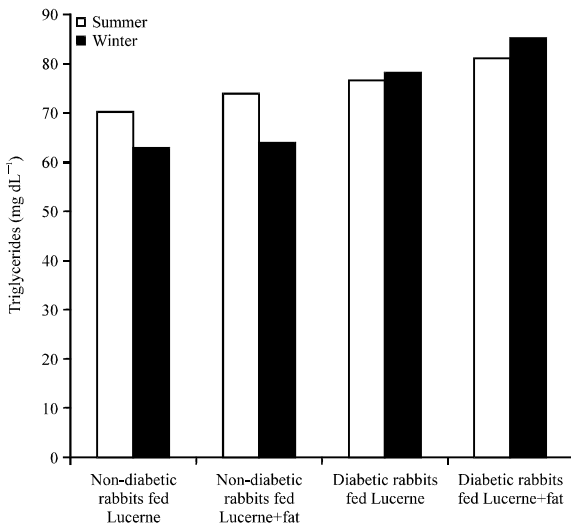


Fig. 4: Effect of supplementation with milk fat (Ghee) and season on serum triglycerides concentration in diabetic and non-diabetic rabbits

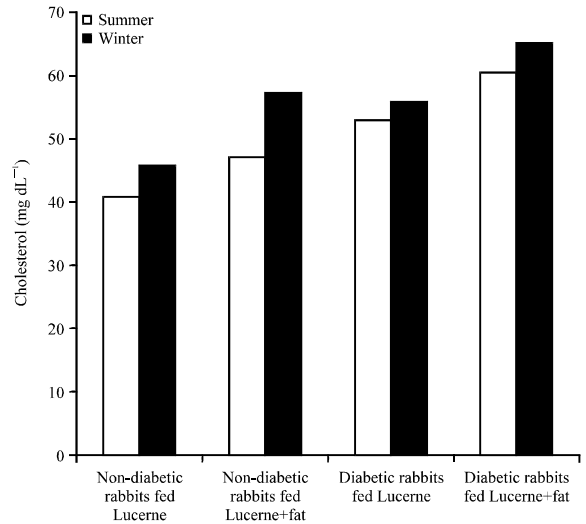


Fig. 5: Effect of supplementation with milk fat (Ghee) and season on serum cholesterol concentration in diabetic and non-diabetic rabbits

and the non-diabetic FS group fed Lucerne. The non-diabetic group fed Lucerne exhibited significantly ($p < 0.05$) lower serum CHOL concentration. Figure 5 shows that serum CHOL concentration in non-diabetic FS rabbits was significantly ($p < 0.05$) higher during winter than summer. However, season had no significant effect on serum cholesterol in other groups.

Serum creatinine (CR): During summer (Table 2), the non-diabetic group of rabbits fed Lucerne had slightly

higher CR value. During winter (Table 3), the effect of FS on serum CR concentration was not significant in diabetic and non-diabetic rabbits. However, the diabetic FS group of rabbits had lower value. Figure 6 shows that serum CR concentration was significantly ($p < 0.05$) higher during summer in non-diabetic group fed lucerne and diabetic FS group.

Serum urea (UR): During summer (Table 2), the diabetic groups of rabbits maintained significantly ($p < 0.001$)

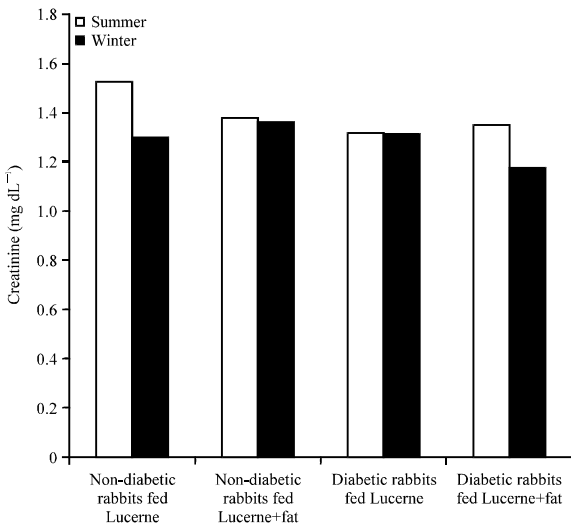


Fig. 6: Effect of supplementation with milk fat (Ghee) and season on serum creatinine concentration in diabetic and non-diabetic rabbits

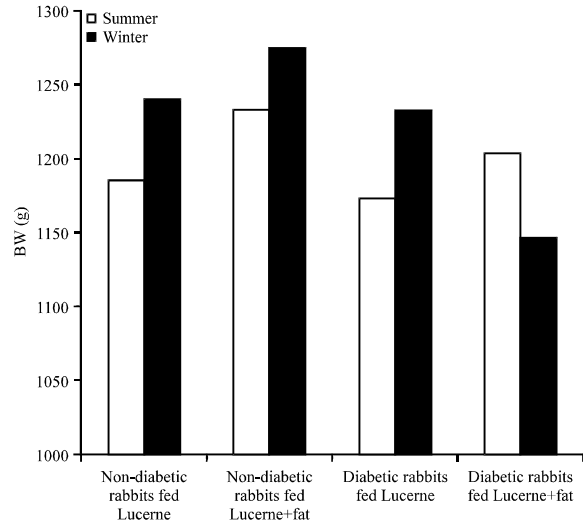


Fig. 8: Effect of supplementation with milk fat (Ghee) and season on body weight (BW) in diabetic and non-diabetic rabbits

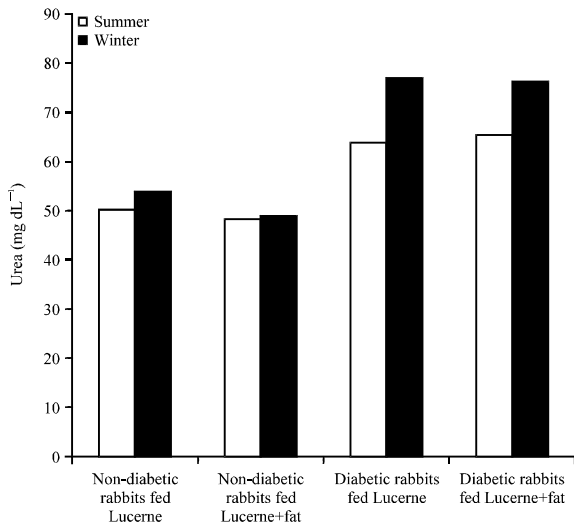


Fig. 7: Effect of supplementation with milk fat (Ghee) and season on serum urea concentration in diabetic and non-diabetic rabbits

higher serum UR concentration compared to non-diabetic groups of rabbits. During winter (Table 3), the diabetic groups of rabbits maintained significantly ($p < 0.001$) higher UR concentration compared to non-diabetic groups of rabbits. There was no significant variation in UR concentration between diabetic groups. Figure 7 shows that the diabetic FS groups of rabbit and their control showed significantly ($p < 0.001$) higher UR concentration during winter compared to the respective values summer values.

Body weight (BW): During summer (Table 2), the diabetic groups had significantly ($p < 0.05$) lower mean BW compared to non-diabetic groups. The non-diabetic SF group of rabbits showed significantly ($p < 0.05$) higher mean BW compared to the other experimental groups of rabbits. During winter (Table 3), the non-diabetic group of rabbits maintained significantly ($p < 0.05$) higher BW compared to the values obtained for the other groups. However, the diabetic FS group of rabbits showed significantly ($p < 0.05$) lower mean BW. Figure 8 shows that the diabetic and non-diabetic group of rabbits fed Lucerne and the non-diabetic FS group had significantly ($p < 0.01$) higher BW during winter compared to the respective summer values. The diabetic FS group had significantly ($p < 0.01$) higher BW during summer.

DISCUSSION

This study aimed to evaluate the effects of supplementation with animal fat (FS) and seasonal change in tropical environment on Body Weight (BW), insulin (INS) level and blood metabolites in alloxan-diabetic and non-diabetic rabbits. The current study used the milk fat (ghee) which generally makes appreciable contribution to saturated fat and cholesterol intake in humans.

The lower INS levels in the diabetic rabbits in the present study is clearly the result of induction of experimental diabetes with alloxan. The tendency for increase in serum INS level in non-diabetic FS rabbits during summer (Fig. 1) in the present study is in agreement with Yin *et al.* (2002) who indicated that the

INS level in rabbits fed a diet high in saturated fat and sucrose increased significantly over the first 3 months of experimental period. However, reductions of 50% in circulating INS level have been reported in high fat fed rats and it has been suggested that these diets impair signal transduction mechanism in pancreatic B-cells to reduce INS secretion (Kim *et al.*, 1996). Yin *et al.* (2003) indicated that there were no significant differences in fasting serum INS levels among rabbits fed high fat and high sucrose diet and their control.

The hyperglycaemia observed in alloxan-treated animals (Table 2, 3) is clearly attributed to INS deficiency. When INS is deficient (absolute or relative), hyperglycaemia develops as a result of increased gluconeogenesis, accelerated glycogenolysis and impaired GL utilization by peripheral tissues (DeFronzo *et al.*, 1994). Increased hepatic GL production is attributed to the high availability of gluconeogenic amino acids precursors associated with enhanced proteolysis (Felig and Wahren, 1971) and lactate (induced by increased muscle glycogenolysis) and glycerol (resulting from increased lipolysis) (Exton, 1987). Conversion of glucose-6-phosphate to glucose is catalyzed by hepatic glucose-6-phosphatase, which is stimulated by an increase in secretion of catabolic hormones and decreased insulin levels (Kitabchi *et al.*, 2001).

The FS diabetic rabbits exhibited significantly higher GL levels than the respective values of diabetic groups fed Lucerne only during summer and winter (Table 2, 3). Ikemoto *et al.* (1995) indicated that in mice, high dietary fat (safflower oil) induced defective glycaemic control and hyperglycaemia. The higher level of hyperglycaemia in FS diabetic rabbits may be related to inhibition of muscle GL uptake and oxidation. Jequier (1998) indicated that GL metabolism may be inhibited at two important steps. First, the increase in cytoplasmic citrate concentration inhibits phosphofructokinase, which results in an increased glucose-6-phosphate concentration; as a consequence, hexokinase inhibition and impairment of GL uptake. Second, inhibition of pyruvate dehydrogenase reduces the entry of pyruvate into oxidative metabolism and thus contributes to inhibition of GL oxidation. Additionally, the expression of the INS-regulatable GL transporter GLUT-4 (mRNA and protein) was dramatically reduced in skeletal muscle after feeding a high-lard-based diet to mice (Kahn and Pedersen, 1993; Zierath *et al.*, 1997). Reduced INS sensitivity in muscle of high-fat-fed animals was attributed partly to changes in INS signaling, which may ultimately produce a lower GL uptake. Yin *et al.* (2003) induced increase in GL level by feeding rabbits with high-fat-high-sucrose diet.

The current results indicate that serum INS and GL levels were influenced by season (Fig. 1, 2). There was a tendency for higher INS level in summer in normal FS and in diabetic groups. However, Donahoo *et al.* (2000) demonstrated that in normal weight humans, GL level was not significantly different between summer and winter.

The data indicate that generally alloxan-treated groups showed higher serum TL and TG levels compared to non-diabetic groups (Table 2, 3). However, FS increased significantly TG levels irrespective of season. The increase in TG is clearly associated with INS deficiency which results in mobilization of fatty acids from adipose tissue. As blood GL is not utilized by tissues, the fatty acids from adipose tissue are mobilized for energy purpose and excess fatty acids which accumulate in the liver are converted to TG (Shih *et al.*, 1997). Suryawanshi *et al.* (2006) indicated that the blood TG levels change according to the glycaemic control.

High levels of TG may be related to obesity, increase in calorie intake and lack of muscular exercise in diabetic patients (Yagi, 1987). High fat meals exacerbate postprandial hypertriglyceridaemia. Impaired INS action in type II diabetes may result in loss of suppression of lipolysis in adipose tissue. This leads to an increased release of free fatty acids into the portal circulation and consequently, increased delivery of FFAs to the liver. The effect of this process is increased production of triglycerides by the liver and a decreased production of HDL-C. In addition, there is impaired clearance of TG from the circulation. The resulting hypertriglyceridaemia alters the activity of other enzymes, which leads to the formation of small dense LDL particles and increased catabolism of HDL (Valabhji and Elkeles, 2003). In contrast to the present results, Zulet *et al.* (1999) indicated that in rats, hypercholesterolaemia was accompanied by a decrease in TG. This reduction in circulating TG could be associated with an inadequate synthesis as Rawashdeh (2002) reported that TG level was reduced after a period of ghee consumption in humans.

Elevated serum TG was noted in rabbits fed high fat/high sucrose diets (Kloeze and Abdellatif, 1975; Yin *et al.*, 2002, 2003). The mobilization of TG seems to be a consequence of INS deficiency. However, in some rabbits the blood lipids remained at low levels during the whole experimental period in spite of an existing INS deficiency and hyperglycaemia. Furthermore, Shafique *et al.* (2002) in studies on alloxan-induced diabetic rabbit, noted increased serum TG in diabetic placebo group.

Diabetic groups of rabbits fed lucerne only had significantly higher CHOL level than the values of the

non-diabetic groups of rabbits (Table 2, 3). The current results are in general agreement with previous studies (Kloeze and Abdellatif, 1975; Khushk *et al.*, 2010) which reported considerable increase in serum CHOL in diabetic rabbits. The diabetic and non-diabetic rabbits had higher serum CHOL levels with FS. A positive relationship between the dietary CHOL intake and serum CHOL was reported in hamsters (Cohen *et al.*, 1994). Rawashdeh (2002) reported that ingestion of ghee significantly increased serum total CHOL level in humans. The hypercholesterolaemic effect of ghee may be related to the high amount of short and medium chain fatty acids which may work as an activator for hepatic hydroxyl-methylgluturate-CoA reductase, the rate limiting enzyme in CHOL biosynthesis. Short chain fatty acids with two other fatty acids, lauric and myristic acids may induce hypercholesterolaemia (Elson, 1992) and may cause high rate of CHOL absorption (Ide *et al.*, 1979). Myristic acid is the most potent CHOL raising saturated fatty acid in humans (Hajri *et al.*, 1998). However, paradoxically Kumar *et al.* (2000) reported that dietary ghee supplementation decreased serum CHOL level that was attributed to increase in the secretion of biliary lipids. The authors reported that dietary ghee did not influence the activity of HMG Co A reductase in liver indicating that it had no influence on biosynthesis of CHOL, but increased significantly the bile secretion and thus reduced CHOL levels.

The current results demonstrated seasonal variation in serum lipid levels. The lower TG level in winter for normal rabbits in the current study (Fig. 4) is in line with previous report in humans (Fuller *et al.*, 1974). However, other researchers reported higher TG level in winter (Donahoo *et al.*, 2000; Kamezaki *et al.*, 2010). Serum CHOL level was higher in winter for non-diabetic rabbits (Fig. 5). This result confirms the hypercholesterolaemia reported in normal subjects consistently in winter by other workers (Donahoo *et al.*, 2000; Ockene *et al.*, 2004; Kamezaki *et al.*, 2010). The current results reported for the first time seasonal variation in CHOL level in alloxan-induced diabetic animals (Fig. 4). Recently, Bardini *et al.* (2012) showed seasonal variation in lipid profile of Type 2 diabetic patients. The mechanisms for seasonal patterns remain largely unknown (Gordon *et al.*, 1987; Woodhouse *et al.*, 1993). It has been suggested that changes in relative plasma volume may explain partly the seasonal differences in lipid profile (Ockene *et al.*, 2004). The climatic data depicted in Table 1 indicate that the mean ambient temperature was 32.71°C in summer and 27.50°C in winter. However, Bardini *et al.* (2012) indicated that in Type 2 diabetic patients, the peak of serum total CHOL during colder months was associated with changes of diet and lifestyle habits.

The UR and CR levels are generally considered as biomarkers of diabetic nephropathy (De Almeida *et al.*, 2012). In the present study, UR level increased significantly in diabetic rabbits but the data does not show marked change in CR level (Table 2, 3). The high UR level could be associated with impaired renal function (Ronco *et al.*, 2010) and may also indicate an increase in tissue catabolism (De Almeida *et al.*, 2012). The higher UR level during winter in diabetic rabbits (Fig. 7) may be related to haemoconcentration and/or an increase in dietary protein intake.

The results indicate that in both seasons, diabetic groups of rabbits had significantly lower Body Weight (BW) than the corresponding mean values in the non-diabetic groups (Table 2, 3). The reported decrease in BW of diabetic rabbits is attributed mainly to breakdown of structural proteins (Franz *et al.*, 2002). The amino acids produced in proteolysis are oxidized because body cells cannot utilize GL for energy (Sekar *et al.*, 2005). Ramesh *et al.* (2006) reported BW loss in streptozotocin diabetic rats. The increase in BW in non-diabetic FS group of rabbits during summer is in agreement with previous studies in animals fed high fat diet (Murray *et al.*, 1999). Sandu *et al.* (2005) reported that in mice exposed to high dietary fat, metabolic processes which lead to BW gain are linked to advanced glycosylation end products. Chen *et al.* (1992) reported that rats fed a high-saturated fat diet consumed more energy and gained more weight compared with animals on two different high-carbohydrate diets. Other researchers indicated that BW in rats were similar in control and hypercholesterolaemic groups, while a marked decrease in abdominal fat was found in rats fed the CHOL and saturated fat enriched diet; this was attributed to reduction in food intake and ketogenic mechanisms (Tomlinson *et al.*, 1992).

We conclude that the metabolic profile of normal and alloxan-diabetic rabbits was influenced by milk fat intake and seasonal climatic change. The seasonal rhythm of serum lipids implies that control of dyslipidaemia and optimal dietary therapy should be adopted in the cold season. Special clinical attention should be paid to hypercholesterolaemic and diabetic subjects consuming diets with high milk fat content.

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