

## Effects of Alloxan-Induced Diabetes Mellitus on Blood Metabolites and Serum Minerals and Hormones in Rabbits (*Lepus cuniculus*) in Relation to Starch Supplementation and Season

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**Abstract:** The objective of the present study was to evaluate the effects of starch supplementation and season (summer vs winter) on blood metabolites and certain minerals and hormones in alloxan-diabetic and normal non-diabetic rabbits. In both seasons, alloxan induced significant lowering of serum insulin level associated with significantly higher plasma glucose levels. Starch supplementation increased insulin and glucose levels significantly in both seasons. Diabetes significantly decreased serum albumin level during winter. Diabetic rabbits had significantly higher serum urea levels in both seasons; non-diabetic rabbits had significantly higher urea level during winter compared to summer values. Non-diabetic rabbits fed Lucerne only had significantly higher creatinine levels that were significantly higher in summer than winter in all experimental groups. In both seasons, diabetic rabbits had higher serum total lipids levels. Starch supplementation caused significant increase in triglyceride level in summer. Diabetic-starch supplemented rabbits had significantly higher serum cholesterol level compared to other groups. With lucerne feeding, diabetic rabbits had significantly higher cholesterol level compared to non-diabetic state. Cholesterol was significantly higher during summer than winter in diabetic starch supplemented rabbits and in non-diabetic rabbits. In both seasons, diabetic rabbits fed Lucerne maintained significantly higher serum Na level compared to other experimental groups; Na level was significantly higher in diabetic- starch supplemented and non-diabetic rabbits during winter compared to respective summer values. Non-diabetic starch supplemented rabbits had significantly higher serum K level in winter compared to summer values. Non-diabetic starch supplemented rabbits had higher cortisol level in summer compared to winter. Diabetic-starch supplemented rabbits had significantly higher cortisol level compared to other groups in winter. Cortisol level was significantly higher in winter compared to summer values in diabetic rabbits. The findings have relevance for understanding pathophysiological changes in human diabetes.

**Key words:** Rabbits • Diabetes mellitus • Starch supplementation • Season • Blood metabolites • Minerals • Hormones

### INTRODUCTION

*Diabetes mellitus* is a chronic metabolic disease characterized by alterations in carbohydrate, protein and lipid metabolism resulting from defects in insulin secretion, insulin action, or both [1]. The disease also causes significant disturbances of water and electrolyte homeostasis [2]. The long-term complications of *diabetes mellitus* include retinopathy, nephropathy, neuropathy and angiopathy [3] associated with oxidative stress and overwhelming free radicals. In recent years, the incidence of *diabetes mellitus* has increased drastically in both developed and underdeveloped countries. In Sudan,

*diabetes mellitus* is currently emerging as an important public health problem, especially in urban areas. Epidemiological studies [4] showed higher prevalence of *diabetes mellitus* in the adult population with wide geographical distribution.

Dietary factors may influence the prognosis of diabetes. Research supports medical nutrition therapy as an effective measure to control glycaemia and lipids [5]. The principles of dietary advice for diabetic patients seem to be similar for insulin dependent *diabetes mellitus* (IDDM) and non-insulin dependent *diabetes mellitus* (NIDDM). The impact of a high carbohydrate diet is mainly seen as an increase in insulin resistance [6].

Carbohydrates appear to be the food components that produce almost all of the blood glucose increase after a meal. Great variations in postprandial glucose level have been shown in dogs [7, 8]. Different kinds of carbohydrate elicit different glucose and insulin levels because their chemical nature, especially the ratio of amylose to amylopectin forms of starch, may affect their rate and speed of digestion ; dietary fibre slows down the rate of passage and the rate of hydrolysis of starchy polysaccharides [9]. Both the quantity and the type or source of carbohydrate found in food influence postprandial glucose level [10]. Diets providing high amounts of simple carbohydrates and/or fructose are associated with insulin resistance and low plasma HDL cholesterol [11]. In contrast, diets providing high amounts of complex carbohydrates and fibre are associated with increased insulin sensitivity [12, 13].

Dietary carbohydrates influence metabolism by mechanisms which include the nature of the monosaccharide absorbed, amount of carbohydrate consumed and rate of absorption and colonic fermentation. Wolever [14] indicated that reducing glycaemic responses by restricting carbohydrate intake increases postprandial free fatty acids (FFA) and does not improve overall glycaemic control in diabetic subjects. Marked seasonal variation in the incidence of diabetes mellitus has been reported; the highest numbers of cases were diagnosed during the cooler months of the year [4, 15]. However, the effects of changes in environmental temperature suggest that heat stress might aggravate diabetic complications; exposure to hot environmental conditions is not recommended for diabetic patients [16]. Experimental induction of *diabetes mellitus* in animal models is useful for elucidating various metabolic and pathophysiological changes and the establishment of new drugs. Therefore, in this study, the rabbit model was used to evaluate the responses of blood metabolites, minerals and hormones to chemically induced *diabetes mellitus* in relation to dietary starch supplementation and seasonal change in thermal environment under tropical conditions.

## MATERIALS AND METHODS

**Animals:** Clinically healthy adult rabbits (*Lepus cuniculus*) were used. All animals were males in order to limit hormonal effects on the responses. The animals were obtained from a private rabbitary 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 house with natural photoperiod at the Department of Physiology. The animals were allowed to adapt to the housing conditions and experimental procedures for two weeks and had free access to fresh Lucerne and tap water. Thorough clinical examination was performed before and during the course of the experiment. Animals were given prophylactic anthelmintic injection (Ivomec: 0.02 ml/kg BW: Alpha Laboratories Ltd, India) and antibacterial injection (Oxytetracycline: 7.5 mg/kg BW: Alpha Laboratories Ltd, India).

**Feeding:** The animals were given fresh Lucerne and a rich source of starch (sorghum grains) for treated groups. The composition of fresh Lucerne and sorghum grains is shown in Table 1 [17].

**Climatic Conditions:** The ambient temperature (Ta) and relative humidity (RH) measurements were obtained from the Meteorological Unit located about 500 meters from the experimental site. The data for the experimental periods under summer and winter conditions are depicted in Table 2.

## Induction of *Diabetes mellitus* and Hyperglycaemia:

The treated groups of rabbits were made diabetic by a single intravenous injection of 150 mg/kg alloxan monohydrate (Sigma, St. Louis, MO) dissolved in 0.9% NaCl. Therapeutic measures were adopted to secure survival of rabbits by administration of glucose to tide over initial hypoglycaemic phase and the injection of insulin during acute phase of hyperglycaemia.

Table 1: The nutrient composition of fresh Lucerne (*Medicago sativa*) and sorghum grains (*S.vulgare caudatum*) (g/kg). (Suliman and Mabrouk, 1999)

Ingredients	Lucerne ( <i>Medicago sativa</i> )	Sorghum grains ( <i>S.vulgare caudatum</i> )
Dry matter	230	945
Ether extract	3.3	25.0
Crude protein	46.1	132.3
Crude fibre	70.0	24.8
Ash	28.2	21.5
NFE	91.4	741.3
Ca	5.1	0.5
P	0.4	3.1
NaCl	3.1	1.1
Mg	0.7	1.7

Table 2: The mean values of ambient temperature (Ta) and relative humidity (RH) prevailing during the experimental period

Experimental period (weeks)	Summer (April- June 2007)				Winter (February, - March 2008)			
	Ta (°C)			RH (%)	Ta (°C)			RH (%)
	Max	Min	Mean		Max	Min	Mean	
1	38.94	19.27	29.10	14.57	30.24	13.45	21.85	24.14
2	39.6	19.07	29.33	15.14	27.51	12.75	20.13	34.00
3	38.4	22.72	30.56	14.57	33.30	17.00	25.15	25.43
4	40.21	25.07	32.64	14.57	32.81	14.71	23.76	23.28
5	40.72	24.02	32.37	14.57	37.87	17.50	27.69	30.42
6	43.11	26.02	34.57	27.42	39.25	20.12	29.37	27.28
7	42.13	29.00	35.56	34.00	35.7	17.98	26.84	25.71
8	43.4	28.57	35.98	26.57	33.7	15.24	24.47	22.71
Mean±SD	40.81	24.22	32.51	20.17	33.79	16.09	24.90	26.62
	±1.88	±3.51	±2.52	±7.37	±3.84	±2.48	±3.05	±3.85

Animals with plasma glucose levels higher than 200 mg/dL were considered to be 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. 1 ml 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 hrs and then centrifuged (Hettich - Germany) at 3000 r.p.m. for 15 min. Haemolysis-free serum samples were stored at -20°C for subsequent analyses.

**Blood Metabolites:** The plasma glucose concentration was determined by colorimetric method using a commercial kit (Spinreact, S.A. Spain). Biuret reagent was used to determine serum total protein concentration [18]. Serum albumin concentration was determined by the colorimetric method [19]. Serum cholesterol concentration was determined by the enzymatic colorimetric method [20]. Serum urea concentration was determined by enzymatic colorimetric test (Berthot) using a kit (Spinreact, S.A., Spain). Serum creatinine concentration was determined by colorimetric method [21].

**Serum Minerals:** The concentrations of Na and K in serum were determined by flame photometer technique (Corning 400- UK) as described by Wootton [22].

**Serum Hormones:** Serum insulin concentration was determined by radioimmuno-assay kit (Beijing Institute of Atomic Research, Beijing, China). Serum cortisol concentration was determined using cortisol (<sup>125</sup>I) radioimmunoassay kit (Institute of Isotopes Ltd).

**Experimental Plan:** Twenty rabbits were used in the study. The animals were assigned to 4 groups of 5 each (A, B, C, D). Groups A and B were made diabetic using alloxan while groups C and D were normal (non-diabetic). Group A was fed Lucerne only and served as a diabetic control, group B was given Lucerne supplemented with starch, group C was fed Lucerne, while group D was fed Lucerne supplemented with starch. Starch was supplemented in the form of ground sorghum grains. Each animal in the supplemented groups received daily 50g of ground sorghum grains at 7.00 a.m. All animals were given free access to tap water. The animals were subjected to the experimental protocol for 8 weeks during typical summer and winter climatic conditions. During the experimental period, blood samples were collected weekly at 9.00 a.m. for analysis.

**Statistics:** For each group of animals, the mean values were computed during the course of the experimental period. The data are presented as mean ± Standard Deviation (SD). The analysis of variance (ANOVA) [23] and Duncans Multiple Range Tests (DMRT) were used to evaluate the effects of diabetes, supplementation with starch and season on the parameters investigated. The differences are considered statistically significant at P value < 0.05.

## RESULTS

**Serum Insulin, Plasma Glucose and Serum Organic Constituents:** Tables 3 and 4 show the effects of starch supplementation on the concentrations of serum insulin, plasma glucose and serum organic constituents in diabetic and non-diabetic rabbits during summer and winter, respectively.

Table 3: Effects of starch supplementation on concentrations of plasma glucose and serum organic constituents in diabetic and non-diabetic rabbits during summer

Parameter	Non-diabetic rabbits fed Lucerne	Non-diabetic rabbits fed Lucerne + starch	Diabetic rabbits fed Lucerne	Diabetic rabbits fed Lucerne + starch	LS
Insulin (mu/L)	16.85 <sup>a</sup> ±0.86	18.87 <sup>a</sup> ±1.32	8.52 <sup>b</sup> ±0.27	8.96 <sup>b</sup> ±1.53	***
Glucose (mg/mL)	96.20 <sup>a</sup> ±14.99	103.49 <sup>c</sup> ±14.87	270.41 <sup>b</sup> ±83.05	337.80 <sup>a</sup> ±56.29	***
Total protein (g/dL)	7.54 <sup>a</sup> ±0.67	7.42 <sup>a</sup> ±0.49	7.62 <sup>a</sup> ±0.34	7.74 <sup>a</sup> ±0.39	NS
Albumin (g/dL)	3.91 <sup>a</sup> ±0.41	3.90 <sup>a</sup> ±0.53	3.86 <sup>a</sup> ±0.41	3.76 <sup>a</sup> ±0.42	NS
Urea (mg/dL)	69.15 <sup>b</sup> ±10.53	53.70 <sup>c</sup> ±8.82	77.83 <sup>a</sup> ±14.32	79.06 <sup>a</sup> ±15.23	***
Creatinine (mg/dL)	1.84 <sup>a</sup> ±0.27	1.75 <sup>ab</sup> ±0.43	1.68 <sup>ab</sup> ±0.51	1.63 <sup>b</sup> ±0.26	*
Triglyceride (mg/dL)	72.15 <sup>b</sup> ±35.55	83.90 <sup>ab</sup> ±22.28	71.10 <sup>b</sup> ±23.11	98.35 <sup>a</sup> ±38.76	**
Cholesterol (mg/dL)	42.60 <sup>b</sup> ±9.78	38.93 <sup>b</sup> ±9.19	44.82 <sup>b</sup> ±11.13	60.96 <sup>a</sup> ±17.80	***

Mean values within the same row bearing different superscripts are significantly different.

LS: level of significance

\*: P<0.05    \*\*: P<01    \*\*\*: P<001

Table 4: Effects of starch supplementation on concentrations of plasma glucose and serum organic constituents in diabetic and non-diabetic rabbits during winter

Parameter	Non-diabetic rabbits fed Lucerne	Non-diabetic rabbits fed Lucerne + starch	Diabetic rabbits fed Lucerne	Diabetic rabbits fed Lucerne + starch	LS
Insulin (mu/L)	14.52 <sup>a</sup> ±2.31	13.03 <sup>a</sup> ±1.32	7.45 <sup>b</sup> ±0.59	8.99 <sup>b</sup> ±1.11	***
Glucose (mg/mL)	95.23 <sup>a</sup> ±14.75	88.99 <sup>a</sup> ±9.37	282.39 <sup>b</sup> ±32.36	306.12 <sup>a</sup> ±49.44	***
Total protein (g/dL)	8.14 <sup>a</sup> ±0.45	7.80 <sup>a</sup> ±0.41	8.04 <sup>a</sup> ±0.37	8.02 <sup>a</sup> ±0.37	NS
Albumin (g/dL)	3.94 <sup>a</sup> ±0.33	3.85 <sup>ab</sup> ±0.27	3.70 <sup>b</sup> ±0.38	3.78 <sup>b</sup> ±0.36	*
Urea (mg/dL)	76.80 <sup>ab</sup> ±14.20	71.9 <sup>b</sup> ±17.51	84.40 <sup>a</sup> ±22.65	82.78 <sup>a</sup> ±17.07	**
Creatinine (mg/dL)	1.21 <sup>a</sup> ±0.38	1.33 <sup>a</sup> ±0.45	1.41 <sup>a</sup> ±0.32	1.36 <sup>a</sup> ±0.57	NS
Triglyceride (mg/dL)	71.05 <sup>a</sup> ±19.67	77.10 <sup>a</sup> ±18.41	71.10 <sup>a</sup> ±16.65	80.55 <sup>a</sup> ±19.25	NS
Cholesterol (mg/dL)	36.60 <sup>b</sup> ±13.02	32.90 <sup>b</sup> ±7.36	49.23 <sup>a</sup> ±10.98	48.68 <sup>b</sup> ±12.32	***

Mean values within the same row bearing different superscripts are significantly different.

LS: level of significance

\*: P<0.05    \*\*: P<01    \*\*\*: P<001    NS: not significant

**Serum Insulin:** During summer (Table 3), diabetic groups had significantly (P<0.001) lower insulin level compared to non-diabetic groups. The pattern indicated that insulin level for the diabetic group supplemented with starch declined at the end of the experimental period. Winter data (Table 4) indicated that diabetic groups maintain significantly (P<0.001) lower insulin level compared to non-diabetic groups. However, starch supplemented rabbits maintained higher insulin level compared to the diabetic group fed Lucerne only. The insulin level was significantly (P<0.001) higher in summer compared to winter value in the non-diabetic group supplemented with starch (Fig. 1).

**Plasma Glucose:** During summer (Table 3), diabetic groups showed higher (P<0.001) plasma glucose level compared to respective values of non-diabetic groups of rabbits. Diabetic rabbits supplemented with starch had higher (P<0.001) glucose levels compared to diabetic rabbits fed Lucerne only. During winter (Table 4), also diabetic groups had higher (P<0.001) glucose levels compared to the non-diabetic groups. Diabetic rabbits supplemented with starch showed higher (P<0.001)

glucose levels compared to diabetic rabbits fed Lucerne only. Fig. 2 shows that plasma glucose level was significantly (P<0.01) higher in summer in diabetic rabbits supplemented with starch. Season had no significant effect in other experimental groups.

**Serum Total Protein:** In both seasons (Tables 3 and 4), for all experimental groups, there was no significant change in serum total protein level related to experimental treatments. Fig.3 indicates that for all groups, total protein level was higher in winter compared to respective summer values; the increase was significant (P<0.05) in non-diabetic rabbits fed Lucerne.

**Serum Albumin:** During summer (Table 3), there was no consistent pattern in albumin level in diabetic and non-diabetic groups of rabbits and there was no significant difference between groups. During winter (Table 4), albumin levels of diabetic groups of rabbits were lower (P<0.05) compared to corresponding values of non-diabetic groups. For diabetic rabbits fed Lucerne, summer value of albumin was slightly higher compared to values obtained in winter (Fig. 4).

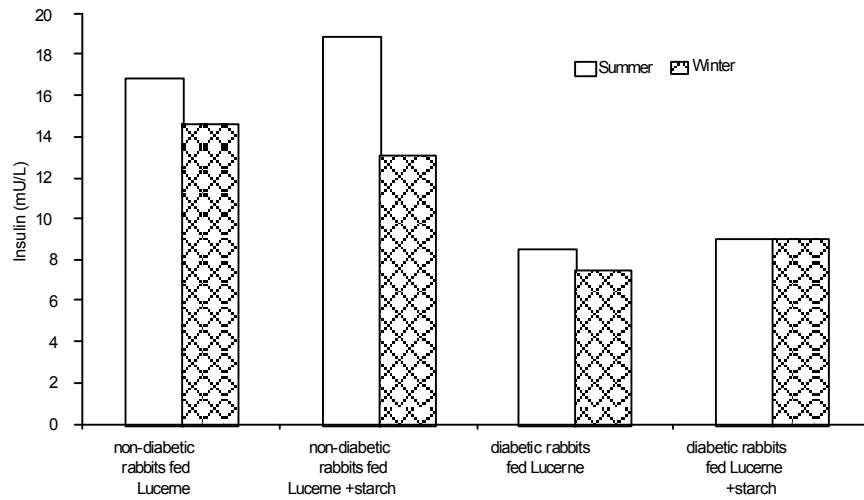


Fig. 1: Effect of starch supplementation and season on serum insulin concentration in diabetic and non-diabetic rabbits

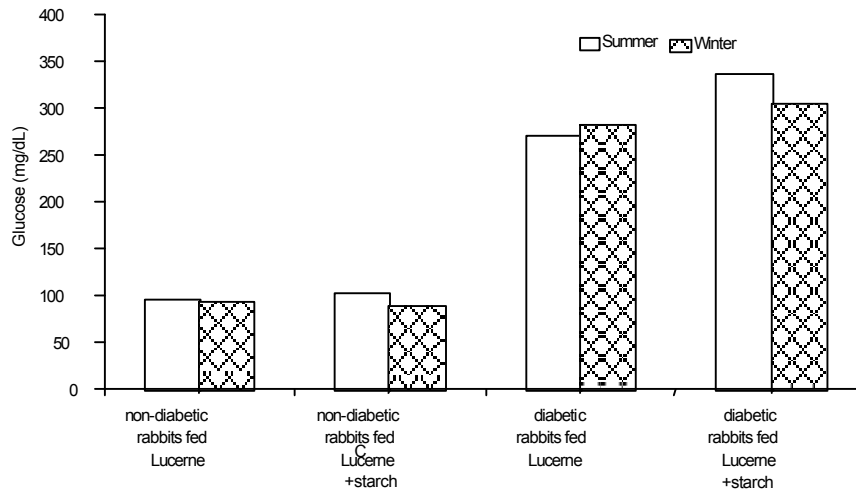


Fig. 2: Effects of starch supplementation and season on plasma glucose level in diabetic and non-diabetic rabbits

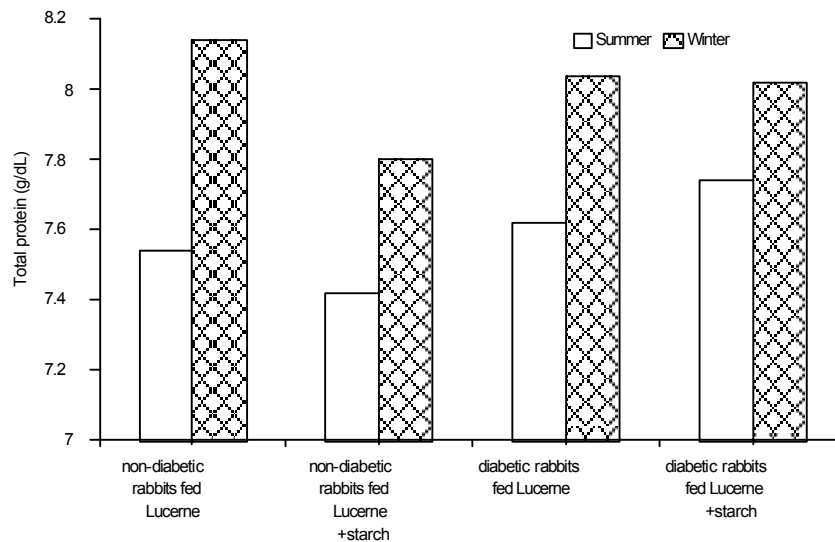


Fig. 3: Effects of starch supplementation and season on serum total protein concentration in diabetic and non-diabetic rabbits

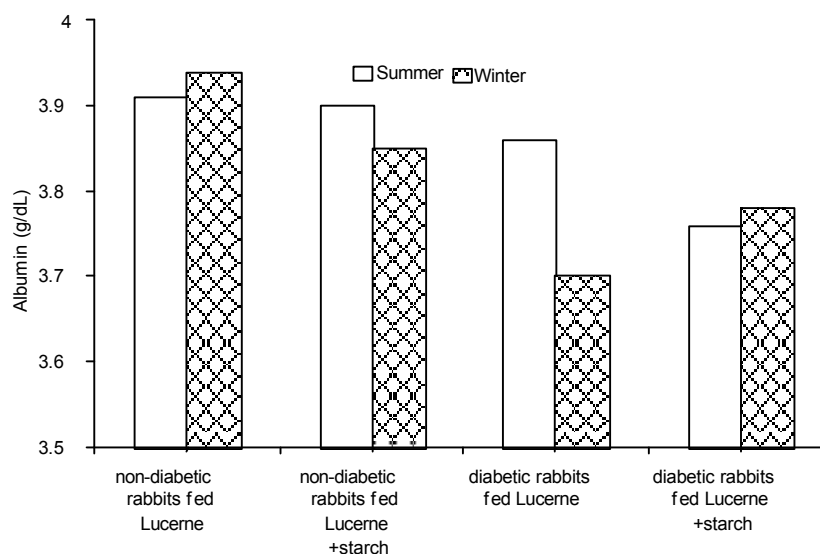


Fig. 4: Effects of starch supplementation and season on serum albumin concentration in diabetic and non-diabetic rabbits

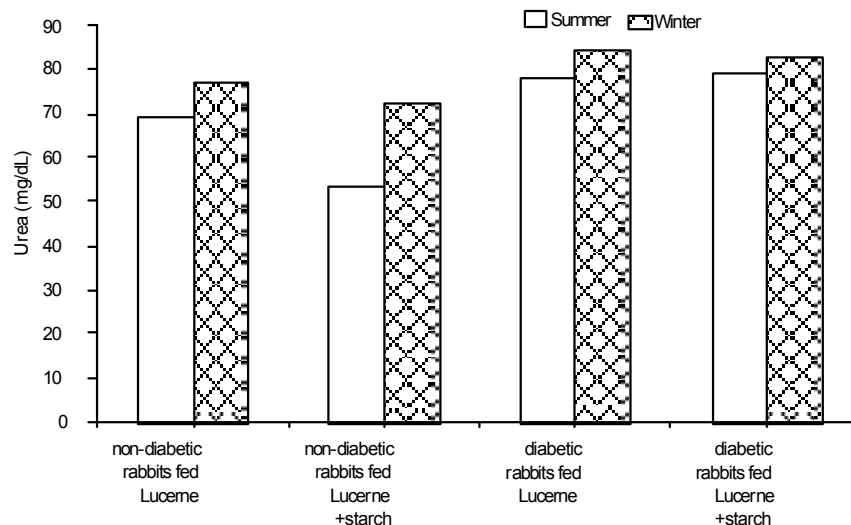


Fig. 5: Effects of starch supplementation and season on serum urea concentration in diabetic and non-diabetic rabbits

**Serum Urea:** Diabetic groups of rabbits maintained higher values of urea during summer ( $P<0.001$ ) and winter ( $P<0.01$ ) compared to respective values of non-diabetic groups (Tables 3 and 4). Fig. 5 shows that for all groups, urea level was higher in winter. Serum urea level was higher in winter compared to respective summer value for non-diabetic group fed Lucerne ( $P<0.05$ ) and non-diabetic group supplemented with starch ( $P<0.01$ ).

**Serum Creatinine:** During summer (Table 3), diabetic rabbits supplemented with starch showed lower values compared to other experimental groups. The non-diabetic rabbits fed Lucerne had higher ( $P<0.05$ ) creatinine level compared to other groups. During winter (Table 4), there

was no significant difference in creatinine between groups. For all experimental groups, creatinine level was relatively higher in summer compared to respective winter values (Fig. 6).

**Serum Triglyceride:** During summer (Table 3), supplementation with starch increased ( $P<0.01$ ) triglyceride level in non-diabetic and diabetic rabbits. The pattern indicates that diabetic rabbits supplemented with starch had the highest triglyceride level. Winter data (Table 4) indicate that in both non-diabetic and diabetic groups of rabbits, starch supplementation was associated with increase in triglyceride level. Season did not elicit significant change in triglyceride level for all experimental groups (Fig. 7).

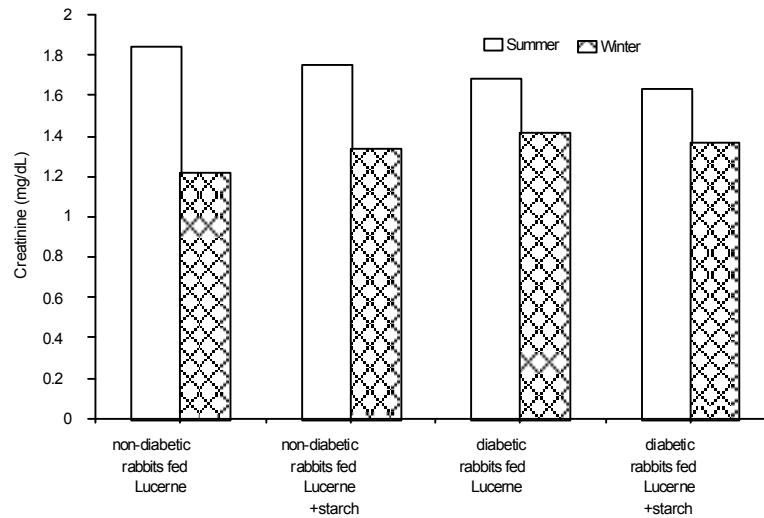


Fig. 6: Effects of starch supplementation and season on serum creatinine concentration in diabetic and non-diabetic rabbits

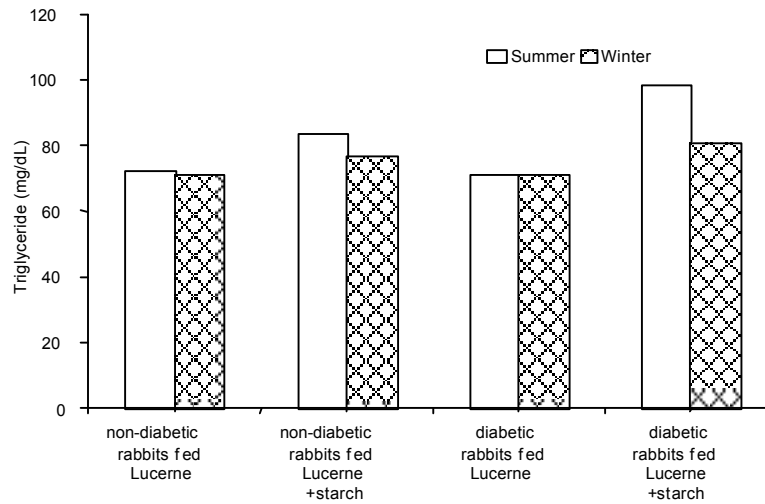


Fig. 7: Effects of starch supplementation and season on serum triglyceride concentration in diabetic and non-diabetic rabbits

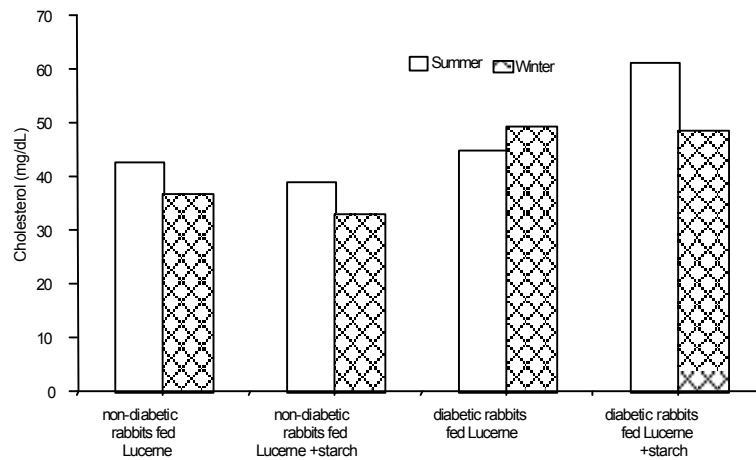


Fig. 8: Effects of starch supplementation and season on serum cholesterol concentration in diabetic and non-diabetic rabbits

Table 5: Effects of starch supplementation on serum minerals and hormones in diabetic and non-diabetic rabbits during summer

Parameter	Non-diabetic rabbits fed Lucerne	Non-diabetic rabbits fed Lucerne + starch	Diabetic rabbits fed Lucerne	Diabetic rabbits fed Lucerne + starch	LS
Na (mEq/L)	160.51 <sup>a</sup> ±10.02	151.18 <sup>b</sup> ±13.81	156.39 <sup>b</sup> ±8.24	153.41 <sup>b</sup> ±12.00	*
K (mEq/L)	6.33 <sup>a</sup> ±0.72	6.03 <sup>a</sup> ±0.90	6.37 <sup>a</sup> ±1.06	5.91 <sup>a</sup> ±0.72	NS
Cortisol (pmol/L)	28.62 <sup>a</sup> ±2.29	33.34 <sup>a</sup> ±3.91	25.62 <sup>a</sup> ±0.65	31.44 <sup>a</sup> ±5.63	NS

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 6: Effects of starch supplementation on serum minerals and hormones in diabetic and non-diabetic rabbits during winter

Parameter	Non-diabetic rabbits fed Lucerne	Non-diabetic rabbits fed Lucerne + starch	Diabetic rabbits fed Lucerne	Diabetic rabbits fed Lucerne + starch	LS
Na (mEq/L)	165.08 <sup>a</sup> ±3.43	162.97 <sup>ab</sup> ±8.70	157.45 <sup>b</sup> ±3.96	160.06 <sup>ab</sup> ±4.21	*
K (mEq/L)	6.42 <sup>a</sup> ±0.58	6.69 <sup>a</sup> ±0.43	6.27 <sup>a</sup> ±0.44	6.37 <sup>a</sup> ±0.64	NS
Cortisol (pmol/L)	34.15 <sup>ab</sup> ±2.29	27.0 <sup>b</sup> ±3.33	39.43 <sup>ab</sup> ±8.19	43.20 <sup>a</sup> ±6.87	*

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

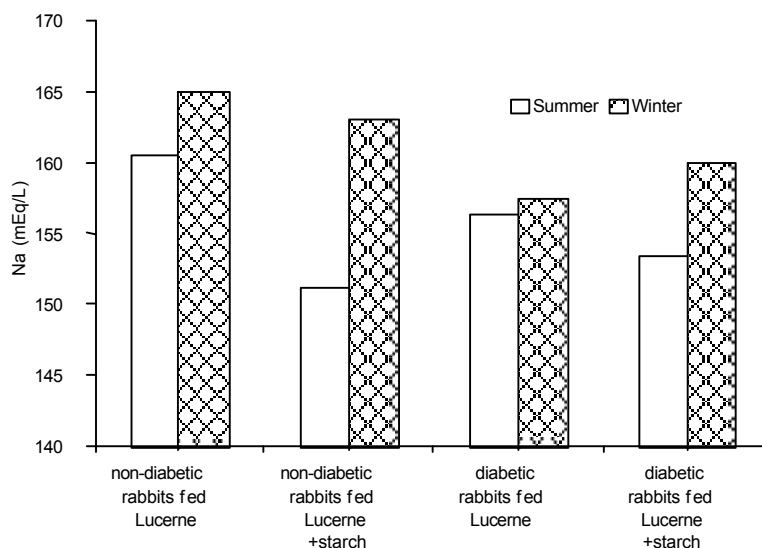


Fig. 9: Effects of starch supplementation and season on serum Na concentration in diabetic and non-diabetic rabbits.

**Serum Cholesterol:** During summer (Table 3), diabetic rabbits supplemented with starch had higher (P<0.001) cholesterol level compared to other experimental groups. During winter (Table 4), diabetic groups had higher (P<0.001) cholesterol level compared to non-diabetic groups. Fig. 8 shows that cholesterol level was significantly higher during summer in the diabetic group supplemented with starch (P<0.001) and in both non-diabetic groups (P<0.05).

**Serum Na, K and Cortisol:** Tables 5 and 6 show the effects of starch supplementation on serum concentrations of Na, K and cortisol in diabetic and non-diabetic rabbits during summer and winter, respectively.

**Serum Sodium (Na):** In both seasons (Tables 5 and 6), non-diabetic rabbits fed Lucerne maintained higher (P<0.05) Na level compared to other experimental groups. Fig. 9 shows that Na level was significantly higher in winter with starch supplementation for non-diabetic rabbits (P<0.01) and diabetic rabbits (P<0.05).

**Serum Potassium (K):** There was no consistent pattern of K level for all experimental groups in summer and winter (Tables 5 and 6). Fig. 10 shows that serum K level was significantly (P<0.05) higher in winter only in non-diabetic rabbits supplemented with starch.

**Serum Cortisol:** Summer results (Table 5) indicate that supplementation with starch was associated with higher



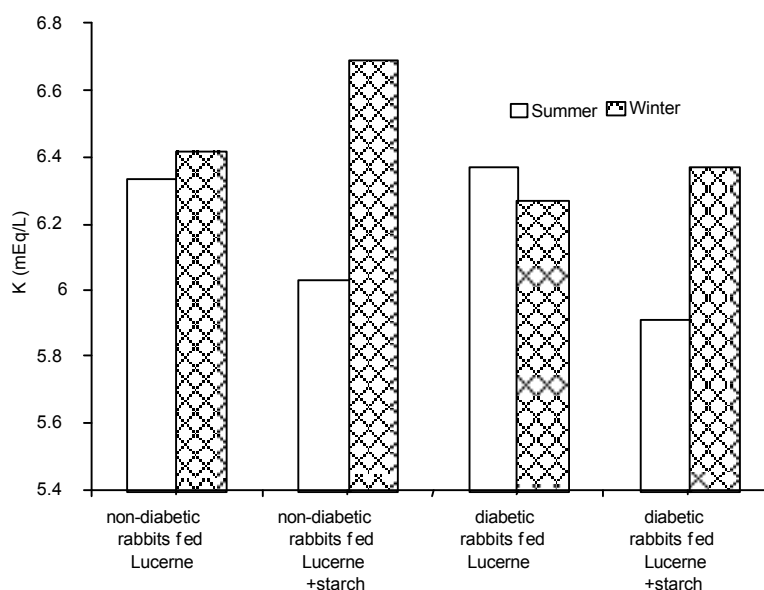


Fig. 10: Effects of starch supplementation and season on serum K concentration in diabetic and non-diabetic rabbits

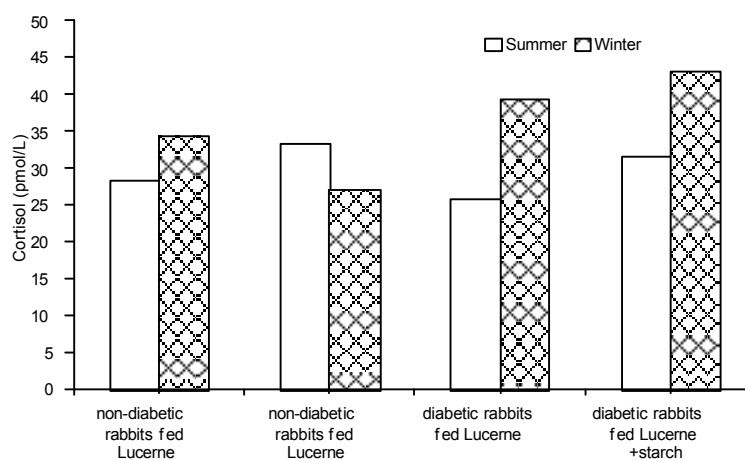


Fig. 11: Effects of starch supplementation and season on serum cortisol concentration in diabetic and non-diabetic rabbits

cortisol levels in non-diabetic and diabetic groups. During winter (Table 6), diabetic rabbits supplemented with starch had higher ( $P < 0.05$ ) cortisol level compared to other experimental groups. Data analysis (Fig. 11) indicates that both diabetic groups of rabbits maintained significantly ( $P < 0.05$ ) higher cortisol level during winter compared to respective summer values. Also a higher cortisol level was obtained during winter compared to summer value for non-diabetic rabbits fed Lucerne only.

## DISCUSSION

In this study, we evaluated the effects of starch supplementation and thermal environment on blood metabolites and certain minerals and hormones in diabetic

and non-diabetic states using the domestic rabbit as a mammalian model. Alloxan induced diabetes by partial destruction of B cells through production of reactive oxygen species [24]. The diabetic state was confirmed by glucose tolerance test (GTT). Diabetes induced in rabbits in this study resembles type 1 diabetes, which is usually induced pharmacologically by selective destruction of B cells of the pancreas.

In both seasons, alloxan treated groups of rabbits had significantly lower values of serum insulin compared to non-diabetic group (Tables 3 and 4). The decrease in insulin level is clearly related to induction of diabetes due to necrosis of B cells of pancreatic islets by the cytotoxin alloxan. A decrease in insulin level in chemically induced diabetes was reported in animal models [25].

The non-significant increase in insulin level with starch supplementation in diabetic groups in both seasons and in non-diabetic rabbits in summer is associated with the reported increase in glucose level related to digestion of cereal starch and absorption of products. An increase in plasma glucose level stimulates insulin secretion [26]. The secretion of insulin in  $\beta$  pancreatic cells in response to glucose requires coordinated change in cellular ion concentrations and membrane depolarization to enable insulin vesicle fusion with cellular membrane [27].

The induction of diabetes was associated with significant increase in plasma glucose level. The hyperglycaemia reported in diabetic groups in both seasons (Tables 3 and 4) is attributed to the reported marked decrease of insulin in the fully developed diabetes mellitus (Tables 3 and 4) as well as reduction of uptake of glucose by muscle and fat cells [28]. In insulin deficiency, glucose formation is promoted, partly due to increased conversion of glycogen to glucose, but mainly by gluconeogenesis. Elevated cyclic AMP which results from unopposed action of glucagon, catecholamines and glucocorticoids causes enhancement of glycogenolysis and gluconeogenesis [29, 30]. It has been indicated that glucagon plays an important role in the maintenance of hyperglycaemia in alloxan-induced diabetic rabbits [31]. The higher glucose level in starch supplemented diabetic group is clearly a reflection of the dietary factor. It has been indicated that total carbohydrate intake is a reliable predictor of postprandial glucose level [32, 33].

The glucose level was significantly higher in summer only in diabetic rabbits supplemented with starch (Fig. 2). Bunout [34] demonstrated fluctuations in glucose and insulin sensitivity in elderly people, with higher levels in the fall or winter, whereas Simon *et al.* [35] reported no seasonal variation in glucose level. The higher glucose level in winter in diabetic rabbits fed Lucerne only is likely to be associated with increase in food consumption in the cool environment. The physical activity has also been reported to diminish in winter months in several settings [36]. The mechanisms by which changes in temperature influence glycated haemoglobin values and glucose level have not been elucidated [37].

The non-significant increase in serum total protein levels in diabetic rabbits (Tables 3 and 4) is in general agreement with studies [38] that reported an increase in serum total protein level in diabetic rats. Conversely, other researchers [39] reported that total protein decreased significantly in alloxan- diabetic rats. However, the results indicated that diabetic groups of rabbits had lower

albumin levels, particularly in winter. Hypoalbuminaemia is a common problem in diabetic animals and is generally attributed to diabetic nephropathy [40]. Microalbuminuria has been frequently used as a diagnostic indicator for the presence of incipient diabetic nephropathy in humans [41]. Furthermore, hepatic levels of albumin and its mRNA were found to be decreased in diabetics [42]. A decline in serum total protein level in diabetics has been attributed to inhibition of oxidative phosphorylation which leads to decrease in protein synthesis, increase in catabolic processes and reduction of protein absorption [38]. The decrease in serum total protein level during summer in diabetic and non-diabetic groups fed Lucerne (Fig. 3) is likely effected by decrease in food intake in the hot environment. A decrease in serum total protein level in rabbits exposed to heat stress has been reported [43, 44].

In both seasons, diabetic groups of rabbits maintained higher serum urea levels (Tables 3 and 4). This response is in agreement with studies which reported increased urea levels in alloxan diabetic rabbits [45, 46] and diabetic subjects [47, 48]. The increase in urea nitrogen in diabetes may be accounted for by enhanced catabolism of both liver and plasma proteins that accompany gluconeogenesis [49]. An increase of urea synthesis in streptozotocin- diabetes in rats was suggested to be due to enzyme induction by glucagon [50]. The higher serum urea level in starch supplemented diabetic rabbits in the current study is in agreement with other studies [51] that reported higher urea level in rabbits maintained with high carbohydrate-low fat diet. The elevation of urea reported during winter in the present study could be associated with a higher level of food intake by rabbits and /or haemoconcentration. Higher serum urea levels were reported during winter in New Zealand White rabbits [52].

The data indicated that during summer, diabetic rabbits had lower values of serum creatinine (Table 3). This finding is in conformity with previous reports [39] which indicated that creatinine level significantly decreased in alloxan -induced diabetic rats. Evaluation of the effects of high carbohydrate-low fat diet in rabbits revealed a significant decrease in serum creatinine level [51]. However, creatininaemia may be attributed to conditions associated with extensive muscle breakdown as in poorly controlled diabetes mellitus [26]. The higher creatinine level in experimental groups during summer (Fig.6) may be related to increase in tissue catabolism associated with increase in the level of glucocorticoid hormones [53]. The current response of creatinine in rabbits confirms previous results [54].

The lipid profile in rabbits was influenced by the treatments (Tables 3 and 4). The higher triglyceride level with impairment of insulin secretion in diabetic rabbits is related to increased lipolysis and decreased activity of endothelial lipoprotein lipase which reduces clearance of triglycerides [55]. Diabetic rabbits supplemented with starch had the highest triglyceride level in both seasons (Tables 3 and 4). This response is in agreement with researchers [51] who found that a high carbohydrate-low fat diet was associated with high triglyceride level.

In both seasons, diabetic rabbits maintained higher serum cholesterol levels (Tables 3 and 4). The rise in cholesterol level which is associated with insulin deficiency, is attributed to increase in plasma concentration of VLDL and LDL which may be due to increased hepatic production of VLDL or decreased removal of VLDL and LDL from the circulation [26, 56]. Similarly previous studies reported an increase in cholesterol level in experimentally induced diabetes in alloxan-diabetic rats [46].

Our results indicated that with Lucerne feeding, serum Na level was significantly lower in diabetic groups of rabbits (Tables 5 and 6). This response could be associated with glucose induced osmotic diuresis and loss of Na in urine. The decrease in Na level may also be attributed to high water consumption and haemodilution associated with hyperglycaemia. A disturbance in water homeostasis in diabetic patients might lead to either hypotonic hyponatraemia or hypernatraemia in response to positive or negative water balance, respectively. Khan *et al.* [57] reported that serum Na level in diabetic rabbits was marginally below the reference range. Other studies [58, 59] demonstrated that Na level was decreased in alloxan- induced diabetic animals. However, in rabbits, high carbohydrate-low fat diet induced a significant increase in serum Na level when compared with control [51]. The lower Na level in summer in all experimental groups is clearly related to haemodilution associated with increase in water consumption and vasodilation.

Our data indicated that there was no significant variation in serum K level related to diabetes and diet in both seasons (Tables 5 and 6). Similarly, previous studies indicated that alloxan induced diabetes did not influence K level significantly in rabbits [60] and rats [59]. However, other studies reported lower K levels in alloxan -diabetic rabbits [58] and streptozotocin diabetic mice [61]. Tzamaloukas and Avasthi [62] noted that hyperkalaemia was associated with hyperglycaemia in diabetic patients; hypokalaemia in diabetes was associated with glycosuria and osmotic diuresis with loss of K and other electrolytes. In poorly controlled diabetes, hyperkalaemia was related

to elevated plasma glucose concentration which causes an osmotic shift of fluid and electrolytes, primarily K, out of cells [63].

The data revealed that during winter, diabetic rabbits supplemented with starch had significantly higher cortisol level compared to other experimental groups (Table 6). This observation implies that both severe hyperglycaemia associated with starch ingestion and cold stress in winter could provoke moderate hypercortisolism in rabbits. Under subtropical conditions, semicaptive rabbits exhibited an annual rhythm of plasma cortisol with peaking in January during winter [64].

Based on the results reported in this study, we concluded that alloxan - induced diabetes in rabbits produces marked alterations in blood metabolites and certain minerals and hormones. The responses are generally influenced by dietary starch and seasonal change in thermal environment. Further investigations are required to elucidate the associated changes in electrolytes and acid-base balance.

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