

## Alterations in Thermoregulation and Haematological Indices in Alloxan - Diabetic Rabbits (*Lepus Cuniculus*) in Relation to Dietary Starch and Season

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**Abstract:** The aim of this study was to evaluate the effects of starch supplementation and season (summer vs winter) on thermoregulation, body weight (BW), water consumption and haematological parameters in alloxan-induced diabetic and non-diabetic rabbits. Starch supplementation increased rectal temperature (Tr) of diabetic groups significantly during summer; in winter, (Tr) in non-diabetic rabbits fed Lucerne was significantly lower than other groups. During summer, non-diabetic starch-supplemented rabbits had significantly higher respiratory rate (RR) than other groups. RR values of experimental groups were significantly higher in summer compared with the respective winter values. In both seasons, diabetic starch-supplemented rabbits had lower body weight (BW) compared to the other groups. During summer, diabetic starch-supplemented rabbits had the lowest (BW). The diabetic group of rabbits fed Lucerne and starch had significantly higher (BW) during winter compared to the respective summer value. In both seasons, diabetic groups of rabbits had significantly higher water consumption compared to the non-diabetic groups. Starch supplementation increased water consumption significantly in diabetic rabbits. Water consumption of non-diabetic group of rabbits fed Lucerne and diabetic groups was significantly higher during summer compared with respective winter values. In both seasons, diabetic groups of rabbits had lower packed cell volume (PCV) and haemoglobin concentration (Hb) compared to the respective values of non-diabetic rabbits. Non-diabetic rabbits fed Lucerne had significantly higher Hb value during winter compared to summer value. During winter, non-diabetic rabbits fed Lucerne had significantly lower total leukocyte count (TLC) compared to values of other groups. Non-diabetic groups had significantly higher (TLC) values during summer compared to winter values. In diabetic groups, winter values of (TLC) were higher compared to summer value. The findings are relevant to the pathophysiological changes in human diabetes.

**Key words:** Rabbits • Diabetes Mellitus • Starch Supplementation • Season • Thermoregulation • Body Weight • Water Consumption • Blood Parameters

### INTRODUCTION

*Diabetes mellitus* is a common chronic metabolic disorder caused by defects in insulin secretion, insulin action, or both, characterized by hyperglycaemia, usually associated with glycosuria, polyuria and polydipsia [1, 2]. The disease causes significant disturbances of water and electrolyte homeostasis [3]. The long-term complications of *diabetes mellitus* include retinopathy, nephropathy, neuropathy and angiopathy [4] associated with oxidative stress and overwhelming free radicals resulting from glucose auto-oxidation and protein glycosylation [5, 6]. 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 [7] showed higher prevalence of *diabetes mellitus* in the adult population with wide geographical distribution.

Dietary factors may influence the prognosis of the disease. Medical nutrition therapy can be adopted as an effective measure to control glycaemia and lipids [8]. 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 [9]. Carbohydrates appear to be the food components that produce almost all of the blood glucose increase after a meal. 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 [10]. Both the quantity and the type or source of carbohydrate found in food influence postprandial glucose level [8]. 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 [7, 15]. However, epidemiologic studies indicate that diabetic subjects have significantly higher rates of heat illness and mortality during heat waves than the general population [16]. The metabolic, cardiovascular and neurologic dysfunctions in diabetes may impair thermoregulatory mechanisms during heat exposure [17]. Heat stress might aggravate diabetic complications as the basal metabolic rate (BMR) is increased in diabetic conditions [18-20]. Also in diabetic conditions, dilatation of skin blood vessels may be impaired which decreases the skin blood flow and heat dissipation [21, 22]. Although *diabetes mellitus* is primarily a metabolic disease, haematological changes have been associated with diabetic conditions. Haematologic abnormalities in diabetes which affect the function, morphology and metabolism of blood cells and the coagulation system [23] are thought to play a role in the development of microangiopathy [24].

Experimental induction of *diabetes mellitus* in animal models is useful for elucidating various metabolic and pathophysiological changes and provides critical insight into human diabetes. We have previously reported on the effects of starch supplementation and season on blood metabolites and serum minerals and hormones in alloxan-

diabetic rabbits [25]. In this report, we present evaluation of the responses of thermoregulation, body weight (BW), water consumption and haematological parameters to alloxan- 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. Male animals were used in order to limit the effects of hormonal changes on the responses. The animals were obtained from a private rabbit 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 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 nutrient composition of fresh Lucerne and sorghum grains is shown in Table 1 [26].

**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. Rabbits showing fasting plasma glucose levels > 200 mg/dL were considered as diabetic.

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

| Ingredients   | Lucerne ( <i>Medicago sativa</i> ) | Sorghum grains ( <i>S. vulgare caudatum</i> ) |
|---------------|------------------------------------|-----------------------------------------------|
| Dry matter    | 230.0                              | 945.0                                         |
| Ether extract | 3.3                                | 25.0                                          |
| Crude protein | 46.1                               | 132.3                                         |
| Crude fibre   | 70.0                               | 24.8                                          |
| Ash           | 28.2                               | 21.5                                          |
| AFE           | 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       | Mean       | Max                             | Min        | Mean       | 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      | 24.00      | 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±1.88                | 24.22±3.51 | 32.51±2.52 | 20.17±7.37 | 33.79±3.84                      | 16.09±2.48 | 24.90±3.05 | 26.62±3.85 |

**Thermoregulation:** The rectal temperature ( $T_r$ ) of rabbit was measured by a digital clinical thermometer (Hartman-United Kingdom). The tip of thermometer was inserted to a depth of approximately 4 cm into the rectum and  $T_r$  was measured with an accuracy of  $\pm 0.1^\circ\text{C}$ . The respiration rate (RR) of animals was measured visually by counting the flank movements for one minute using a stopwatch.

**Body Weight and Water Consumption:** The rabbits were weighed during the experiments to the nearest  $\pm 2.0$  g using an electronic digital balance (Every - United Kingdom). A known amount of water was measured and offered to each animal in individual container. One container was left for measurement of water lost by evaporation. Next morning, the water left in the container was measured. The sum of remaining water and water lost by evaporation was subtracted from the initial amount offered to the animals; the difference was considered as water consumption.

**Blood Analysis:** Blood samples (4 ml) were withdrawn aseptically from the jugular vein by plastic syringes into clean, dry test tubes containing dipotassium ethylene diamine tetra acetate (K2-EDTA) as anticoagulant. The haemogram parameters were determined according to the standard methods [27].

The PCV was measured in plain capillary tubes using a microhaematocrit centrifuge (Hawksley, London). The Hb concentration was determined by cyanomethaemoglobin technique using Drabkin's solution. Improved Neubauer haemocytometer was used to perform total leukocytes count (TLC) using Turks solution as a dilution fluid.

**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, the rectal temperature ( $T_r$ ) and respiration rate (RR) were measured weekly at 8.00 a. m. Blood samples were collected weekly at 9.00 a. m. The body weights (BW) of animals were measured weekly. The water consumption was determined daily.

**Statistics:** For each group of animals, the mean values were computed during the course of the experimental period. The data are presented as mean  $\pm$  Standard Deviation (SD). The analysis of variance (ANOVA) [28] and Duncan's 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  $< 0.05$ .

## RRESULTS

### Thermoregulation, Body Weight (BW) and Water Consumption:

Tables 3 and 4 illustrate the effects of starch supplementation on rectal temperature (Tr), respiratory rate (RR), body weight (BW) and water consumption in diabetic and non-diabetic groups of rabbits during summer and winter, respectively.

**Rectal Temperature (Tr):** During summer (Table 3), the diabetic groups had higher (Tr) value compared to the non-diabetic groups. Supplementation with starch had no significant effect on (Tr) of non-diabetic groups of rabbits. Diabetic rabbits fed Lucerne and starch had significantly ( $P<0.05$ ) higher (Tr) compared to other groups. During winter (Table 4), the mean value of (Tr) in non-diabetic rabbits fed Lucerne was significantly ( $P<0.001$ ) lower compared to other groups.

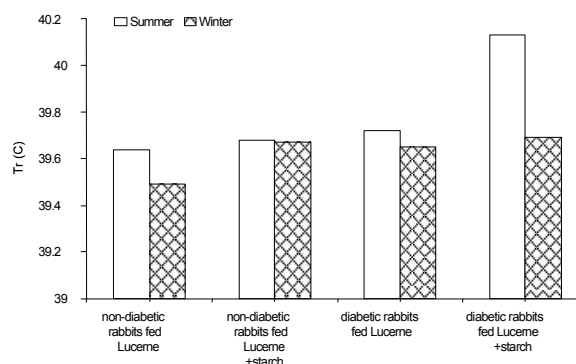


Fig. 1: Effect of starch supplementation and season on rectal temperature (Tr) in diabetic and non-diabetic rabbits

Fig. 1 shows that for experimental groups, the mean (Tr) value was higher during summer compared to the respective winter value. The diabetic group of rabbits fed Lucerne and starch had significantly ( $P<0.001$ ) higher (Tr) in summer compared with winter. Season did not influence significantly (Tr) values in other experimental groups.

**Respiratory Rate (RR):** During summer (Table 3), non-diabetic rabbits fed Lucerne and starch had significantly ( $P<0.05$ ) higher (RR) value compared to the other groups. During winter (Table 4), there was no significant difference between among experimental groups. The mean (RR) values of all experimental groups were significantly ( $P<0.001$ ) higher in summer compared with the respective winter values (Fig. 2).

Table 3: Effects of starch supplementation on rectal temperature (Tr), respiration rate (RR), body weight (BW) and water consumption 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 | P value |
|-------------------------------|----------------------------------|-------------------------------------------|------------------------------|---------------------------------------|---------|
| Tr (°C)                       | 39.64 <sup>b</sup> ±0.44         | 39.67 <sup>b</sup> ±0.35                  | 39.7 <sup>b</sup> ± 0.78     | 40.13 <sup>a</sup> ± 0.49             | *       |
| RR (breaths/min)              | 183.70 <sup>ab</sup> ±30.11      | 194.48 <sup>a</sup> ±30.33                | 178.88 <sup>b</sup> ± 32.94  | 183.78 <sup>ab</sup> ±32.94           | *       |
| Body weight (g)               | 1248.28 <sup>b</sup> ±61.87      | 1417.65 <sup>a</sup> ±110.75              | 1221.24 <sup>b</sup> ±145.28 | 1156.18 <sup>c</sup> ±194.08          | ***     |
| Water consumption (ml/animal) | 89.00 <sup>c</sup> ±21           | 79.00 <sup>c</sup> ±22                    | 200.00 <sup>b</sup> ±43      | 259.00 <sup>a</sup> ±98               | ***     |

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

\*:  $P<0.05$

\*\*\*:  $P<0.001$

Table 4: Effects of starch supplementation on rectal temperature (Tr), respiration rate (RR), body weight (BW) and water consumption 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 | P value |
|------------------------|----------------------------------|-------------------------------------------|------------------------------|---------------------------------------|---------|
| Tr (°C)                | 39.49 <sup>b</sup> ± 0.45        | 39.68 <sup>a</sup> ± 0.29                 | 39.65 <sup>a</sup> ± 0.31    | 39.69 <sup>a</sup> ± 0.32             | *       |
| RR (breaths/min)       | 119.30 <sup>a</sup> ±22.07       | 120.80 <sup>a</sup> ± 21.63               | 111.75 <sup>a</sup> ± 25.23  | 119.12 <sup>a</sup> ± 25.88           | NS      |
| Body weight (g)        | 1288.00 <sup>b</sup> ±253.80     | 1469.38 <sup>a</sup> ±190.87              | 1255.25 <sup>b</sup> ±129.39 | 1263.25 <sup>b</sup> ±111.03          | ***     |
| Water consumption (ml) | 68.28 <sup>c</sup> ±29.72        | 69.37 <sup>c</sup> ±27.93                 | 97.93 <sup>b</sup> ±25.87    | 128.88 <sup>a</sup> ±21.03            | ***     |

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

\*:  $P<0.05$

\*\*\*:  $P<0.001$

NS: not significant

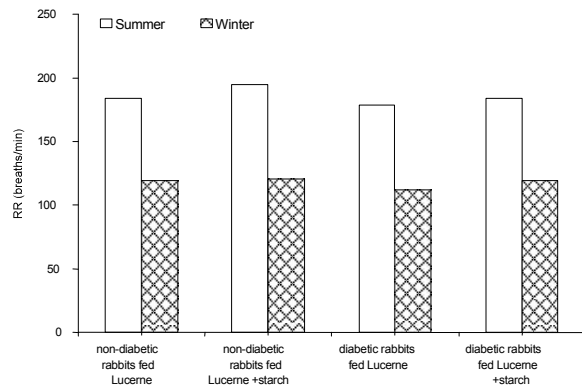


Fig. 2: Effect of starch supplementation and season on respiratory rate (RR) in diabetic and non-diabetic rabbits

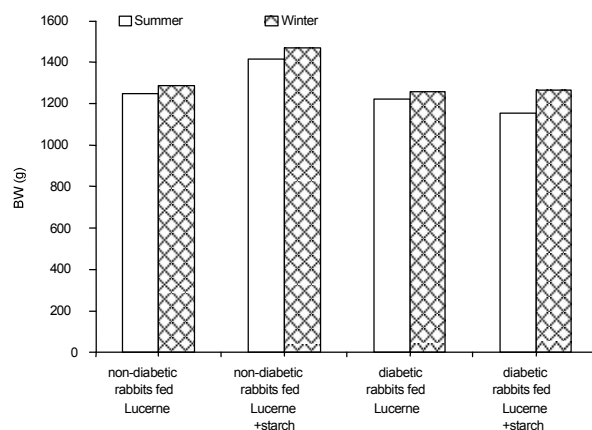


Fig. 3: Effect of starch supplementation and season on mean body weight (BW) in diabetic and non-diabetic rabbits

**Body Wweight (BW):** During summer (Table 3), the non-diabetic rabbits fed Lucerne and starch had significantly ( $P<0.001$ ) higher mean (BW) compared to the other groups. During winter (Table 4), non-diabetic rabbits fed Lucerne and starch maintained significantly ( $P<0.001$ ) higher (BW) compared to the other groups. Fig. 3 shows that the diabetic group of rabbits fed Lucerne and starch had significantly ( $P<0.05$ ) higher (BW) during winter compared to the respective summer value. However, for the other experimental groups, there was no significant seasonal variation.

**Water Consumption:** During summer (Table 3), the general pattern indicates that the diabetic groups of rabbits had significantly ( $p<0.001$ ) higher water consumption compared to the non-diabetic groups. Starch supplementation was associated with a significant ( $P<0.001$ ) increase in water consumption in diabetic

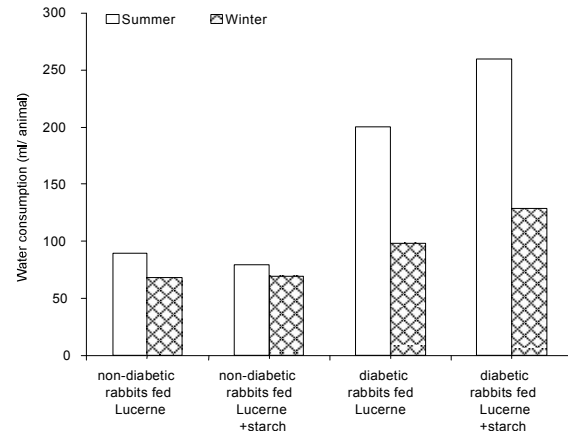


Fig. 4: Effect of starch supplementation and season on mean water consumption in diabetic and non-diabetic rabbits

rabbits. During winter (Table 4), the pattern indicates that the diabetic groups of rabbits had significantly ( $p<0.001$ ) higher water consumption compared to the non-diabetic groups of rabbits. Diabetic rabbits fed Lucerne and starch had significantly ( $p<0.001$ ) higher water consumption than diabetic rabbits fed Lucerne only. Fig. 4 shows that non-diabetic group of rabbits fed Lucerne had significantly ( $P<0.05$ ) higher values of water consumption during summer compared to the winter value; non-diabetic group of rabbits supplemented with starch had no significant seasonal variation. The mean water consumption of diabetic groups was significantly ( $P<0.001$ ) higher during summer compared to respective winter value.

**Haematological Parameters:** Tables 5 and 6 show the effects of starch supplementation on haematological values in diabetic and non-diabetic rabbits during summer and winter, respectively.

**Packed Cell Volume (PCV):** During summer (Table 5), generally, the diabetic groups of rabbits had lower values of (PCV) compared to the respective values of the non-diabetic rabbits. Diabetic group fed Lucerne and starch had significantly ( $P<0.001$ ) lower (PCV) than the diabetic group fed Lucerne only and the non-diabetic groups. During winter (Table 6), diabetic groups had significantly ( $P<0.001$ ) lower (PCV) values compared to the respective values of the non-diabetic groups. Fig. 5 shows that there were no seasonal variation in (PCV) for diabetic groups and the non-diabetic group of rabbits fed lucerne and starch. However, the non-diabetic rabbits fed lucerne had significantly ( $P<0.001$ ) higher (PCV) during winter compared to summer.

Table 5: Effects of starch supplementation on packed cell volume (PCV), haemoglobin concentration (Hb) and total leukocyte count (TLC) in diabetic and non-diabetic rabbits during summer

| Parameter                  | Non-diabetic rabbits<br>fed Lucerne | Non-diabetic rabbits<br>fed Lucerne + starch | Diabetic rabbits<br>fed Lucerne | Diabetic rabbits<br>fed Lucerne + starch | P value |
|----------------------------|-------------------------------------|----------------------------------------------|---------------------------------|------------------------------------------|---------|
| PCV (%)                    | 37.65 <sup>b</sup> ±2.87            | 40.20 <sup>a</sup> ±3.90                     | 37.29 <sup>b</sup> ±3.78        | 35.50 <sup>c</sup> ±4.30                 | ***     |
| Hb (g/dL)                  | 9.55 <sup>b</sup> ±0.97             | 10.38 <sup>a</sup> ±1.30                     | 9.32 <sup>b</sup> ±1.15         | 8.71 <sup>c</sup> ±1.26                  | ***     |
| TLC (x10 <sup>3</sup> /μL) | 6.75 <sup>a</sup> ±1.48             | 6.94 <sup>a</sup> ±1.82                      | 6.55 <sup>a</sup> ±1.73         | 6.54 <sup>a</sup> ±2.05                  | NS      |

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

\*\*\*: P<0.01

NS: not significant

Table 6: Effect of starch supplementation on packed cell volume (PCV), haemoglobin concentration (Hb) and total leukocyte count (TLC) in diabetic and non-diabetic rabbits during winter

| Parameter                  | Non-diabetic rabbits<br>fed Lucerne | Non-diabetic rabbits<br>fed Lucerne + starch | Diabetic rabbits<br>fed Lucerne | Diabetic rabbits<br>fed Lucerne + starch | P value |
|----------------------------|-------------------------------------|----------------------------------------------|---------------------------------|------------------------------------------|---------|
| PCV (%)                    | 42.70 <sup>a</sup> ±3.07            | 40.68 <sup>b</sup> ±3.18                     | 36.50 <sup>c</sup> ±3.10        | 37.40 <sup>c</sup> ±2.38                 | ***     |
| Hb (g/dL)                  | 10.89 <sup>a</sup> ±0.16            | 10.29 <sup>b</sup> ±1.05                     | 9.07 <sup>c</sup> ±0.86         | 9.07 <sup>c</sup> ±0.67                  | **      |
| TLC (x10 <sup>3</sup> /μL) | 5.98 <sup>b</sup> ±1.03             | 6.29 <sup>a</sup> ±1.10                      | 6.87 <sup>a</sup> ±1.51         | 6.92 <sup>a</sup> ±2.12                  | **      |

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

\*\* : P<0.1

\*\*\*: P<0.01

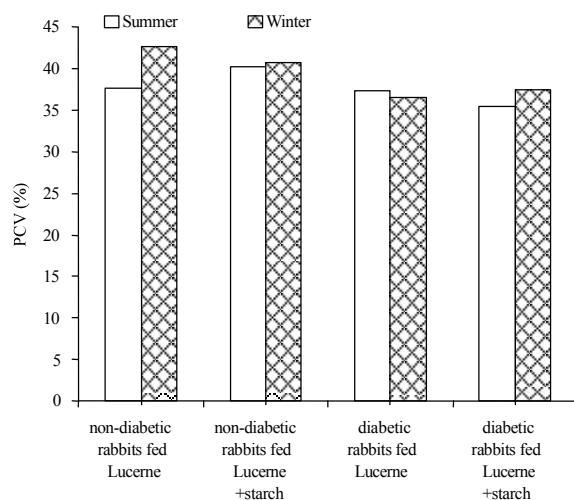


Fig. 5: Effect of starch supplementation and season on packed cell volume (PCV) in diabetic and non-diabetic rabbits

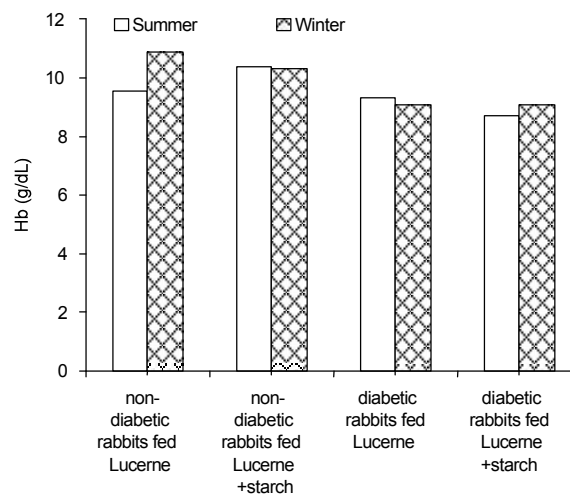


Fig. 6: Effect of starch supplementation and season on haemoglobin (Hb) concentration in diabetic and non-diabetic rabbits

**Haemoglobin Concentration (HB):** During summer (Table 5), generally the diabetic groups of rabbits showed lower values of (Hb) concentration compared to the respective values of the non-diabetic rabbits. Table 5 shows that non-diabetic groups of rabbits had significantly (P<0.001) higher (Hb) value than other groups, while diabetic group fed lucerne and starch had significantly (P<0.001) lower (Hb) values. During winter (Table 6), the diabetic groups of rabbits showed lower

(Hb) values compared to respective non-diabetic values. Table 6 shows that diabetic groups had significantly (P<0.01) lower (Hb) values compared to the respective values of the non-diabetic groups. Fig. 6 indicates that diabetic groups of rabbits and non-diabetic rabbits fed lucerne and starch had no significant seasonal variation in (Hb) concentration. Non-diabetic rabbits fed lucerne had significantly (P<0.001) higher (Hb) value in winter compared to summer value.

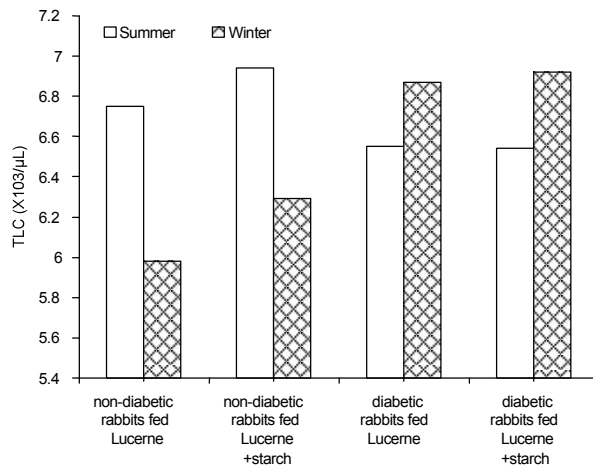


Fig. 7: Effect of starch supplementation and season on total leukocyte count (TLC) in diabetic and non-diabetic rabbits

**Total Leukocyte Count (TLC):** During summer (Table 5), there was no significant difference in (TLC) between experimental groups. During winter (Table 6), diabetic groups of rabbits had higher (TLC) values compared to the non-diabetic groups. Non-diabetic group of rabbits fed lucerne had significantly ( $P < 0.01$ ) lower (TLC) in contrast to the respective values of other groups. Fig. 7 shows that non-diabetic group of rabbits had significantly ( $P < 0.001$ ) higher (TLC) values during summer compared to winter values. The diabetic groups of rabbits had no significant seasonal variation in (TLC). However, winter values were higher in diabetic groups.

## DISCUSSION

In this study, the effects alloxan-induced *Diabetes mellitus*, starch supplementation and seasonal change in thermal environment on thermoregulation, body weight (BW) and haematological parameters have been investigated using the rabbit as a mammalian model. Alloxan monohydrate (150 mg/kg BW) administered intravenously was used to induce diabetes. The diabetic state was confirmed using glucose tolerance test (GTT). The diabetes induced in rabbits in this study resembles Type I diabetes. Alloxan accumulates in B- cells via GLUT 2 glucose transporters [29] and it generates hydroxyl radicals responsible for destruction of B-cells which have a particularly low antioxidative defence capacity [30].

The data indicate that during summer, diabetic groups of rabbits (diabetic control and diabetic supplemented with starch) tended to have higher rectal temperature (Tr) compared to the non-diabetic groups

(Fig. 1). This response in body temperature is indicative of imbalance between heat production and heat loss that cannot be modulated by thermoregulatory mechanisms. Shalaby *et al.* [31] indicated that in streptozotocin-induced diabetic rats kept at 35°C, the observed hyperthermia could be related to increase in metabolic rate not accompanied by greater peripheral vasodilatation. Owu *et al.* [32] reported that the basal metabolic rate (BMR) was significantly higher in alloxan-induced-diabetic rats compared with control. On the other hand, the rate of heat loss from diabetic rabbits might have been compromised. In diabetic subjects, reduction in heat dissipation was related to less microvascular blood flow and thinner skin than that in controls [33, 34]. The release of nitric oxide from the endothelium plays an important role in regulation of vascular tone [35]. The decrease in skin blood flow in diabetes was attributed to reduction in nitric oxide release or the sensitivity of nitric oxide receptors in smooth muscles which reduces vasodilation [36]. In diabetic subjects, the increase in body temperature appeared to be associated with failure of sweating; sweating was lower at any skin temperature in diabetic patients compared with control, thus both type I and type 2 diabetic patients were reported to be more susceptible to heat stress [37].

The mean values of (Tr) of rabbits were higher during summer compared to the respective winter values for all experimental groups (Fig.1). This is attributed to decrease in thermal gradient and consequent decline in sensible heat loss at high environmental temperature during summer (Table 2). The increase in (Tr) at high ambient temperature is in agreement with previous findings in rabbits [38,39,40]. In studies performed on rabbits [41], exposure to high ambient temperature, despite reduction in food intake, was associated with an increase in body temperature.

The data indicate that respiration rate (RR) tended to be lower in diabetic groups of rabbits in both seasons (Tables 3, 4). However, in humans, particularly in chronic diabetes, acidosis stimulates respiration, producing rapid, deep respiration called Kussmaul breathing [42]. Supplementation with starch had no significant effect on (RR) in non- diabetic groups of rabbits, while it was associated with higher (RR) in the diabetic groups of rabbits. This response could be related to availability of energy substrate and increase in oxygen demand. The higher mean values of (RR) in all experimental groups during summer compared with the respective winter values (Fig. 2), indicate that respiratory evaporation is a major channel of heat loss at high ambient temperature.

In rabbits, the sweat glands are not functional and evaporation of water through the skin is not great due to the fur insulation [39]. Therefore, during summer, heat is dissipated by altering the breathing rate to increase vaporization through the enhanced respiratory ventilation. Panting is controlled by alteration in temperature of preoptic region in anterior hypothalamic, spinal cord and mean skin temperature [43]. Neurons that are temperature sensitive are located throughout the animal's body and send information to the hypothalamus, which invokes numerous physiological and behavioural changes in an attempt to maintain heat balance [44].

Supplementation with starch increased (BW) of non-diabetic rabbits in both seasons (Tables 3, 4). In diabetic rabbits, starch supplementation was associated with significant (BW) loss in summer, in winter there was no significant change. The (BW) loss in diabetic-starch supplemented rabbits was associated with increased degree of hyperglycaemia in this group. Following carbohydrate ingestion, the blood glucose level rises even higher, because in absence of insulin, storage of glycogen in the liver is inhibited. Loss of weight, muscle wasting and hunger are characteristic features in diabetes mellitus [45].

The diabetic groups of rabbits had lower (BW) compared to non-diabetic groups both during summer and winter (Tables 3, 4). The observed decrease in (BW) in experimental *diabetes* is in agreement with previous studies on rodents [32, 46, 47]. This response is linked to the effects of inability to use carbohydrates, including lipolysis, glycogenolysis and acidosis [48]. A greater increase in whole-body protein breakdown than protein synthesis occurs also occurs resulting in a net protein loss [20]. The reported greater loss of (BW) in diabetic rabbits supplemented with starch indicates that this group experienced relatively more severe catabolic and other complications of the disease.

The (BW) of diabetic rabbits supplemented with starch was significantly higher during winter compared to summer. However, in all experimental groups, summer was associated with lower (BW) compared to winter values. This is partially attributed to the reported increase in body core temperature during summer which leads to depression of appetite and food intake. Exposure of rabbits to hot environment decreased the (BW) due to decrease in food intake [49]. Impairment of appetite occurs as a result of stimulation of peripheral thermal receptors and transmission of suppressive nerve impulses to the appetite centre in the hypothalamus [50].

The data showed that in both seasons (Tables 3, 4), the diabetic groups of rabbits maintained higher water consumption compared to the non-diabetic groups. This is clearly associated with hyperglycaemia of diabetes and osmotic diuresis. Thirst results from intracellular dehydration that occurs as blood glucose levels rise and water is pulled out of body cells [45], consequently water consumption increases. The higher water consumption in diabetic group of rabbits fed Lucerne and starch in both seasons (Fig. 4) is attributed to osmotic diuresis related to hyperglycaemia augmented by supplementation with starch.

The water consumption of diabetic rabbits was significantly higher during summer compared with the respective winter values while the non-diabetic groups maintained slightly higher values during summer than winter values (Fig. 4). This is attributed to body water deficit and rise in osmolality associated with evaporative water loss in the hot environment. Water intake is stimulated by increased effective osmotic pressure of plasma which acts via osmoreceptors located in the anterior hypothalamus [42].

The diabetic groups of rabbits had lower (PCV) and (Hb) values compared to the respective values of the non-diabetic rabbits during summer and winter (Tables 5, 6). Similarly, decreases in (PCV) and (Hb) levels in diabetic rats have been reported [51, 52] indicating suppression of haemopoiesis. Diabetes-related anaemia was reported in advanced uraemia of diabetic nephropathy; however, diabetes affects the haematologic system in several ways [53]. Previous studies related anaemia to reduction in erythropoietin level in type 1 and type 2 diabetes even without advanced nephropathy [54-56]. The anaemia in *diabetes mellitus* could also be related to increased non-enzymatic glycosylation of erythrocyte membrane proteins, which correlates with hyperglycaemia [57]. Oxidation of these glycosylated membrane proteins and hyperglycaemia in *diabetes mellitus* cause an increase in the production of lipid peroxides causing haemolysis. Meral *et al.* [58] demonstrated an increase in serum lipid peroxide level in diabetic rabbits.

The data indicate that in summer (Table 5), diabetic groups of rabbits had slightly lower (TLC) compared to the non-diabetic groups. However, values obtained in winter (Table 6) indicate that the non-diabetic group fed Lucerne only had significantly lower (TLC) compared to other groups. Previous studies indicated that the (TLC) was lower in diabetic than in non-diabetic subjects

[58-60]. The pattern of response of (TLC) in winter suggests that the diabetic rabbits were more stressed by the cold environment and hyperglycaemia aggravated by starch supplementation that could induce nephropathy. The mechanism responsible for leukocytosis in diabetes is largely unknown. However, current evidence suggests association of inflammatory markers, including leukocyte count, with development of diabetic microvascular and macrovascular complications [61]. The non-diabetic rabbits had significantly higher (TLC) during summer compared to the winter values (Fig. 7). This increase could be associated with thermal stress. Exposure to heat stress stimulates the release of glucocorticoids which usually increases the leukocytes in peripheral blood [62].

In conclusion, alloxan-induced diabetes in rabbits influenced thermoregulation, BW, water consumption and basic haematologic indices in rabbits. The responses were variably modulated by dietary starch supplementation and seasonal change in thermal environment. The findings have clinical implications in both veterinary and human medicine. Dietary and environmental factors should be adequately considered in assessment of the pathophysiology of *diabetes mellitus*.

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