

# Oral glucose supplementation improved semen quality and constituents of seminal and blood plasma of NZW buck rabbits in the subtropics

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**Abstract:** The effect of different levels of oral glucose supplementation on reproductive performance of New Zealand white buck rabbits was studied on 12 bucks aged 6–7 months, randomly divided among four groups from February to September. The treatments consisted of supplementing drinking water with 0 (control), 2.5, 5, and 10 g of glucose/L, respectively. Semen was collected twice weekly from April through September. Three samples of blood and seminal plasma were collected for each treatment during August. Semen quality, biochemical constituents of seminal and blood plasma, and testosterone were studied. Oral glucose supplementation of 5 or 10 g/L of drinking water significantly increased semen volume, sperm motility, sperm concentration, live sperm percentage, total sperm output, and total live sperm output and significantly decreased abnormal sperm percentage as compared to the control group. Addition of glucose at 5 g/L water significantly increased blood plasma total protein, albumin, glucose, alanine aminotransferase, and testosterone hormone compared to the control group.

**Keywords:** rabbit, glucose, semen quality, seminal and blood plasma

## Introduction

Rabbit meat (or other) is increasingly becoming a source of animal protein. The negative effects of heat stress on rabbit bucks include decreased semen quality, essential biochemical constituents of seminal, and blood plasma, and increased respiration rate, temperature of ear, rectum and skin, and water consumption.<sup>1–3</sup> These effects have been attributed to the decrease in hormone profile eg, androgen and thyroid hormone,<sup>4,5</sup> and focal degeneration in both seminiferous tubules and interstitial cells.<sup>6</sup>

Glucose is the principal metabolic fuel used by the growing embryo, fetus, and neonate;<sup>7</sup> the principal precursor for milk lactose and de novo synthesis of milk fat;<sup>8</sup> and the precursor of vitamin C, which is well known as an antistressor.<sup>9</sup> On the other hand, rabbits raised during summer season in Egypt (average temperature  $31 \pm 5^\circ\text{C}$ ) exhibited a significant decrease in serum glucose concentration.<sup>5</sup>

Productive and reproductive performance of rabbits during heat stress has been investigated extensively. However, no study to the best of our knowledge has addressed the possibility that glucose supplementation could improve productive and reproductive performance of rabbits exposed to heat stress due to decreased availability of glucose as a principal fuel for different metabolic and functional body processes. Thus, the present work aimed to study the effect of different concentrations of oral glucose diluted in drinking water on productive and reproductive performance of New Zealand White (NZW) buck rabbits.

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## Material and methods

### Animal feeding and treatments

Twelve NZW bucks aged 6–7 months and with an average live body weight of  $3432 \pm 9.7$  g were used for this study. The bucks were divided into four experimental groups, with an equal number in each ( $n = 3$ ). The first group was kept without oral glucose supplementation and served as a control. The other three experimental groups were given drinking water supplemented with glucose (2.5, 5, and 10 g glucose/L). The experimental period spanned February, March, April (moderate climate:  $16.1^{\circ}\text{C}$ ; 59.4% relative humidity (RH)), May, June, July, August, and September (hot climate:  $27.9^{\circ}\text{C}$ ; 53.2% RH). The total number of semen samples in all treatment groups was 576 samples. Semen was collected twice weekly from April through September by using an artificial vagina.

### Housing condition

The animals were raised in flat deck batteries (Italian type) with universal specifications. Batteries were kept under hygienic control and accommodated with feeders to provide animals with pelleted rations and automatic fresh-water drinkers. All batteries were located in a naturally ventilated windowed house. The buck rabbits were fed on a basal pelleted ration with principal chemical-nutritional characteristics (on a dry matter basis) of: 17% crude protein, 2,600 kcal of digestible energy/kg, and 14% crude fibre. Feed and fresh water were offered ad libitum to the rabbits.

### Criteria of response

Semen volume, sperm motility, sperm concentration, abnormal sperm percentage, total sperm output, and total live sperm output were studied.<sup>10</sup>

Three samples of blood and seminal plasma were collected for each treatment in August, four months after starting the experiment. Blood samples (3–5 mL) were collected in the morning, before feeding, from the marginal ear vein of each experimental buck, under vacuum in heparinized tube. Blood and semen samples were immediately centrifuged at 3,000 rpm for 10 minutes and plasma was separated, frozen at under  $-20^{\circ}\text{C}$ , and kept for assaying using a calorimetric assay kit following the methodology suggested by the procedures. Plasma total protein, albumin, and glucose (bioMérieux, Marcy l'Etoile, France); total lipid and cholesterol (Boehringer Ingelheim GmbH, Ingelheim, France); alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (BioSystems, Barcelona, Spain);

and testosterone (Biocon, Bangalore, India) were determined. Plasma globulin was determined from the difference between plasma total protein and plasma albumin.

### Statistical analysis

The data were analyzed by least squares maximum likelihood method of analysis,<sup>11</sup> using SPSS software (version 8; SPSS Inc., Chicago, IL) according to the following model:

$$Y_{ij} = \mu + \alpha_j + e_i$$

where  $Y$  = the dependent variable;  $\mu$  = general mean;  $\alpha$  = glucose effect;  $e$  = random error.

All percentages were subjected to logarithmic transformation ( $\log_{10} x + 1$ ) to normalize data distribution. Duncan's new multiple range test<sup>12</sup> was used for the multiple comparisons.

## Results

### Biochemical constituents of seminal plasma

Oral glucose supplementation had no significant effect on total lipids and AST and ALT enzymes, showing that glucose up to 10 g glucose/L did not induce cell injury (Table 1).

### Semen quality traits

A 5 g/L concentration of oral glucose supplementation significantly improved ( $P < 0.01$ ) the semen quality of NZW buck rabbits (Table 2). Sperm motility (%), sperm concentration ( $\times 10^6/\text{mL}$ ), total sperm output ( $\times 10^6/\text{ejaculate}$ ), and total live sperm output increased significantly ( $P < 0.01$ ), while abnormal sperm and dead spermatozoa (%) decreased significantly in the semen of bucks administered with 5 g glucose/L water compared to those of the control group. In addition, 10 g glucose/L water induced similar effects, showing that 5 g glucose/L water was sufficient. However, ejaculate volume was not affected by administration of 5 and 10 g glucose/L water compared to the control (Table 2).

A negative effect was quoted in ejaculate volume ( $-33.3\%$ ), total sperm output ( $-35.4\%$ ), and total live sperm output ( $-31.3\%$ ) for bucks supplemented with 2.5 g glucose/L as compared to the unsupplemented control. In addition, the reduction in these criteria of semen was significant compared to the other levels of glucose. On the other hand, 2.5 g glucose/L water resulted in similar ( $P < 0.05$ ) sperm motility and sperm concentration to those of the unsupplemented control and 10 g glucose/L water, indicating

**Table 1** Biochemical constituents of seminal plasma by different glucose level of NZW buck rabbits (LSM  $\pm$  SE)

	Control	Glucose concentration (g/L)			P-value
		2.5	5	10	
Total protein (g/dL)	5.63 <sup>b</sup> $\pm$ 0.41	6.13 <sup>b</sup> $\pm$ 0.32	8.23 <sup>a</sup> $\pm$ 0.78	9.17 <sup>a</sup> $\pm$ 0.73	0.05
Albumin (g/dL)	2.63 $\pm$ 0.23	2.77 $\pm$ 0.22	3.27 $\pm$ 0.19	3.30 $\pm$ 0.26	NS
Globulin (g/dL)	3.00 <sup>b</sup> $\pm$ 0.55	3.37 <sup>b</sup> $\pm$ 0.52	4.97 <sup>ab</sup> $\pm$ 0.96	5.87 <sup>a</sup> $\pm$ 0.61	0.05
Total lipid (g/L)	1.30 $\pm$ 0.12	1.37 $\pm$ 0.09	1.73 $\pm$ 0.22	1.57 $\pm$ 0.09	NS
Cholesterol (mg/dL)	74.6 <sup>b</sup> $\pm$ 3.8	79.2 <sup>b</sup> $\pm$ 4.21	104.2 <sup>a</sup> $\pm$ 8.6	117.5 <sup>a</sup> $\pm$ 3.6	0.01
AST (U/L)	17.47 $\pm$ 2.29	15.40 $\pm$ 1.04	12.57 $\pm$ 1.41	12.57 $\pm$ 0.45	NS
ALT (U/L)	30.73 $\pm$ 1.01	29.10 $\pm$ 1.23	26.00 $\pm$ 2.20	26.90 $\pm$ 1.51	NS
Testosterone (ng/mL)	12.43 <sup>b</sup> $\pm$ 0.63	13.20 <sup>b</sup> $\pm$ 0.70	17.93 <sup>a</sup> $\pm$ 0.95	18.43 <sup>a</sup> $\pm$ 0.75	0.01

**Notes:** Means within the same row not having similar superscripts (<sup>a,b,c</sup>) are significantly different ( $P < 0.05$ ).

**Abbreviations:** LSM, least squares mean; SE, standard error; NZW, New Zealand White; NS, not significant; AST, aspartate aminotransferase; ALT, alanine aminotransferase; U/L, units per litre.

that 2.5 g glucose/L did not affect these characteristics and was less efficiently compared to 5 g glucose/L. Glucose significantly reduced abnormal sperm and dead spermatozoa, while it did not affect sperm concentration compared to the unsupplemented control. This effect was linear up to 5 g glucose/L water and stabilized afterwards.

## Biochemical constituents of blood plasma

Plasma globulin and cholesterol levels did not increase significantly compared with the control (Table 3). It was found that 5 and 10 g glucose/L water significantly increased plasma albumin, total protein, glucose, total lipid, AST, and ALT enzyme compared to the control group. On the other hand, plasma albumin level was increased significantly ( $P < 0.01$ ) due to 2.5 g glucose/L water when compared to the unsupplemented group, and this increment was not significant afterwards. However, 2.5 g glucose/L water did not significantly affect plasma total protein, glucose, total lipids, AST, and ALT plasma level. However, there was a stepwise increase in plasma testosterone with increasing level of glucose supplementation.

## Discussion

Supplementation of 5 or 10 g glucose/L in drinking water significantly improved semen qualities and increased seminal plasma total protein, cholesterol, and testosterone, while only 10 g glucose significantly increased semen globulin level. This indicated that 5 g glucose/L water was adequate. The increase in seminal plasma testosterone due to 5 and 10 g glucose/L water disagrees with the results reported by Attia et al<sup>13</sup> who indicated that plasma testosterone was not affected by energy allotments to broiler breeder males. Similarly, Hotzel et al<sup>14</sup> found that in rams infused with gonadotropin-releasing hormone (GnRH), gonadotrophin secretion was not affected by feeding high or low-diet for maintenance of body weight, and the patterns of secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were similar to those in saline-infused rams fed the high diet. However, Brown<sup>15</sup> reported that reproductive functions in young animals appear to be more susceptible to dietary restrictions of energy and protein than in the adult, and severe feed restriction may even result in permanent damage to gonadal and neural tissue. Martin and Walkden-Brown<sup>16</sup> concluded

**Table 2** Semen quality traits by different oral glucose administration of NZW buck rabbits (LSM  $\pm$  SE)

	Control	Glucose concentration (g/L)			P-value
		2.5	5	10	
Ejaculate volume (mL)	0.60 <sup>a</sup> $\pm$ 0.003	0.40 <sup>b</sup> $\pm$ 0.003	0.65 <sup>a</sup> $\pm$ 0.005	0.63 <sup>a</sup> $\pm$ 0.004	0.01
Sperm motility (%)	74.2 <sup>b</sup> $\pm$ 3.2	70.4 <sup>b</sup> $\pm$ 2.3	82.5 <sup>a</sup> $\pm$ 0.8	77.5 <sup>ab</sup> $\pm$ 2.4	0.01
Abnormal spermatozoa (%)	18.9 <sup>a</sup> $\pm$ 0.7	15.3 <sup>b</sup> $\pm$ 0.8	10.8 <sup>c</sup> $\pm$ 0.7	9.39 <sup>c</sup> $\pm$ 0.63	0.01
Dead spermatozoa (%)	25.9 <sup>a</sup> $\pm$ 0.9	22.5 <sup>b</sup> $\pm$ 1.1	15.7 <sup>c</sup> $\pm$ 1.1	15.2 <sup>c</sup> $\pm$ 1.0	0.01
Sperm concentration ( $\times 10^6$ /mL)	193.4 <sup>c</sup> $\pm$ 12.1	198.6 <sup>bc</sup> $\pm$ 10.5	279.6 <sup>a</sup> $\pm$ 11.2	229.7 <sup>b</sup> $\pm$ 10.4	0.01
Total sperm output ( $\times 10^6$ /ejaculate)	117.9 <sup>b</sup> $\pm$ 8.6	76.24 <sup>c</sup> $\pm$ 6.86	179.6 <sup>a</sup> $\pm$ 14.0	142.7 <sup>b</sup> $\pm$ 10.9	0.01
Total live sperm output ( $\times 10^6$ /ejaculate)	87.1 <sup>b</sup> $\pm$ 6.7	59.9 <sup>c</sup> $\pm$ 6.0	153.4 <sup>a</sup> $\pm$ 12.5	129.0 <sup>b</sup> $\pm$ 10.9	0.01

**Notes:** Means within the same row not having similar superscripts (<sup>a,b,c</sup>) are significantly different ( $P < 0.05$ ).

**Abbreviations:** LSM, least squares mean; SE, standard error; NZW, New Zealand White.

**Table 3** Biochemical constituents of blood plasma by different oral glucose administration of NZW buck rabbits (LSM  $\pm$  SE)

	Control	Glucose concentration (g/L)			P-value
		2.5	5	10	
Total protein (g/dL)	6.37 <sup>b</sup> $\pm$ 0.26	7.13 <sup>ab</sup> $\pm$ 0.34	8.23 <sup>a</sup> $\pm$ 0.50	8.58 <sup>a</sup> $\pm$ 0.76	0.05
Albumin (g/dL)	4.02 <sup>b</sup> $\pm$ 0.05	4.16 <sup>a</sup> $\pm$ 0.06	4.98 <sup>a</sup> $\pm$ 0.27	5.61 <sup>a</sup> $\pm$ 0.38	0.01
Globulin (g/dL)	2.35 $\pm$ 0.24	2.97 $\pm$ 0.31	3.25 $\pm$ 0.25	2.97 $\pm$ 0.41	NS
Glucose (mg/dL)	76.4 <sup>b</sup> $\pm$ 2.49	80.3 <sup>b</sup> $\pm$ 5.29	100.0 <sup>a</sup> $\pm$ 3.59	107.4 <sup>a</sup> $\pm$ 2.80	0.01
Total lipid (g/L)	3.32 <sup>b</sup> $\pm$ 0.01	3.94 <sup>b</sup> $\pm$ 0.37	3.56 <sup>ab</sup> $\pm$ 0.32	4.54 <sup>a</sup> $\pm$ 0.28	0.05
Cholesterol (mg/dL)	112.2 $\pm$ 1.71	122.5 $\pm$ 3.17	122.7 $\pm$ 1.10	123.7 $\pm$ 2.31	NS
AST (U/L)	10.88 <sup>b</sup> $\pm$ 0.61	12.26 <sup>ab</sup> $\pm$ 1.22	12.26 <sup>ab</sup> $\pm$ 0.23	13.84 <sup>a</sup> $\pm$ 0.87	0.01
ALT (U/L)	19.28 <sup>b</sup> $\pm$ 0.92	20.78 <sup>b</sup> $\pm$ 0.47	25.32 <sup>a</sup> $\pm$ 1.17	26.78 <sup>a</sup> $\pm$ 1.22	0.01
Testosterone (ng/mL)	3.50 <sup>c</sup> $\pm$ 0.26	4.11 <sup>bc</sup> $\pm$ 0.49	4.92 <sup>ab</sup> $\pm$ 0.11	5.57 <sup>a</sup> $\pm$ 0.45	0.05

**Notes:** Means within the same row not having similar superscripts (<sup>a,b,c</sup>) are significantly different ( $P < 0.05$ ).

**Abbreviations:** LSM, least squares mean; SE, standard error; NZW, New Zealand White; NS, not significant; AST, aspartate aminotransferase; ALT, alanine aminotransferase; U/L, units per litre.

that energetic components of the diet, rather than the protein content, seem to be responsible for affecting gonadotrophin secretion in rams. In the gonads, the gametogenic tissue responds rapidly to changes in nutrition, but the endocrine compartments are less affected.

The decrease in abnormal sperm and dead spermatozoa due to oral glucose administration suggests that glucose is a limiting factor (fuel) in the sperm maturation and development, and glucose administration improves carbohydrate status.<sup>17,18</sup> Rigau et al<sup>19</sup> reported that glucose increased sperm motility, cyclic adenosine 5-phosphate, and ATP and decreased the respiration rate. In chickens, Bramwell et al<sup>20</sup> following the early research of Attia et al<sup>13,21</sup> concluded that decreasing energy allotments decreased sperm concentration and total live sperm per mL of ejaculate, whereas it had no significant effect on ejaculate volume or percentage dead sperm. These results indicated that NZW bucks could tolerate 5 and 10 g glucose/L water. Glucose tolerance was reported to be affected by strain, species, health condition, pancreatic functions, and pregnancy.<sup>22</sup> In this respect, Kamuro et al<sup>23,24</sup> found that liver glycogen content in animals that received no glucose was significantly lower than that of the 0.2 g/kg/h and the results suggested that intraoperative glucose supplementation is effective in preventing glycogen depletion. To avoid glucose overloading, the optimal dose is 0.1–0.2 g/kg/h. Furthermore, the positive impact of glucose on semen quality could be attributed to its role as a precursor of vitamin C, which is well known as an antistressor, and decreased gluconeogenesis.<sup>9</sup> In addition, Attia et al<sup>13</sup> reported that ejaculate volume was generally improved in high (above the requirements) and intermediate energy (adequate) allotments groups when compared to the low energy (below the requirements) allotment group, although there was no clear

trend in packed cell volume (PCV) of semen and plasma testosterone concentration of broiler breeder males. However, Attia and Badawy<sup>25</sup> found that semen quality (semen volume, viscosity, dead, abnormality, liveability, mass motility, PCV, and percent male producing semen) of Silver and Golden Montazah chicken breeder males were not significantly affected by dietary energy levels. This could be attributed to the strain of birds as heavy broiler breeders were used in the first study and light strains were used in the second one.

The increase in serum glucose observed here is in agreement with the results of Rubio et al<sup>26</sup> who reported that intraperitoneal glucose administration increased serum glucose (50% dextrose) and insulin, while having no effect on serum growth hormone. However, Matsuno et al<sup>27</sup> indicated that plasma glucose and insulin values were not significantly affected by dietary glucose level in rat diets. In partial agreement with the present results, Atta<sup>28</sup> analyzed the blood plasma of broiler chicks and reported that D-glucose did not affect plasma albumin and total lipid. The increase in serum testosterone due to increasing glucose level is in contrast to the results reported by Attia et al<sup>13</sup> who indicated that plasma testosterone was not affected by different dietary energy allotments in broiler breeder males. Along the same line, Hotzel et al<sup>14</sup> found that in GnRH-infused rams, gonadotrophin secretion was not affected by high or low-diet for maintenance of body weight and the patterns of secretion of LH and FSH were similar to those in saline-infused rams fed the high diet. However, Brown<sup>15</sup> concluded that restricted feed intake in adult animals can reduce androgen secretion. Moreover, Martin and Walkden-Brown<sup>16</sup> concluded that in the gonads, the gametogenic tissue responds rapidly to changes in nutrition, but the endocrine compartments are less affected. This contradiction to nutrition in androgen response could be attributed to species differences.

## Conclusion

Glucose supplementation at 5 g glucose/L water in NZW buck rabbits significantly increased total protein and testosterone in serum and seminal plasma and these results concurred with the improving production and quality of semen observed under the subtropical conditions of Egypt.

## Disclosure

The authors report no conflicts of interest in this work.

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