Effect of Gestation and Season on the Haematological and Biochemical Parameters in Domestic Rabbit (*Oryctolagus cuniculus*)

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ABSTRACT

The aim of this study was to investigate the effect of gestation and season on the haematological and biochemical parameters in domestic rabbit (*Oryctolagus cuniculus*) to improve the conditions of breeding and conservation in Saudi Arabia. For this study, 30 non-pregnant and 30 pregnant rabbits on day 24 to 26 of gestation were examined. The animals weighed between 2.6 and 3.6 kg and were 1.8-2.6 years old. Blood samples were collected from pregnant and non–pregnant rabbits in January (winter season), April (spring season), July (summer season) and October (autumn season) for analyzing hematological and biochemical parameters. It was revealed for the study that the value of hematological parameters i.e., erythrocyte, leukocyte, lymphocyte numbers and haemoglobin concentrations of pregnant rabbits were significantly (p<0.05) decreased compared with non-pregnant rabbit. Whereas, mean corpuscular volume was significantly (p<0.05) increased in pregnant rabbits compared with non-pregnant ones. The value of biochemical parameters i.e., total protein, albumin, triglyceride, cholesterol, calcium, and phosphorus were significantly (p<0.05) decreased in pregnant rabbits compared with non-pregnant. Whereas, glucose level was significantly (p<0.05) increased in pregnant rabbits compared with non-pregnant rabbits. Most of hematological parameters significantly (p<0.05) decreased in July. In contrast only few
significant changes recorded in biochemical parameters. In conclusion, the rabbits suffer of heat stress during summer season and this causes deterioration in some, hematological constituents and biochemical parameters. These changes can be reflected on the activities, performance and fetus of pregnant rabbits under hot environmental conditions of Saudi Arabia. Hence, it can be concluded that winter is the best season for breeding and conservation of domestic rabbits.

Keywords: Oryctolagus cuniculus, gestation, season, haematology, biochemical parameters;

ABBREVIATIONS

HCT: Hematocrit ratio, MCH: mean corpuscular hemoglobin, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration.

1. INTRODUCTION

Several studies have found that conducting researches on rabbits are beneficial for farmer’s requirements and animal’s welfare. Hence, haematology and serum chemistry are becoming increasingly important diagnostic tools. Blood parameters in rabbits are used as an aid tool for the diagnosis of organic, infectious and several parasitic diseases. In addition to assess the metabolic condition of animals, haematological and biochemical parameters could be affected by many factors including: sex, age, reproductive status and seasonal variations (Al-Eissa et al., 2008; Wells et al., 1999; Gill and Wanska, 1978; Mira and Mathias, 1994; Cetin et al., 2009). On the other hand, it was reported that haematological parameters were not influenced by sexuality (Schalm et al., 1975) and gestation (Egbe-nwiyi et al., 2000). In various studies, red blood corpuscle numbers, haemoglobin and hematocrit parameters were reported to reach the highest levels during winter months in different rodents (Rewkiewicz-Dziarska, 1975). In contrast, these parameters were reported to be at the lowest level during winter months in large animals such as horses (Gill and Wanska, 1978) and cows (Rusoff et al., 1954).

Previous studies reported that there are no significant differences in haematological parameters between non-pregnant and pregnant rabbits (Brewer, 2006). Furthermore, haematological parameters for different spices of rabbits are reported by many researchers (Chineke et al., 2006; Cetin et al., 2009; Ahamefule et al., 2006; Solomon et al., 1998; Barlet, 1980), but no data have been published for the domestic rabbits.

Since rabbits are usually used as indicators to detect the differences in reference parameters of haematological and biochemicals parameters, the present study was undertaken to investigate the influence of season and pregnancy on haematological and biochemical parameters in domestic rabbits to know the best conditions for breeding and conservation of this species.
2. MATERIALS AND METHODS

2.1 EXPERIMENTAL ANIMALS

A total of 60 domestic rabbits female were used in this study. 30 non-pregnant and 30 pregnant rabbits on day 24 to 26 of gestation were examined. The animals weighed between 2.6 and 3.6 kg and were 1.8-2.6 year old. The rabbits received water and a standard pelleted diet during the whole period. On a dry matter basis, the diet contained 17.9% crude protein, 15.57% crude fiber, 2.45% ether extract, 58.5 nitrogen free extract, and 6.29% ash.

2.2 BLOOD CHEMISTRY

Blood samples were collected from pregnant and non-pregnant rabbits in January (winter season) (9-20.2°C), April (spring season) (20.3-33.3°C), July (summer season) (35.4-43.5°C) and October (autumn season) (20.9-35.0°C). The duration of rabbit gestation period is about one month. From the finding data of non-pregnant rabbit we can choose the best season for breeding. So the effect of season on pregnant rabbits was not studied. The blood obtained from the ear vein into two vacutainer tubes for each animal, one with Ethylene Diamine Tetra Acetic Acid (EDTA) for haematological analysis and the other without EDTA. Another set of tubes were centrifuged for separating serum and kept in deep-frozen for serum biochemical analyses.

Haematological parameters were analyzed within 24 hours after collecting blood. Hematocrit ratio (HCT) was determined by spinning 75UL of blood in heparinized capillary tubes and centrifuged for 5 min. Red blood cell (RBC) and white blood cell (WBC) numbers were estimated. Hemoglobin concentration (HBC) was estimated by using Cyanmethaemoglobin method while the mean corpuscular hemoglobin (MCH), mean corpuscular volume(MCV), mean corpuscular hemoglobin concentration (MCHC) were calculated as described by Jain (1986).

Total erythrocyte numbers were scored by using Thoma Chamber. Haemoglobin concentration was determined by using a Shale Hemometer (Pejrilova et al., 2004). Biochemical investigations were performed using the biochemistry analyser VetScan VS2 (Abaxis Veterinary Diagnostics, Union City, CA 94587, USA). A total of 60 Serum samples were collected then centrifuged at 1300 x g for 15 min, and divided into eppendorf tubes. Isolated sera were stored at -20°C. Total protein, albumin, total cholesterol (TC), triglyceride, glucose, calcium, phosphorus and magnesium levels were measured by using spectrophotometric methods. Flame photometer was used to detect potassium level (Hassabo, 2008; Peter et al., 1982).

2.3 STATISTICAL ANALYSIS

Data were analyzed by using the SPSS (SPSS Inc., Chicago, IL, USA). The significant differences in seasons were analyzed by (ANOVA) test. Statistical significances between pregnant and non-pregnant animals were determined by t-test.
3. RESULTS

3.1 EFFECT OF GESTATION

The hematological parameters of pregnant and non-pregnant rabbits are illustrated in Table 1. The erythrocyte, leukocyte and lymphocyte numbers, haemoglobin concentration were significantly (p<0.05) decreased in the pregnant rabbits as compared to non-pregnant rabbits.

All biochemical parameters except magnesium were significantly (p<0.05) decreased when compared to those of non-pregnant rabbits (Table 2). Glucose level was significantly (p<0.05) increased in pregnant rabbits compared with non-pregnant rabbits.

Table 1. The hematological parameters of non-pregnant and pregnant rabbits

| Parameter                  | Non-pregnant rabbit | Pregnant rabbit |
|----------------------------|---------------------|----------------|
| Erythrocyte (x10⁹/mm³)    | 5.30 ± 0.40         | 3.72 ±0.11     |
| Haemoglobin (g/100 ml)    | 9.00 ± 0.20         | 7.66 ± 0.33    |
| Hematocrit (%)            | 32.99 ± 1.15        | 30.41 ± 0.62   |
| MCV(fl)                   | 56.30 ± 4.00        | 66.33 ± 2.22   |
| MCH (pg)                  | 15.81 ± 1.00        | 17.82 ± 0.52   |
| MCHC (g/dl)               | 28.27 ± 1.21        | 27.55 ± 0.72   |
| Leukocyte (x10³/mm³)      | 5.55 ± 0.75         | 4.10 ± 0.69    |
| Lymphocyte (%)            | 68.82 ± 2.22        | 59.10 ± 3.41   |

*Significantly different from non-pregnant at p<0.05. The data presented are the mean ±SE

MCV(fl): mean corpuscular volume; MCH (pg): mean corpuscular hemoglobin; MCHC (g/dl): mean corpuscular hemoglobin concentration

Table 2. The biochemical parameters of non-pregnant and pregnant rabbits

| Parameter                  | Non-pregnant rabbit | Pregnant rabbit |
|----------------------------|---------------------|----------------|
| Total Protein (g/dl)       | 5.60 ± 6.84         | 5.35 ± 1.26    |
| Albumin (g/dl)             | 4.2 ± 0.16          | 3.3 ± 0.24     |
| Triglyceride (mg/dl)       | 160.55 ± 5.74       | 135.00 ± 2.99  |
| Cholesterol (mg/dl)        | 127.66 ± 5.13       | 54.85 ± 0.51   |
| Calcium (mg/dl)            | 16.35 ± 0.32        | 13.90 ± 0.14   |
| Magnesium (mg/dl)          | 2.22 ± 0.02         | 2.32 ± 0.02    |
| Phosphorus (mg/dl)         | 5.65 ± 0.30         | 4.28 ± 0.22    |
| Glucose (mg/dl)            | 103.16 ± 3.05       | 113.94 ± 1.18  |

*Significantly different from non-pregnant at p<0.05. The data present are the mean ±SE

3.2 EFFECT OF SEASON

The effect of season on hematological parameters of non-pregnant rabbits are showed in Table 3.

The effect of season on biochemical parameters of non-pregnant rabbits are showed in Table 4. The highest value of albumin level was observed in July. The lowest value of
glucose was found in July. Cholesterol level was significantly (p<0.05) decreased in April and increased in October.

Erythrocyte numbers and hemoglobin concentration were significantly (p<0.05) decreased in July, whereas hematocrit level and mean corpuscular volume were significantly (p<0.05) increased at the same month. Leukocyte and lymphocyte numbers were significantly (p<0.05) decreased in July and October. Erythrocyte and leukocyte numbers and MCH concentration were significantly (p<0.05) increased in January.

Table 3. The effect of seasons on the haematological parameters of non-pregnant rabbits

| Parameter                | January      | April        | July         | October      |
|--------------------------|--------------|--------------|--------------|--------------|
| Erythrocyte (x106/mm3)   | 6.00 ± 0.41  | 5.90 ± 0.04  | 5.00 ± 0.02  | 5.52 ± 0.14  |
| Haemoglobin (g/dl)       | 10.00 ± 0.23 | 10.06 ± 0.01 | 9.82 ± 0.02  | 9.77 ± 0.12  |
| MCH (pg)                 | 16.15 ± 0.70 | 15.73 ± 0.04 | 14.62 ± 0.54 | 15.18 ± 0.44 |
| MCHC (g/dl)              | 28.00 ± 1.37 | 27.43 ± 0.33 | 25.40 ± 1.24 | 26.60 ± 1.14 |
| Hematocrit (%)           | 36.26 ± 0.86 | 36.85 ± 1.14 | 41.00 ± 0.85 | 35.88 ± 0.33 |
| MCV (fl)                 | 56.54 ± 3.94 | 56.96 ± 0.01 | 62.04 ± 0.33 | 57.94 ± 0.54 |
| Leukocyte (x109/mm3)     | 11.55 ± 0.55 | 10.66 ± 1.31 | 8.44 ± 0.50  | 9.02 ± 1.00  |
| Lymphocyte               | 58.20 ± 4.14 | 57.00 ± 0.58 | 53.00 ± 0.45 | 54.32 ± 0.45 |

* Significantly different from reference at p<0.05. The data present are the mean ±SE

Table 4. The effect of seasons on the biochemical parameters of non-pregnant Rabbits

| Parameter                | January      | April        | July         | October      |
|--------------------------|--------------|--------------|--------------|--------------|
| Albumin (mg/dl)          | 2.66 ± 0.21  | 2.80 ± 0.00  | 5.12 ± 0.00  | 2.10 ± 0.11  |
| Total Protein (g/dl)     | 4.44 ± 1.22  | 3.80 ± 2.07  | 3.85 ± 3.01  | 3.89 ± 2.34  |
| Triglyceride (mg/dl)     | 145.17 ± 4.54| 147.21 ± 2.33| 151.17 ± 2.54| 148.23 ± 2.55|
| Cholesterol (mg/dl)      | 81.44 ± 8.02 | 72.35 ± 1.05 | 75.68 ± 7.05 | 86.47 ± 2.33 |
| Glucose (mg/dl)          | 115.30 ± 1.74| 113.60 ± 0.45| 95.30 ± 0.35 | 113.20 ± 0.41|
| Calcium (mg/dl)          | 16.88 ± 1.35 | 16.22 ± 0.58 | 15.11 ± 0.14 | 16.75 ± 0.33 |
| Phosphorus (mg/dl)       | 6.50 ± 0.18  | 6.82 ± 0.32  | 6.24 ± 0.43  | 6.53 ± 0.11  |
| Magnesium (mg/dl)        | 2.21 ± 0.05  | 2.42 ± 0.03  | 2.33 ± 0.45  | 2.66 ± 0.07  |

* Significantly different from reference at p<0.05. The data present are the mean ±SE

5. DISCUSSION

The data in this study provided an alternative set of reference values that can be used in the clinical evaluation of domestic rabbits. Pregnancy of a rabbits and season influenced on haematological and biochemical parameters of rabbits. The present data showed that the reduction in erythrocyte numbers and haemoglobin parameters were observed in pregnant rabbits. This finding agreed with previous study on New Zealand rabbits (Kim et al., 2002; Wells et al., 1999). This reduction may be related to the physiological anemia occurring due to hemodilution (Ozegbe, 2001). The increase in MCV suggests an increase in the number...
of immature erythrocytes, which is in accordance with findings by many researchers in case of pregnant rabbits (Kim et al., 2002; Lurie, 1993). In our study there were fewer leukocytes in pregnant rabbits than in non-pregnant, which corresponded with the results obtained by (Wells et al., 1999; Kim et al., 2002).

Some studies demonstrated that haematological and biochemical parameters reached the highest value during winter months in rodents (Rewkiewicz-Dziarska, 1975), whereas, these parameters reached the lowest level in large animals such as horses and cows (Rusoff et al., 1954; Gill and Wanska, 1978). These differences may be due to from different adaptation skills of animal species against temperature changes. In addition, the summer season may decrease the oxygen-carrying capacity of blood by changing erythrocyte numbers and hemoglobin concentration. Scelza and Knoll (1982) reported that in desert rodent's erythrocyte numbers was at the highest level during summer months whereas it progressed to lower levels during winter months. Moreover, cold weather displays an effect providing a better blood circulation. Increased in the amount of haemoglobin during winter months may be considered as a pathway of increasing the oxygen-carrying capacity. Study on the erythrocyte numbers revealed that it became higher in summer but hemoglobin and hematocrit parameters remained the same in both summer and winter (Rietkerk et al., 1994).

In this study, leukocyte and lymphocyte numbers were lower during summer and autumn months than in winter months. These findings were in agreement with the results obtained in Chinchillas (crepuscular rodents) as reported by Egbe-nwiyi et al. (2000). Differences in lymphocyte numbers and albumin levels were observed in summer, which may be related to animal species and the effect of the seasons (Dobrowolska and Gromadzka-Ostrowska, 1983).

The reason for the increase of albumin amount during summer months is due to the increase in the osmotic pressure exerted by albumin (Egbe-nwiyi et al., 2000). Total protein, glucose and albumin parameters in Nubian Goats were found to be the maximum in winter and were minimum in wet summer (Abdelatif et al., 2009). Whereas cholesterol, triglyceride, calcium, magnesium and phosphorus levels were not influenced by seasons (Cetin et al., 2009). The differences may originate from animal, species, environment temperature, age, sex, hormonal changes and the seasonal changes in food intake as mentioned by Al-Eissa and Alhomida (1997) in gazelle.

6. CONCLUSION

In conclusion, under our experimental conditions, the heat stress in summer season negatively reflected on the hematological and biochemical parameters. So according to the obtained data winter is the best season for breeding and conservation of domestic rabbit (Oryctolagus cuniculus) in Saudi Arabia.

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