

## Effect of Different Dietary Protein Levels and Age on Testicular Development and Ultrastructure in Karadi Ram Lambs

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### Abstract

This study was undertaken to investigate the effect of two dietary crude protein levels and age on testicular development and ultrastructure in Karadi ram lambs. Twenty five lambs [6 months old;  $28.6 \pm 1.19$  kg (average body weight  $\pm$  SE)] were randomly assigned to one of the two experimental diets containing either 12% (n=12) or 16% (n=13) dietary crude protein. Two ram lambs were slaughtered from each dietary group at the beginning of the experiment at a monthly interval except for five animals, which were slaughtered when they attained 11 months of age. The right testicular tissue was then removed from each lamb and processed for histomorphometric analysis. The body weight, testicular measurements, and testicular histology was not significantly ( $p > 0.05$ ) affected by the dietary protein levels. The body weight gain and testicular growth rate were increased ( $p < 0.05$ ) linearly between 6 to 11 months of age. Most of the increase in the testicular weight (345%) was attained by lambs between 6 to 9 months of age. Greater and lower ( $p < 0.05$ ) total seminiferous and spermatogenic cells were noticed in ram lambs at age of 11 and 6 months respectively. Moreover, the diameter of the seminiferous tubule and the height of the epithelial cells were increased by two folds ( $p < 0.01$ ) in ram lambs at 11 months of age in comparison to those at 6 months of age. Sperm was observed in the lumen of the testicular seminiferous tubule for the first time when the old ram lambs were 6 months old. In conclusion, the testicular measurements and component weights of Karadi ram lambs were not affected by the dietary crude protein levels. However, the physiological puberty was attained approximately at the age of 10 months. This may enable the use of Karadi ram lambs for breeding purpose in early age, when they have attained physiological puberty as found in the study.

**Keywords:** *dietary protein, age, testes, Karadi rams lambs.*

### 1. Introduction

The Karadi breed which comprises 18-20% of the Iraqi sheep population is native to the north-eastern mountains, villages, and undulating dry farming plains of the Kurdistan region of Iraq. The Karadi lambs are white in color with a black open face and pendulous ears. The black color often extends to the shoulders and other parts of the body. Rams

and ewes are polled. Occasionally two wattles are found under the throat. It is fat tailed animal with an excessively large tail. The weight of rams and ewes are about 70kg and 60kg, respectively. The fleece is coarse and weighs about 1.8kg (Alkass and Juma, 2005). The reproductive well-being and performance of farm animals is largely dependent

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on their 15 nutritional status. It is well documented that adequate nutritional management is crucial for successful gonadal development and mating in sheep flocks (Smith and Akinbamijo, 2000; Fernandez et al., 2004; Kheradmand et al., 2006). Studies (Murray et al., 1990, Martin and Walkden-Brown, 1995; Al-Haboby et al., 1999; Smith and Akinbamijo, 2000) have shown that dietary protein metabolism and deficiency may impair spermatogenesis, libido, and fertility in lambs and rams. However, a study (Madhat, 1977) has not found any effect of dietary crude protein on testicular and epididymal characteristics of Awassi ram lambs.

Using of younger ram lambs in breeding programs may reduce production costs, accelerate benefits of genetic selection, and allow earlier progeny and libido testing. Benefits of advanced puberty in ram lambs may be accentuated in the year-round breeding programs that can take advantage of early fertility in the ram lambs (Wheaton and Godfrey, 2003). The selection of a fertile sheep can be accomplished through correlating characters in young rams such as testis size (Land and Carr, 1975). In the same manner, Koyuncu et al., (2005) found that the effects of lambs age and live weight on the whole testicular measurements were significant. He also pointed out that selection of breeding rams based only on the scrotal and the testicular measurements were not sufficient, and further studies on testicular ultrastructure and spermatogenic activity were needed for confirmed selection.

To the best of our knowledge, the effects of dietary crude protein levels and age on testicular ultrastructure and spermatogenesis have not been studied in Karadi ram lambs. It was therefore decided to investigate these factors in Karadi ram lambs.

## 2. Materials and Methods

### 2.1 Animals

A total of twenty five Karadi lambs [6 months old;  $28.6 \pm 1.19$  kg (average body weight  $\pm$  SE)] were randomly assigned to one of the two experimental diets containing either 12% (n=12) or 16% (n=13) dietary crude protein (Table 1). The two concentrate diets consisted mainly of barley, wheat

bran, soybean meal as well as mixture of salts, vitamins, and minerals. Urea (0.4%) was added to 16% crude protein diet only as non-protein nitrogen (NPN) source to correct the nitrogenous content. The two diets had approximately equal metabolizable energy contents (2.428 and 2.458 Mcal/kg respectively). Barley straw was introduced as roughage to the two experimental groups, 9% and 7.5% respectively. Two ram lambs were slaughtered from each group at the beginning of the experiment and then at a monthly interval at the farm abattoir, except for five animals who were slaughtered when they had attained 11 months of age. The scrotal circumference was measured using cloths tape, pre-slaughter as described by Alkass (1979). All lambs were weighed at the start of the experiment and at weekly interval, just prior to the morning feed.

**Table 1**

Formulation and chemical compositions of the experimental diets\*

| Ingredient (%)    | Groups      |             |
|-------------------|-------------|-------------|
|                   | 12% protein | 16% protein |
| Barley            | 51          | 48          |
| Wheat bran        | 35          | 31          |
| Soya bean meal    | 4           | 12          |
| Barley Straw      | 9           | 7.5         |
| Salt              | 0.5         | 0.5         |
| Vitamins          | 0.5         | 0.5         |
| Urea              | -           | 0.4         |
| Crude protein (%) | 12.18       | 16.03       |
| ME (Mcal/kg)*     | 2.428       | 2.458       |

\* NRC (2001)

Following slaughter, the epididymis was dissected from the testes and weighed. The testis was weighed and the circumference at the maximum diameter was measured after removing excess connective tissue. The volume of each testicle was estimated by measuring the volume of water displaced when it was gently plunged into a volumetric measuring cylinder filled with water up to the brim (Alkass, 1979).

**Table 2**

Effect of dietary protein levels and age of animals on live body weight and some testicular traits of Karadi ram lambs (Mean  $\pm$  SE)

| Effects       | No. of Animals | Live body weight (kg)         | Scrotal circumference (cm)   | Testes weight (g)               | Epididymal weight (g)         | Testicular volume (cm <sup>3</sup> ) | Testicular circumference (cm)  | Relative testicular weight (g/kg) |
|---------------|----------------|-------------------------------|------------------------------|---------------------------------|-------------------------------|--------------------------------------|--------------------------------|-----------------------------------|
| Overall mean  | 25             | 42.61 $\pm$ 2.59              | 24.58 $\pm$ 0.96             | 154.00 $\pm$ 18.4               | 27.85 $\pm$ 3.78              | 152.56 $\pm$ 19.2                    | 12.88 $\pm$ 0.65               | 0.33 $\pm$ 0.03                   |
| Protein level |                |                               |                              |                                 |                               |                                      |                                |                                   |
| 12%           | 12             | 40.77 $\pm$ 3.9 <sup>a</sup>  | 23.83 $\pm$ 1.6 <sup>a</sup> | 151.7 $\pm$ 28.31 <sup>a</sup>  | 27.03 $\pm$ 5.4 <sup>a</sup>  | 149.61 $\pm$ 24.0 <sup>a</sup>       | 12.56 $\pm$ 0.93 <sup>a</sup>  | 0.34 $\pm$ 0.04 <sup>a</sup>      |
| 16%           | 13             | 44.30 $\pm$ 3.5 <sup>a</sup>  | 25.26 $\pm$ 1.2 <sup>a</sup> | 156.1 $\pm$ 25.03 <sup>a</sup>  | 28.61 $\pm$ 5.5 <sup>a</sup>  | 155.75 $\pm$ 31.4 <sup>a</sup>       | 13.17 $\pm$ 0.94 <sup>a</sup>  | 0.32 $\pm$ 0.04 <sup>a</sup>      |
| Age (month)   |                |                               |                              |                                 |                               |                                      |                                |                                   |
| 6             | 4              | 23.62 $\pm$ 1.2 <sup>d</sup>  | 19.5 $\pm$ 3.4 <sup>b</sup>  | 42.8 $\pm$ 21.16 <sup>c</sup>   | 8.04 $\pm$ 2.9 <sup>d</sup>   | 40.75 $\pm$ 19.8 <sup>c</sup>        | 8.76 $\pm$ 1.58 <sup>b</sup>   | 0.18 $\pm$ 0.090 <sup>c</sup>     |
| 7             | 4              | 35.25 $\pm$ 2.9 <sup>c</sup>  | 21.5 $\pm$ 0.3 <sup>ab</sup> | 72.8 $\pm$ 8.51 <sup>c</sup>    | 12.1 $\pm$ 2.7 <sup>cd</sup>  | 71.25 $\pm$ 9.4 <sup>bc</sup>        | 10.87 $\pm$ 0.52 <sup>b</sup>  | 0.21 $\pm$ 0.030 <sup>bc</sup>    |
| 8             | 4              | 34.87 $\pm$ 4.2 <sup>c</sup>  | 23.0 $\pm$ 2.6 <sup>ab</sup> | 126.3 $\pm$ 40.60 <sup>bc</sup> | 18.3 $\pm$ 2.5 <sup>bc</sup>  | 144.5 $\pm$ 59.4 <sup>abc</sup>      | 11.81 $\pm$ 1.77 <sup>ab</sup> | 0.3 $\pm$ 0.080 <sup>abc</sup>    |
| 9             | 4              | 49.32 $\pm$ 0.8 <sup>b</sup>  | 26.75 $\pm$ 1.4 <sup>a</sup> | 190.6 $\pm$ 1.42 <sup>ab</sup>  | 19.92 $\pm$ 0.52 <sup>b</sup> | 175.25 $\pm$ 22.4 <sup>ab</sup>      | 14.62 $\pm$ 0.87 <sup>a</sup>  | 0.4 $\pm$ 0.007 <sup>abc</sup>    |
| 10            | 4              | 51.12 $\pm$ 2.1 <sup>ab</sup> | 27.12 $\pm$ 1.6 <sup>a</sup> | 210.6 $\pm$ 32.03 <sup>ab</sup> | 50.48 $\pm$ 1.3 <sup>a</sup>  | 214.50 $\pm$ 25.4 <sup>a</sup>       | 15.06 $\pm$ 1.02 <sup>a</sup>  | 0.41 $\pm$ 0.070 <sup>ab</sup>    |
| 11            | 5              | 57.70 $\pm$ 2.6 <sup>a</sup>  | 28.60 $\pm$ 0.8 <sup>a</sup> | 255.5 $\pm$ 28.94 <sup>a</sup>  | 52.65 $\pm$ 2.80 <sup>a</sup> | 245.80 $\pm$ 32.3 <sup>a</sup>       | 15.50 $\pm$ 0.63 <sup>a</sup>  | 0.45 $\pm$ 0.070 <sup>a</sup>     |

a, b, c, d: within each column, differing superscripts indicate significant differences among dietary protein levels and ages ( $p < 0.05$ )

## 2.2 Histological Examination

Small pieces of the right testicular and epididymal tissue were fixed in 10% formalin solution, dehydrated in graded ethanol and embedded in paraffin wax (Denza and Sheehan, 1983). Sections (10µm) were cut in rotator microtome (Lecia, ARM 2235, Germany), stained with eosin and counter stained with hematoxylin (Luna, 1968). Histomorphometric analyses (Pease, 1964) of tubular and interstitial tissues were performed. Volume density (%), absolute (g), and relative (g/kg body weight) weights of testicular components were calculated according to Weibel (1979) procedure. Absolute and relative weights of certain testicular components were calculated by multiplying their volume density to testicular absolute and relative weights respectively. Weibel grid (Weibel, 1979) comprising 228 points was imposed on the screen of Visopan Projection Microscope (Reichert Visopan, Artarmon, NSW; 400 X) to calculate the number of points that fall on a particular component. Volume density of any component was calculated by dividing number of points on that component on total points in the reference area (228 points). The seminiferous tubule, spermatogonia, spermatocyte, spermatid, sperm, total spermatogenic cells, basement membrane, sertoli cells, and the lumen and tubule vacuoles were considered. By using the same screen microscope, the tubule and the lumen diameter and the germinal epithelial cell height were calculated by the examination of nearly round five tubules.

## 2.3 Statistical Analyses

General Linear Model (GLM) procedure of SAS (2005) was used to determine the factors (dietary protein level, age) affecting the studied traits. The statistical model for analysis of variance was:

$$Y_{ij} = \mu + P_i + A_j + PA_{ij} + e_{ij}$$

Where:  $Y_{ij}$  = dependent variable (testicular measurements, testicular seminiferous tubule components absolute and relative weights).  $\mu$  = overall mean.  $P_i$  = effect of dietary protein level ( $P = 12\%$  and  $16\%$ ).  $A_j$  = effect of age ( $A = 6, 7, 8, 9, 10$ , and  $11$  months).  $PA_{ij}$  = effect of interaction between  $i^{th}$  dietary protein level and  $j^{th}$  age.  $e_{ij}$  = Error term.

Duncan's multiple range test (Duncan, 1955) was used to compare differences among means. The

data was considered statistically significant when for all the statistical analyses performed when  $p < 0.05$ .

## 3. Results

### 3.1 Body Weight and Testicular Measurements

All the studied measurements are presented in Table 2. The body weight gain and testicular growth rate were increased ( $p < 0.05$ ) linearly between 6 to 11 months of age (Table 2) in the ram lambs. The rate of increase of testicular weight (497%) between 6 to 11 months of age was 3.4 times greater than that of the body weight (144%). This increase (345%) was attained by the ram lambs mostly during 6-9 months of age. A similar trend of increase was noted in the epididymal weight (555%) and the testicular volume (503%). Also an increase of 47% and 77% was recorded in the scrotal circumference and the testicular circumference respectively. The body weight and the testicular measurements were not altered significantly ( $p > 0.05$ ) by the dietary crude protein levels.

### 3.2 Testicular Histology

All the studied measurements of testicular histology are presented in Tables (3-5). However, all the testicular and the interstitial components in terms of volume density, absolute weight, and relative weight (g/kg) were not significantly ( $p > 0.05$ ) affected by the dietary crude protein levels (12% vs. 16%). The average of absolute and relative testicular weights of the seminiferous tubule component was  $148.3 \pm 20.6$ g and 3.2g/kg respectively (Tables 3 and 4). Furthermore, testicular growth rate increased dramatically between 180 and 270 days of age, and this increase accounted five folds in terms of absolute and relative weight (517% and 166 % respectively).

The weight of spermatogenic cells was highest (66.1g absolute weight and 1.4g/kg relative weight). The sertoli cells mass represented  $21.2 \pm 3.3$ g and 0.4g/kg absolute and relative weights, respectively (Tables 3 and 4). Excluding the data of basement membrane, all other components in term of absolute weight were affected significantly ( $p < 0.05$ ) by lamb's age.

**Table 3**

Absolute weight (g) of testicular seminiferous tubule components of Karadi ram lambs at different ages and fed two levels of dietary proteins (Mean  $\pm$  SE)

| Effect       | No. of Animals | Absolute testicular weight     | Spermato-gonia               | Spermato-cyte               | Spermatid                     | Sperm                       | Total sper-matogenic cells   | Sertoli cells                  | Vacuoles                      | Lumen                         | Basement membrane            | Total seminiferous tubules    |
|--------------|----------------|--------------------------------|------------------------------|-----------------------------|-------------------------------|-----------------------------|------------------------------|--------------------------------|-------------------------------|-------------------------------|------------------------------|-------------------------------|
| Overall mean | 22             | 148.3 $\pm$ 20.6               | 16.0 $\pm$ 1.9               | 16.2 $\pm$ 2.3              | 16.7 $\pm$ 3.1                | 17.1 $\pm$ 2.9              | 66.1 $\pm$ 9.8               | 21.2 $\pm$ 3.3                 | 0.7 $\pm$ 0.1                 | 9.8 $\pm$ 2.1                 | 12.0 $\pm$ 5.3               | 106.0 $\pm$ 15.1              |
| Protein      |                |                                |                              |                             |                               |                             |                              |                                |                               |                               |                              |                               |
| 12%          | 11             | 148.6 $\pm$ 30.8 <sup>a</sup>  | 15.7 $\pm$ 2.7 <sup>a</sup>  | 15.8 $\pm$ 2.9 <sup>a</sup> | 16.4 $\pm$ 4.2 <sup>a</sup>   | 15.6 $\pm$ 3.4 <sup>a</sup> | 63.7 $\pm$ 12.9 <sup>a</sup> | 21.5 $\pm$ 5.0 <sup>a</sup>    | 0.7 $\pm$ 0.2 <sup>a</sup>    | 8.5 $\pm$ 2.0 <sup>a</sup>    | 8.1 $\pm$ 2.2 <sup>a</sup>   | 103.5 $\pm$ 21.0 <sup>a</sup> |
| 16%          | 11             | 148.0 $\pm$ 28.9 <sup>a</sup>  | 16.2 $\pm$ 2.8 <sup>a</sup>  | 16.7 $\pm$ 3.7 <sup>a</sup> | 17.0 $\pm$ 4.7 <sup>a</sup>   | 18.5 $\pm$ 4.8 <sup>a</sup> | 68.6 $\pm$ 15.5 <sup>a</sup> | 20.9 $\pm$ 4.5 <sup>a</sup>    | 0.7 $\pm$ 0.2 <sup>a</sup>    | 11.1 $\pm$ 3.7 <sup>a</sup>   | 15.9 $\pm$ 10.5 <sup>a</sup> | 108.5 $\pm$ 22.9 <sup>a</sup> |
| Age (month)  |                |                                |                              |                             |                               |                             |                              |                                |                               |                               |                              |                               |
| 6            | 4              | 42.7 $\pm$ 21.1 <sup>c</sup>   | 7.3 $\pm$ 2.6 <sup>d</sup>   | 4.9 $\pm$ 2.3 <sup>b</sup>  | 2.9 $\pm$ 1.2 <sup>d</sup>    | 2.0 $\pm$ 1.0 <sup>b</sup>  | 17.3 $\pm$ 7.2 <sup>b</sup>  | 7.3 $\pm$ 3.5 <sup>b</sup>     | 0.1 $\pm$ 0.1 <sup>a</sup>    | 1.6 $\pm$ 1.1 <sup>b</sup>    | 3.1 $\pm$ 1.6 <sup>a</sup>   | 29.7 $\pm$ 13.4 <sup>b</sup>  |
| 7            | 4              | 72.7 $\pm$ 8.5 <sup>c</sup>    | 10.2 $\pm$ 0.9 <sup>dc</sup> | 7.1 $\pm$ 0.7 <sup>b</sup>  | 5.5 $\pm$ 1.8 <sup>dc</sup>   | 8.1 $\pm$ 1.4 <sup>b</sup>  | 31.0 $\pm$ 3.3 <sup>b</sup>  | 9.7 $\pm$ 1.9 <sup>b</sup>     | 0.2 $\pm$ 0.1 <sup>b</sup>    | 4.5 $\pm$ 1.8 <sup>b</sup>    | 3.9 $\pm$ 0.2 <sup>a</sup>   | 51.8 $\pm$ 6.5 <sup>b</sup>   |
| 8            | 3              | 108.7 $\pm$ 51.7 <sup>bc</sup> | 9.8 $\pm$ 3.7 <sup>dc</sup>  | 9.9 $\pm$ 5.2 <sup>b</sup>  | 10.4 $\pm$ 7.9 <sup>bdc</sup> | 10.2 $\pm$ 7.1 <sup>b</sup> | 40.8 $\pm$ 23.9 <sup>b</sup> | 16.2 $\pm$ 6.9 <sup>b</sup>    | 0.4 $\pm$ 0.2 <sup>ab</sup>   | 5.7 $\pm$ 3.1 <sup>b</sup>    | 5.2 $\pm$ 2.0 <sup>a</sup>   | 68.4 $\pm$ 35.9 <sup>b</sup>  |
| 9            | 4              | 210.7 $\pm$ 31.9 <sup>a</sup>  | 22.2 $\pm$ 4.3 <sup>ab</sup> | 27.1 $\pm$ 4.6 <sup>a</sup> | 25.6 $\pm$ 8.09 <sup>ab</sup> | 23.7 $\pm$ 5.0 <sup>a</sup> | 98.8 $\pm$ 21.6 <sup>a</sup> | 27.46 $\pm$ 8.37 <sup>ab</sup> | 1.58 $\pm$ 0.57 <sup>a</sup>  | 8.5 $\pm$ 4.5 <sup>ab</sup>   | 36.9 $\pm$ 27.9 <sup>a</sup> | 146.2 $\pm$ 27.4 <sup>a</sup> |
| 10           | 3              | 191.9 $\pm$ 0.5 <sup>ab</sup>  | 17.6 $\pm$ 1.7 <sup>bc</sup> | 21.3 $\pm$ 0.8 <sup>a</sup> | 21.0 $\pm$ 0.5 <sup>abc</sup> | 23.0 $\pm$ 3.6 <sup>a</sup> | 83.0 $\pm$ 4.7 <sup>a</sup>  | 27.51 $\pm$ 2.72 <sup>ab</sup> | 1.68 $\pm$ 0.48 <sup>a</sup>  | 18.83 $\pm$ 0.06 <sup>a</sup> | 8.7 $\pm$ 1.03 <sup>a</sup>  | 139.8 $\pm$ 10.8 <sup>a</sup> |
| 11           | 4              | 264.0 $\pm$ 35.7 <sup>a</sup>  | 27.5 $\pm$ 2.2 <sup>a</sup>  | 26.7 $\pm$ 2.4 <sup>a</sup> | 34.2 $\pm$ 4.5 <sup>a</sup>   | 35.2 $\pm$ 4.3 <sup>a</sup> | 123.7 $\pm$ 6.2 <sup>a</sup> | 39.38 $\pm$ 7.58 <sup>a</sup>  | 0.73 $\pm$ 0.46 <sup>ab</sup> | 20.74 $\pm$ 5.06 <sup>a</sup> | 11.8 $\pm$ 5.5 <sup>a</sup>  | 199.3 $\pm$ 14.3 <sup>a</sup> |

a, b, c, d: within each column, differing superscripts indicate significant differences among dietary protein levels and ages ( $p < 0.05$ )

**Table 4**

Relative weight (g/kg) of testicular seminiferous tubule components of Karadi ram lambs at different ages and fed two levels of dietary proteins (Mean  $\pm$  SE)

| Effect       | No. of Animals | Relative weight              | Spermato-gonia              | Spermato-cyte                | Spermatid                     | Sperm                         | Total sper-matogenic cells    | Sertoli cells                 | Vacuoles                       | Lumen                         | Basement membrane           | Total seminiferous tubules   |
|--------------|----------------|------------------------------|-----------------------------|------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|-----------------------------|------------------------------|
| Overall mean | 22             | 3.2 $\pm$ 0.3                | 0.3 $\pm$ 0.0               | 0.3 $\pm$ 0.0                | 0.3 $\pm$ 0.0                 | 0.3 $\pm$ 0.0                 | 1.4 $\pm$ 0.1                 | 0.4 $\pm$ 0.0                 | 0.01 $\pm$ 0.00                | 0.2 $\pm$ 0.0                 | 0.1 $\pm$ 0.0               | 2.3 $\pm$ 0.2                |
| Protein      |                |                              |                             |                              |                               |                               |                               |                               |                                |                               |                             |                              |
| 12%          | 11             | 3.4 $\pm$ 0.54 <sup>a</sup>  | 0.3 $\pm$ 0.05 <sup>a</sup> | 0.3 $\pm$ 0.04 <sup>a</sup>  | 0.3 $\pm$ 0.07 <sup>a</sup>   | 0.3 $\pm$ 0.06 <sup>a</sup>   | 1.4 $\pm$ 0.2 <sup>a</sup>    | 0.5 $\pm$ 0.09 <sup>a</sup>   | 0.01 $\pm$ 0.00 <sup>a</sup>   | 0.2 $\pm$ 0.0 <sup>a</sup>    | 0.1 $\pm$ 0.0 <sup>a</sup>  | 2.3 $\pm$ 0.3 <sup>a</sup>   |
| 16%          | 11             | 3.1 $\pm$ 0.5 <sup>a</sup>   | 0.3 $\pm$ 0.04 <sup>a</sup> | 0.3 $\pm$ 0.06 <sup>a</sup>  | 0.3 $\pm$ 0.08 <sup>a</sup>   | 0.3 $\pm$ 0.08 <sup>a</sup>   | 1.4 $\pm$ 0.27 <sup>a</sup>   | 0.4 $\pm$ 0.08 <sup>a</sup>   | 0.01 $\pm$ 0.00 <sup>a</sup>   | 0.2 $\pm$ 0.07 <sup>a</sup>   | 0.1 $\pm$ 0.0 <sup>a</sup>  | 2.2 $\pm$ 0.3 <sup>a</sup>   |
| Age (month)  |                |                              |                             |                              |                               |                               |                               |                               |                                |                               |                             |                              |
| 6            | 4              | 1.8 $\pm$ 0.9 <sup>b</sup>   | 0.3 $\pm$ 0.11 <sup>a</sup> | 0.20 $\pm$ 0.09 <sup>c</sup> | 0.12 $\pm$ 0.05 <sup>c</sup>  | 0.08 $\pm$ 0.04 <sup>c</sup>  | 0.7 $\pm$ 0.3 <sup>c</sup>    | 0.3 $\pm$ 0.1 <sup>ab</sup>   | 0.006 $\pm$ 0.00 <sup>b</sup>  | 0.07 $\pm$ 0.04 <sup>b</sup>  | 0.1 $\pm$ 0.0 <sup>a</sup>  | 1.2 $\pm$ 0.5 <sup>b</sup>   |
| 7            | 4              | 2.1 $\pm$ 0.3 <sup>b</sup>   | 0.2 $\pm$ 0.03 <sup>a</sup> | 0.2 $\pm$ 0.0 <sup>c</sup>   | 0.1 $\pm$ 0.04 <sup>bc</sup>  | 0.2 $\pm$ 0.05 <sup>bc</sup>  | 0.8 $\pm$ 0.10 <sup>bc</sup>  | 0.2 $\pm$ 0.05 <sup>b</sup>   | 0.00 $\pm$ 0.003 <sup>b</sup>  | 0.2 $\pm$ 0.04 <sup>ab</sup>  | 0.11 $\pm$ 0.0 <sup>a</sup> | 1.50 $\pm$ 0.2 <sup>b</sup>  |
| 8            | 3              | 2.9 $\pm$ 1.1 <sup>ab</sup>  | 0.2 $\pm$ 0.07 <sup>a</sup> | 0.2 $\pm$ 0.1 <sup>bc</sup>  | 0.2 $\pm$ 0.1 <sup>abc</sup>  | 0.26 $\pm$ 0.16 <sup>bc</sup> | 1.0 $\pm$ 0.50 <sup>bc</sup>  | 0.44 $\pm$ 0.14 <sup>ab</sup> | 0.01 $\pm$ 0.005 <sup>ab</sup> | 0.15 $\pm$ 0.06 <sup>ab</sup> | 0.14 $\pm$ 0.0 <sup>a</sup> | 1.8 $\pm$ 0.7 <sup>b</sup>   |
| 9            | 4              | 4.1 $\pm$ 0.1 <sup>ab</sup>  | 0.4 $\pm$ 0.09 <sup>a</sup> | 0.5 $\pm$ 0.09 <sup>a</sup>  | 0.5 $\pm$ 0.16 <sup>ab</sup>  | 0.4 $\pm$ 0.1 <sup>ab</sup>   | 1.9 $\pm$ 0.46 <sup>ab</sup>  | 0.5 $\pm$ 0.17 <sup>ab</sup>  | 0.03 $\pm$ 0.01 <sup>ab</sup>  | 0.15 $\pm$ 0.08 <sup>ab</sup> | 0.24 $\pm$ 0.0 <sup>a</sup> | 2.88 $\pm$ 0.5 <sup>ab</sup> |
| 10           | 3              | 3.9 $\pm$ 0.08 <sup>ab</sup> | 0.3 $\pm$ 0.04 <sup>a</sup> | 0.4 $\pm$ 0.0 <sup>abc</sup> | 0.4 $\pm$ 0.01 <sup>abc</sup> | 0.4 $\pm$ 0.08 <sup>ab</sup>  | 1.6 $\pm$ 0.13 <sup>abc</sup> | 0.5 $\pm$ 0.04 <sup>ab</sup>  | 0.03 $\pm$ 0.01 <sup>a</sup>   | 0.38 $\pm$ 0.12 <sup>a</sup>  | 0.17 $\pm$ 0.0 <sup>a</sup> | 2.8 $\pm$ 0.2 <sup>ab</sup>  |
| 11           | 4              | 4.80 $\pm$ 0.8 <sup>a</sup>  | 0.4 $\pm$ 0.05 <sup>a</sup> | 0.4 $\pm$ 0.0 <sup>ab</sup>  | 0.62 $\pm$ 0.1 <sup>a</sup>   | 0.6 $\pm$ 0.06 <sup>a</sup>   | 2.2 $\pm$ 0.20 <sup>a</sup>   | 0.7 $\pm$ 0.13 <sup>a</sup>   | 0.01 $\pm$ 0.00 <sup>ab</sup>  | 0.37 $\pm$ 0.09 <sup>a</sup>  | 0.2 $\pm$ 0.1 <sup>a</sup>  | 3.6 $\pm$ 0.4 <sup>a</sup>   |

a, b, c: within each column, differing superscripts indicate significant differences among dietary protein levels and ages ( $p < 0.05$ )

**Table 5**

Effect of dietary protein levels and age on seminiferous tubules diameter, epithelial height, and lumen diameter of Karadi Ram Lambs (Mean  $\pm$  S.E.)

| Effect        | No. of Animals | Seminiferous tubules diameter ( $\mu$ m) | Epithelial Cells height ( $\mu$ m) | Lumen diameter ( $\mu$ m)       |
|---------------|----------------|------------------------------------------|------------------------------------|---------------------------------|
| Overall mean  | 22             | 192.53 $\pm$ 12.23                       | 72.66 $\pm$ 5.40                   | 97.30 $\pm$ 7.07                |
| Protein level |                |                                          |                                    |                                 |
| 12%           | 11             | 194.85 $\pm$ 17.14 <sup>a</sup>          | 67.86 $\pm$ 8.70 <sup>a</sup>      | 99.94 $\pm$ 11.90 <sup>a</sup>  |
| 16%           | 11             | 190.20 $\pm$ 18.27 <sup>a</sup>          | 77.47 $\pm$ 21.51 <sup>a</sup>     | 94.67 $\pm$ 8.18 <sup>a</sup>   |
| Age (month)   |                |                                          |                                    |                                 |
| 6             | 4              | 120.65 $\pm$ 31.62 <sup>b</sup>          | 47.25 $\pm$ 6.51 <sup>a</sup>      | 61.47 $\pm$ 19.77 <sup>a</sup>  |
| 7             | 4              | 163.10 $\pm$ 11.12 <sup>ab</sup>         | 60.20 $\pm$ 11.77 <sup>b</sup>     | 94.20 $\pm$ 7.86 <sup>a</sup>   |
| 8             | 3              | 193.03 $\pm$ 42.37 <sup>ab</sup>         | 51.63 $\pm$ 12.29 <sup>b</sup>     | 105.80 $\pm$ 39.20 <sup>a</sup> |
| 9             | 4              | 227.90 $\pm$ 5.80 <sup>a</sup>           | 91.87 $\pm$ 2.58 <sup>a</sup>      | 110.00 $\pm$ 5.40 <sup>a</sup>  |
| 10            | 3              | 228.60 $\pm$ 8.48 <sup>a</sup>           | 98.86 $\pm$ 6.56 <sup>a</sup>      | 104.43 $\pm$ 4.93 <sup>a</sup>  |
| 11            | 4              | 231.05 $\pm$ 20.62 <sup>a</sup>          | 87.47 $\pm$ 9.56 <sup>a</sup>      | 111.85 $\pm$ 8.70 <sup>a</sup>  |

<sup>a, b</sup>: within each column, differing superscripts indicate significant differences among dietary protein levels and ages ( $p < 0.05$ ).

The greatest absolute weight of total seminiferous tubules, total spermatogenic cells, sertoli cells, vacuole, and lumen was recorded when the ram lambs were 11 months old. Whereas the lowest absolute weight of the total seminiferous tubule, total spermatogenic cells, vacuole, and lumen was recorded when the ram lambs were 6 months old. Similarly, relative weight of total seminiferous tubule and total spermatogenic cells were significantly ( $p < 0.05$ ) affected by lamb's age. The highest and lowest total seminiferous tubules and total spermatogenic cells were recorded in the ram lambs at the age of 11 months and 6 months respectively (Table 4).

A significant ( $p < 0.01$ ) two folds increase was noticed in the seminiferous tubule diameter (231.05 $\mu$ m vs. 120.65 $\mu$ m) and the epithelial cells height (87.47 $\mu$ m vs. 47.25 $\mu$ m) of the ram lambs when they were 11 months old as compared to those when the ram lambs were 6 months old (Table 5). This increase was expected, as seminiferous tissue is composed mostly of germ cells.

#### 4. Discussion

Dietary protein plays a major role in developing male characteristics, including the attainment of

body weight and onset of puberty. In Iraq, Madhat (1977) in his study did not find any significant effect of 25% dietary crude protein supplementation on testicular and epididymal weights of Awassi ram lambs. In contrast, Fernandez et al., (2004) observed that scrotal circumference and testicular volume of mature Assaf rams were lower for low protein diet than for medium and high protein diets. Similarly, Elmaz et al., (2007) pointed out that live body weight, testicular diameter, testicular circumference, testicular length, and testicular volume of ram lambs in high protein diet group (18% crude protein) were greater than those in low protein diet group (12% crude protein). However, the present data did not show any prominent effect of increased dietary crude protein of up to 16% on body weight, testicular measurements, and histology. The dietary crude protein source, breed, feeding period, and management practices may be the reasons behind different findings amongst the studies. Moreover, certain amount of protein intake will not have any additional benefit on testicular growth. The mechanism by which the dietary proteins affect testicular growth rate and body weight gain is well documented (Boukhliq, 1993; and Martin and Walkden-Brown, 1995). There are three types of responses to supplementary crude protein, the first of which is the stimulation of pulsatile

luteinizing hormone (LH) secretion. This response is expressed as a 2 to 3 fold increase in LH pulse frequency and can be obtained after a period as short as 2-3 days, but thereafter it declines and becomes undetectable after 3-4 weeks. The second response is that of follicle-stimulating hormone (FSH) which usually starts to be detectable 1-week to 10 days after supplementation has started and is maintained for several weeks. The third response, an increase in testicular size is rarely detectable before 2-weeks but continues for at least 5-weeks (Boukhliq, 1993). This suggests that the testes are capable of growing with minimal or absent gonadotrophic support. Such finding challenges our understanding that testicular growth is solely driven by gonadotrophins (Lincoln, 1979 and Jeffcoate et al., 1982) and indicates that somatotrophic and gonadotrophic axes may probably interact to regulate testicular growth. It is possible that under some circumstances, testicular growth could be more sensitive to changes in the somatotrophic axis and less sensitive to changes in the gonadotrophic axis. In other words, those factors that are associated with the regulation of somatic growth could exert more important and direct effect on the testis than gonadotrophins.

Compatible with the current results, Emsen (2005) found an increase (2-4 fold) in scrotal circumference (215%) and testicular volume (442%) between 1 and 360 days of age, higher than that of body weight (114%). The trend of testicular growth of Karadi ram lambs observed in this study was similar to that described by Al-Haboby et al., (1994) in Iraqi Awassi and Hamdani ram lambs. A rapid testicular growth and germinal epithelium division were noted around puberty in ram lambs concomitant with increased body weight. Such testicular growth could be due to the positive correlation with the mean and (or) baseline concentrations of LH and testosterone (Yarney and Sanford, 1990). A similar pattern of epididymal growth at 7-10 months was also described in Iraqi Awassi ram lambs (Al-Haboby et al., 1994). Increasing epididymal growth around this time may reflect the increase in testosterone production by Leydig cells.

This study is the first to describe the histomorphometric testicular changes of Karadi ram lambs. Moreover, this technique is considered a better tool to evaluate testicular components and to describe

spermatogenic process and is widely used to evaluate spermatogenesis in rams (Hochereau-de-Reviere et al., 1992). In ram, early pubertal changes are associated with changes in the hypothalamic-pituitary-testicular axis which drives changes in testicular function (Amann, 1983). Full testicular growth was accomplished around 270 days in relation to hormonal levels (Yarney and Sanford, 1989). Al-Haboby et al., (1997) indicated that testicular growth was accomplished by 150-200 days in Awassi ram lambs in his study.

In the present study, no sperm was observed in the epididymal ducts of slaughtered lambs at the age 6, 7, 8, and 9 months. However, the sperms were clearly observed in caudal epididymis of ram lambs at the age of 11 months, in spite of the presence of sperms in the lumen of the seminiferous tubule as early as 6 months of age. Hence, the completion of spermatogenic cycle is estimated to take 49-days in the ram, and the period for the transport of spermatozoa through the epididymis in the ram to take about 11-14 days (Ortavant, 1977). Therefore, such result is expected. The ram lambs which have spermatozoa in their epididymis when slaughtered, had therefore, by definition, attained physiological puberty which was approximately at the age of 10 months. Many factors may affect age at puberty in ram lambs, including breed (Belibasaki and Kouimtzi, 2000), nutrition (Brown, 1994) and lambing season (Foster et al., 1986). Previous study on Iraqi Awassi sheep, Madhat (1977) indicated that the chronological age at puberty occurs in the first half of 8 months of age while the physiological age at puberty occurs at 7 months of age. Moreover, Al-Hassan (2009) reported average puberty age to be 182.5-days and 195.4-days and average body weight to be 38.6kg and 47.2kg in Awassi and Hamdani ram lambs respectively. However, such a difference in the results between Iraqi sheep breeds is expected since a considerable evidence of genetic variation between and within breeds exist (Dyrmondsson, 1973). On the other hand, in Karadi ram lambs, no previous studies were conducted related to the effect of lambing season on age at puberty. However, some studies (Barwary, 2008) concerning seasonal variations in semen characteristics in adult Karadi ram lambs found that this breed is not seasonal as in case of many tropical and subtropical breeds. In the present study, all ram lambs were born in January

and February. Apparently, studies are needed to investigate the effect of season including photoperiod on age at puberty of this breed.

The testicular circumference could provide a useful estimate of testicular growth size since its correlations with other testicular measurements were highly significant. These results were in agreement with the findings of Celis et al., (1987); Foster et al., (1989); Salhab et al., (2001); and Alkass et al., (1987).

## 5. Conclusions

In conclusion, post-weaning, nutrition management with good dietary crude protein supplementation had a strong influence on lamb's weight gain, testicular growth, and consequently on the onset of

puberty in Karadi ram lambs, in our study. However, 12% dietary crude protein could be of economic impact for sheep producers in Iraq to improve their animal's reproductive performance. The suitability of scrotal size management as criterion for early selection of tropical ram lambs is emphasized. Further studies are warranted with more data concerning the effect of other protein sources on testicular development and ultra-structure in Karadi ram lambs.

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