

Some Productive, Reproductive and Physiological Effects of Using Different Dietary Protein Levels in Rabbit Does

Research Article

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ABSTRACT

Three groups of 11 New Zealand White (NZW) rabbit does, each were reared from 12 weeks of age on iso-energetic diets containing 16, 14 or 12% crude protein (CP) were used to study influences on productive performance, digestibility, some carcass traits, haematological, biochemical and hormonal parameters of rabbit does. The results showed rabbits fed on 16% protein diet had heavier body weights (BW) and body weight gains (WG) than the other groups. Daily feed intake (DFI) decreased and daily protein intake (DPI) increased by increasing dietary CP level whilst the feed conversion ratio (FCR) slightly improved as dietary CP increased. Dressing percentage increased slightly with increasing dietary protein level, while, rabbits fed 12% protein diet had a lower carcass weights compared to others. Moreover, the assessment results of mated does clarified significant increase in average daily or total FI and FCR, but significant decrease in kindling (%) in group fed 14% CP and in fertility and prolificacy% in 12 and 14% CP groups assessment of reproductive parameters, low protein levels had an adverse effect on litter size and kidney and liver weights of kids, while bunny weight decreased in the group fed 14% CP. However, plasma biochemical analysis showed a decrease in blood urea nitrogen (BUN) levels and activity of alanine-aminotransferase (ALT) and increase in creatinine in both two CP levels while no significant change in the concentration of total protein (TP), albumin (A), globulin (G), A/G ratio, calcium (Ca), phosphorous (Ph), iron (Ir) and aspartate-aminotransferase (AST) activity among three experimental groups was recorded. Furthermore, hormonal assay revealed a decrease in insulin.

KEY WORDS carcass, digestibility, performance, protein level, rabbit does, reproduction.

INTRODUCTION

Dietary protein level has a major impact on rabbit performance and was found to be age dependant. Therefore, an attempt should be directed to detect the exact levels of protein without lowering the does performance. The nutritional requirements for rabbits' various production functions (growth, gestation and lactation) are limited. Published reports on the requirement of rabbits for protein levels vary considerably. Some investigators reported that optimum

growth rate cannot be achieved when low-protein diets are fed to rabbits (Maertens *et al.* 1997; Parigi Bini *et al.* 1988; Yamani *et al.* 1991; Dias *et al.* 2000). However, other investigators concluded that increasing protein level in the rabbit diets improve productive efficiency (Abdel-Malak, 2000; Rohilla *et al.* 2002; Fu-chang *et al.* 2002). It is expected that great efforts will be directed to maximize the utilization of low-protein diet for rabbits. Several factors such as biological type, physical environmental conditions and nutrition are known to affect the reproductive perfor-

mance of rabbits (Robinson, 1996). Malnutrition was found to block growth, to delay puberty, to lower conception rate, to reduce ovarian follicular growth and finally the retardation of both maternal and fetal growth through fetal/maternal competition for growth (Mello and Cury, 1989; Armstrong *et al.* 2001). IGF-1 is considering one of the important nutritional markers as animal fed adequate nutrition have the highest concentration of serum IGF-1 while, fasting causes marked reduction in its concentration (Lucy *et al.* 1999; Brameld *et al.* 2000).

The objective of the present study was to investigate the response of rabbit does to different protein levels during pregnancy till obtaining two generations on the productive and reproductive performance and biochemical and hormonal changes in pregnant does.

MATERIALS AND METHODS

A total of 33 New Zealand White female rabbits (NZW) of about 3 months old were randomly distributed according to their body weight into three equal groups (11 rabbits each). Rabbits were housed in individual batteries provided with continuous feeders and automatic drinkers with nipples. All rabbits were raised on control diet (16% CP) for two weeks for adaptation, after that they were fed the tested diets prior to mating. Diets shown in Table 1 were formulated and pelleted to include three different crude protein levels (16, 14 and 12%). Experimental diets were formulated according to the rabbit requirements of NRC (1977), except the protein level, and fed to the female rabbits during pregnancy till two generations. Mating was assured after 2 successful trials and the day of mating was designated at first day of pregnancy (McNeilly and Friesen, 1978). All mated does were palpated 9 days post mating to determine pregnancy or to repeat mating in case of failure. Diets and fresh water offered ad-libitum for 6 months period and were kept under similar managerial and hygienic conditions. Rabbits were weighted bi-monthly intervals and the amount of feed intake was recorded to calculate WG and DEI and FCR values during the experimental period.

A digestibility trial was carried out using 6 rabbits from each treatment and individual metabolic cages that allow separating faeces and urine. The rabbits were subjected for a 7 consecutive day collection period for dry matter determination and chemical analysis.

Parameters measured for productive performance included:

1. Body weight (BW), weight gain (WG) and feed conversion ratio (FCR) were measured bi-monthly while, daily feed intake (DFI) and daily protein intake (DPI) were measured daily.

2. BW of each mated does in three groups was calculated weekly and FI was calculated during pregnancy according to Fathy (2000) to calculate: weight at mating (at the day of conception), weight at kindling (at 27th day of gestation), total BG and cumulative FI (during gestation period).

Parameters measured for reproductive parameters were: The percent of each conception, kindling, fertility, prolificacy and gestation period. In addition, litter size and weight (at birth) and the weights of both kidney and liver of kids at 7th day of age.

Blood samples were collected from marginal ear vein of does 3 times during pregnancy starting from the day of mating (9th, 18th and 27th) from each treatment into heparinized tubes to determine some biochemical and hormonal studies. The biochemical parameters included: total protein (TP), albumin (A), globulin (G), A/G ratio, glucose (Glu), alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), blood urea nitrogen (BUN) and creatinine (Cr). Also, calcium (Ca), Inorganic phosphorus (Ior-P) and iron (Fe) were measured using kits. Insulin like Growth Factor-1 (IGF-1) was measured using radioimmunoassay (RIA) kits according to Bistraw *et al.* (1990). At the line of slaughter, four rabbits from each treatment were randomly chosen for body weight and carcass analysis to determine some slaughter traits.

The complete chemical analysis of feed and feces were determined according to AOAC (1980) and the data were subjected to one way analysis of variance for productive performance, digestion, blood and carcass parameters. Two analysis of variance were used (protein levels and generation) for analyzed of kids parameters.

Categorical data including percent of conception, kindling, fertility and prolificacy parameters were analyzed using chi-square for counted data. Such statistical analysis was performed according to Snedecor and Cochran (1982) using SPSS program and significant differences were verified by Duncan's New Multiple Range test (Duncan, 1955).

RESULTS AND DISCUSSION

Productive performance parameters

Live body weight (LBW) and body weight gain (BWG)

Performance data of (NZW) rabbits as influenced by dietary protein levels are presented in Table 2. It is evident that rabbits fed 16% CP recorded significant ($P < 0.5$) higher value of LBW and BWG followed by those fed 14% CP, while those fed 12% CP gave the lowest values. Analysis of variance showed significant differences among treatment groups at all of the tested periods ($P < 0.5$). Moreover, no significant difference was detected for BW between group fed diet contained 16% CP and those fed 14% CP ($P > 0.5$). This may be attributed to the amount of CP

Table 1 Ingredients and chemical analysis of the experimental diets

| INGREDIENTS (%) | TREATMENTS | | |
|-------------------------------|-------------|-------------|-------------|
| | 16% Protein | 14% Protein | 12% Protein |
| Yellow corn | 25.00 | 33.00 | 36.50 |
| Soybean meal (44%) | 13.00 | 9.00 | 3.00 |
| Wheat bran | 23.00 | 21.00 | 24.00 |
| Clover hay | 25.00 | 19.00 | 19.50 |
| Clover straw | 11.00 | 15.00 | 14.00 |
| Di-Calcium phosphate | 2.00 | 2.00 | 2.00 |
| Sodium chloride | 0.50 | 0.50 | 0.50 |
| Vitamins and mineral premix* | 0.30 | 0.30 | 0.30 |
| DL-Methionine | 0.15 | 0.15 | 0.15 |
| Lysine | 0.05 | 0.05 | 0.05 |
| Total | 100.00 | 100.00 | 100.00 |
| Calculated analysis** | | | |
| Digestible energy (Kcal/kg) | 2585 | 2556 | 2551 |
| Crude protein (%) | 16.02 | 14.01 | 12.03 |
| Calcium (%) | 0.84 | 0.75 | 0.70 |
| Total phosphorus (%) | 0.87 | 0.83 | 0.84 |
| Lysine (%) | 0.61 | 0.51 | 0.35 |
| Methionine (%) | 0.30 | 0.28 | 0.26 |
| Chemical analysis on DM basis | | | |
| Dry matter (%) | 90.89 | 89.08 | 88.75 |
| Organic matter (%) | 89.81 | 89.43 | 89.12 |
| Crude protein (%) | 16.86 | 14.63 | 12.75 |
| Ether extract (%) | 2.80 | 2.84 | 3.04 |
| Crude fiber (%) | 13.57 | 13.09 | 12.39 |
| Ash (%) | 10.19 | 10.57 | 11.09 |
| Nitrogen free extract (%) | 56.58 | 58.87 | 60.73 |

*Vitamins and minerals premix per kilogram contains: Vit. A, 10,000 IU; Vit. D3, 900 IU; Vit. E, 50.0 mg; Vit. K, 2.0 mg; Vit. B1, 2.0 mg; Vit. B2, 6.0 mg; Vit. B6, 2.0 mg; Vit. B12, 0.01 mg; Biotin, 0.2 mg; Choline, 1200 mg; Niacin, 50 mg; Zinc, 70 mg; Cu., 0.1 mg; Mn., 8.5 mg; Fe., 75.0 mg; Folic acid, 5 mg and Pantothenic acid, 20.0 mg.

** According to NRC (1977) for rabbits.

Table 2 Body weight and body weight gain ($X \pm SE$) of rabbits as affected by dietary Protein levels*

| ITEMS | TREATMENTS | | |
|-------------------------|-------------------------------|-------------------------------|-------------------------------|
| | 16% Protein | 14% Protein | 12% Protein |
| Body weight (g/wk) | | | |
| 12 th wk | 2013 \pm 43.74 ^a | 2015 \pm 28.66 ^a | 1990 \pm 30.01 ^a |
| 20 th wk | 2490 \pm 36.82 ^a | 2395 \pm 30.49 ^b | 2350 \pm 23.36 ^b |
| 28 th wk | 2868 \pm 35.85 ^a | 2763 \pm 63.30 ^b | 2618 \pm 21.34 ^c |
| 36 th wk | 3168 \pm 40.60 ^a | 3090 \pm 33.59 ^a | 2900 \pm 21.32 ^b |
| Body weight gain (g/wk) | | | |
| 12-20 wk | 477 \pm 33.28 ^a | 379 \pm 14.62 ^b | 359 \pm 16.26 ^b |
| 21-28 wk | 377 \pm 23.71 ^a | 368 \pm 19.39 ^a | 268 \pm 6.82 ^b |
| 29-36 wk | 300 \pm 15.08 ^{ab} | 327 \pm 12.37 ^a | 281 \pm 8.98 ^b |
| 12-36 wk | 1154 \pm 36.59 ^a | 1075 \pm 24.07 ^b | 909 \pm 17.60 ^c |

The means within the same row for the same item that have at least one common letter, do not have significant difference ($P > 0.05$).

consumed (Table 3) since the protein is the most essential nutrient for *Abou Zeid et al. (1999)*, *Dias et al. (2000)*, *Zanaty and Aahmed (2000)*, *Ayyat et al. (2002)* and *Rohilla et al. (2002)*.

Feed intake (FI) and feed conversion ratio (FCR)

Regarding to FI and FCR, a significant increase ($P<0.05$) in daily feed intake but poorer FCR was observed in the group fed diet containing 12% CP than in the other two experimental groups (14 and 16% CP) (Table 3). Furthermore, dietary protein levels of 14 and 16% had nearly the same effect during the tested periods. It is observed that the highest protein intake was recorded with diets containing high level of protein (16%) and the lowest with that contained low level of protein. This may be attributed to the rabbits attempted to meet the requirements of protein. These results agree with those obtained by *El-Hindawy et al. (1993)*, *Abdel-Malak (2000)*, *Dias et al. (2000)* and *Ayyat et al. (2002)*. Generally, increasing dietary protein level improved the growth performance traits.

Digestibility Coefficient and feeding values

The results of digestibility coefficients and nutritive values are shown in Table 4. The results obtained revealed that rabbits fed diets containing higher level of protein (16% CP) showed increased ($P<0.05$) digestibility coefficient of dry matter (DM), crude protein (CP), ether extract (EE) and nitrogen free extract (NFE), while non-significant differences were detected in digestibility coefficients of organic matter (OM) and crude fiber (CF) ($P>0.5$). However, CP digestibility was gradually improved ($P<0.05$) due to increase protein level in the diets. This improvement was more augmented with the lower dietary protein levels (14 or 12%). These findings are in agreement with those of *Hemid and Tharwat (1995)*, *Zanaty et al. (1999)*, *Zanaty and Ahmed (2000)* and *Rohilla et al. (2002)*, while *Gad-Alla et al. (2002)* and *Radwan (2002)* reported that there were no significant differences in CF and OM digestibilities ($P>0.5$).

With respect to nutritive value (Table 4), it is noted that the highest values of total digestible nutrients (TDN), digestible crude protein (DCP) and digestible energy (Kcal/kg feed DM) were significantly ($P<0.05$) scored with high protein diet (16%) whereas, DCP was significantly ($P<0.05$) decreased with the decrease in dietary protein levels and in this case, it was similar to the findings of *Sankhyan et al. (1990)*. Also, *Hemid and Tharwat (1995)*, *El-Hady (2001)* and *Gad-Alla et al. (2002)* reached the same conclusion.

Carcass traits

Various carcass traits are presented in Table 5. It was reported earlier in this study that rabbits fed the high

protein level had significantly heavier body weights at the tested period. The same trend was observed where; the enhancement of carcass traits was related to an improved utilization of high protein diet. Carcass weight, dressed weight and dressing percentage were significantly increased ($P<0.05$) by the higher level of CP in the diet. Also, increasing dietary CP level from 14 to 16% improved the various carcass traits. Similar results were reported by *Zanaty et al. (1999)*. However, liver weight was not affected by dietary protein levels. The same results were reported by *Omole (1977)*, *Sankhyan et al. (1990)* and *Sonbol et al. (1992)* who reported that liver weight were not affected by CP levels. Therefore, using the mentioned protein level (16% CP) did not adversely affect neither dressing, carcass weight nor digestive system.

Concerning BW at mating (Table 6), the results of BW at mating, kindling, average daily gain (ADG) and BWG showed no significant change as compared to control ($P>0.05$) while DFI, cumulative FI and FCR showed significant increase ($P<0.05$) in groups fed 14% and 12% CP compared with the control. These results are in agreement with those reported in rabbits by *Yamani et al. (1991)* and *Badawy et al. (2002)*. In support to our finding, the reduction in WG by malnourished mothers occurred only during last two days of pregnancy (*Snoeck et al. 1990*). Also, significant increase ($P<0.05$) in DFI, cumulative FI and FCR in two groups fed low protein levels. This result agrees with those obtained by *Yamani et al. (1991)*, *Levy and Jackson (1993)* and *Tirapegui et al. (1996)*. Difference in FI among does seems to be related to FI capacity of individual does, which is dependent on body size (*Rommers et al. 2004*). Values of plasma TP, A, G and A/G ratio (Table 7) revealed non-significant changes between the two experimental groups and control ($P>0.5$). This result is closely associated with those previously recorded in rabbits by *Smith et al. (1994)* and *Weidel et al. (1994)*. *Tirapegui et al. (1996)* clarified that plasma TP and A were closely related to the protein content of the diet as well as feed intake, and their levels were associated with their synthesis by hepatic cells in relation to the concentration of serum amino acids. So, with reference to previous explanation, mild protein deficiency in our study may be a cause of this lack of change in protein profile.

Our result showed a significant decrease ($P<0.05$) in ALT activity in low protein fed groups through the three stages of pregnancy when compared to the control. This finding is supported by the observation of *Oprzade et al. (2000)* who suggested that the variability of liver enzymes activity is dependent on many environmental factors and physiological conditions. *Obatolu et al. (2003)* mentioned that ALT decreased in the case of feeding rats isocaloric protein free diet. Moreover, *Davidson (1994)* reported that, the function

Table 3 Feed intake, feed conversion and protein intake (X±SE) of rabbits as affected by dietary protein levels*

| ITEMS | TREATMENTS | | |
|-----------------------------|--------------------------|--------------------------|--------------------------|
| | 16% Protein | 14% Protein | 12% Protein |
| Daily feed intake (g/wk) | | | |
| 12-20 wk | 89.04±2.15 ^b | 92.52±2.20 ^b | 99.46±2.10 ^a |
| 21-28 wk | 100.28±1.70 ^c | 112.05±1.53 ^b | 116.95±1.40 ^a |
| 29-36 wk | 111.68±0.97 ^c | 121.29±1.86 ^b | 128.42±0.81 ^a |
| 12-36 wk | 100.38±1.14 ^c | 108.62±1.12 ^b | 114.94±1.09 ^a |
| Feed conversion ratio | | | |
| 12-20 wk | 11.65±0.69 ^c | 14.81±0.57 ^b | 16.75±0.61 ^a |
| 21-28 wk | 16.44±0.84 ^b | 18.71±0.89 ^b | 26.14±0.63 ^a |
| 29-36 wk | 22.88±1.13 ^b | 22.54±0.85 ^b | 27.39±0.68 ^a |
| 12-36 wk | 14.73±0.45 ^c | 17.05±0.36 ^b | 21.30±0.34 ^a |
| Daily protein intake (g/wk) | | | |
| 12-20 wk | 15.01±0.36 ^a | 13.54±0.32 ^b | 12.86±0.27 ^b |
| 21-28 wk | 16.91±0.29 ^a | 16.39±0.22 ^a | 14.91±0.18 ^b |
| 29-36 wk | 18.83±0.16 ^a | 17.74±0.27 ^b | 16.37±0.10 ^c |
| 12-36 wk | 16.92±0.19 ^a | 15.89±0.16 ^b | 14.66±0.13 ^c |

*The means within the same row for the same item that have at least one common letter, do not have significant difference (P>0.05).

Table 4 Digestion coefficients and nutritive value (X±SE) of rabbits as affected by dietary protein levels*

| ITEMS | TREATMENTS | | |
|---------------------------|-------------------------|--------------------------|-------------------------|
| | 16% Protein | 14% Protein | 12% Protein |
| Digestibility (%) | | | |
| Dry matter (%) | 65.63±0.68 ^a | 64.86±0.28 ^a | 59.01±0.56 ^b |
| Organic matter (%) | 64.61±1.23 ^a | 63.52±0.68 ^a | 61.54±1.09 ^a |
| Crude protein (%) | 71.26±0.65 ^a | 68.09±0.52 ^b | 59.28±1.21 ^c |
| Ether extract (%) | 71.21±0.90 ^a | 69.79±0.94 ^{ab} | 67.97±0.51 ^b |
| Crude fiber (%) | 29.17±0.34 ^a | 29.18±1.66 ^a | 31.65±0.84 ^a |
| Nitrogen free extract (%) | 68.64±0.37 ^a | 67.26±0.70 ^{ab} | 65.35±1.00 ^b |
| Nutritive value | | | |
| DE(kcal/kg feed DM) | 2621±9.83 ^a | 2546±16.25 ^b | 2427±22.89 ^c |
| DCP | 12.01±0.11 ^a | 9.97±0.07 ^b | 7.56±0.15 ^c |
| TDN | 59.29±0.22 ^a | 58.03±0.39 ^b | 55.87±0.53 ^c |

*The means within the same row for the same item that have at least one common letter, do not have significant difference (P>0.05).

Table 5 Carcass traits (X±SE) of rabbits as affected by dietary protein levels*

| ITEMS | TREATMENTS | | |
|----------------------|-------------------------|--------------------------|--------------------------|
| | 16% Protein | 14% Protein | 12% Protein |
| Live body weight (g) | 3150±86.60 ^a | 3083±72.65 ^a | 2633±101.38 ^b |
| Carcass weight (g) | 1755±73.93 ^a | 1655±46.78 ^a | 1415±60.05 ^b |
| Dressed weight (g) | 2046±79.60 ^a | 1930±48.81 ^a | 1654±60.48 ^b |
| Dressing (%) | 64.94±0.82 ^a | 62.62±0.47 ^{ab} | 62.85±0.48 ^b |
| Liver weight (g) | 87.62±0.66 ^a | 80.68±50.83 ^a | 73.37±6.00 ^a |
| Heart weight (g) | 9.63±0.22 ^a | 9.79±0.74 ^a | 6.39±0.47 ^b |
| Kidneys weight (g) | 22.9±1.29 ^a | 23.72±0.88 ^a | 18.28±1.00 ^b |
| Spleen weight (g) | 2.23±0.06 ^a | 2.09±0.30 ^{ab} | 1.47±0.20 ^b |
| Digestive system (g) | | | |
| full | 313±7.26 ^a | 331±9.94 ^a | 320±5.77 ^a |
| empty | 113±3.33 ^c | 138±7.26 ^b | 170±5.77 ^a |

*The means within the same row for the same item that have at least one common letter, do not have significant difference (P>0.05).

Table 6 Protein profile and glucose level of New Zealand female rabbits fed on different dietary protein levels during various stages of pregnancy*

| parameters | Proteins (g/dL) | | | | | | | | | Glucose (mg/dL) | | | | | |
|------------------------------------|-----------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|-----------------|---------------|---------------|------------------------------|--------------------------------|------------------------------|
| | Total | | | Albumin | | | T. Globulins | | | A:G ratio | | | | | |
| | 16% | 14% | 12% | 16% | 14% | 12% | 16% | 14% | 12% | 16% | 14% | 12% | 16% | 14% | 12% |
| 1 st stage of pregnancy | 7.95 ±0.38 | 8.02 ±0.20 | 7.84 ±0.28 | 4.68 ±0.16 | 4.62 ±0.18 | 4.50 ±0.30 | 3.30 ±0.36 | 3.37 ±0.15 | 3.26 ±0.32 | 1.37 ±0.20 | 1.33 ±0.10 | 1.35 ±0.22 | 126.40 ±9.40 ^a | 123.20 ±9.29 ^{ab} | 114.10 ±8.71 ^b |
| 2 nd stage of pregnancy | 8.01 ±0.26 | 7.78 ±0.25 | 7.87 ±0.66 | 4.70 ±0.21 | 4.59 ±0.37 | 4.62 ±0.20 | 3.31 ±0.35 | 3.30 ±0.32 | 3.31 ±0.51 | 1.38 ±0.20 | 1.37 ±0.23 | 1.35 ±0.22 | 126.40 ±6.18 ^a | 116.80 ±8.28 ^b | 109.30 ±3.68 ^c |
| 3 rd stage of pregnancy | 7.31 ±0.41 | 7.49 ±0.58 | 7.34 ±0.72 | 4.53 ±0.24 | 4.56 ±0.30 | 4.44 ±0.25 | 2.78 ±0.25 | 2.81 ±0.41 | 2.80 ±0.34 | 1.60 ±0.16 | 1.55 ±0.28 | 1.55 ±0.31 | 118.50± 6.05 ^a | 113.60 ±10.67 ^{ab} | 107.50 ±5.54 ^b |
| Overall mean | 7.75 ±0.47 | 7.76 ±0.43 | 7.68 ±0.62 | 4.63 ±0.21 | 4.58 ±0.29 | 4.52 ±0.25 | 3.19 ±0.40 | 3.17 ±0.38 | 3.12 ±0.45 | 1.45 ±0.21 | 1.41 ±0.23 | 1.41 ±0.25 | 123.76 ±8.06 ^a | 117.86 ±9.99 ^b | 110.30 ±6.73 ^c |

Values represent Means±SD 16%, 14%, 12% are the protein levels (Group fed on 16% protein level is considered as control).

*The means within the same row for the same item that have at least one common letter, do not have significant difference (P>0.05).

Table 7 Activities of transferases (ALT and AST) and some renal function parameters of New Zealand female rabbits fed on different dietary protein levels during various stages of pregnancy*

| parameters | ALT (IU/L) | | | AST (IU/L) | | | Creatinine (mg/dL) | | | BUN (mg/dL) | | |
|------------------------------------|------------------------------|------------------------------|------------------------------|-----------------|-----------------|-----------------|-----------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|------------------------------|
| | 16% | 14% | 12% | 16% | 14% | 12% | 16% | 14% | 12% | 16% | 14% | 12% |
| 1 st stage of pregnancy | 59.80 ±9.43 ^a | 40.90 ±11.15 ^b | 28.80 ±7.80 ^c | 39.77 ±12.74 | 30.20 ±7.56 | 36.20 ±8.45 | 0.89 ±0.009 ^a | 1.23 ±0.15 ^b | 1.31 ±0.14 ^b | 19.67 ±1.41 ^a | 17.38 ±3.27 ^b | 18.43 ±1.94 ^{ab} |
| 2 nd stage of pregnancy | 59.30 ±17.62 ^a | 35.70 ±4.78 ^b | 40.00 ±8.57 ^b | 36.17 ±3.73 | 39.70 ±5.01 | 36.08 ±7.48 | 0.91 ±0.17 ^a | 0.94 ±0.12 ^a | 1.12 ±0.002 ^b | 17.31 ±1.40 ^a | 15.46 ±3.08 ^b | 14.45 ±0.55 ^b |
| 3 rd stage of pregnancy | 52.30 ±12.32 ^a | 40.40 ±10.14 ^b | 34.90 ±11.04 ^b | 46.30 ±19.75 | 52.10 ±16.03 | 43.58 ±10.30 | 0.97 ±0.20 ^a | 0.93 ±1.75 ^a | 1.16 ±0.20 ^b | 16.75 ±2.16 | 16.34 ±1.65 | 18.03 ±1.28 |
| Overall mean | 57.13 ±13.54 ^a | 39.00 ±9.13 ^b | 34.56 ±10.6 ^c | 40.74 ±13.93 | 40.66 ±13.73 | 38.62 ±9.23 | 0.928 ±0.16 ^a | 1.03 ±0.02 ^b | 1.20 ±0.16 ^b | 17.91 ±2.08 ^a | 16.39 ±2.78 ^b | 16.97 ±2.25 ^b |

Values represent Means±SD 16%, 14%, 12% are the protein levels (Group fed on 16% protein level is considered as control).

*The means within the same row for the same item that have at least one common letter, do not have significant difference (P>0.05).

of ALT enzyme is to transfer amino group from amino acid to synthesis another one and to play an important role in gluconeogenesis. Meanwhile, [Olbrich *et al.* \(1972\)](#) recorded that an increase in ALT is a response to an increase need for gluconeogenesis. In our result decreased ALT activity, followed the process of gluconeogenesis, maybe due to the protein deficiency.

However, no alteration was detected in the activity of AST in two low protein fed groups and this is in agreement with [Zaleska and Priebe \(1991\)](#) and [Oprzade *et al.* \(2000\)](#). An opposite result was recorded by [Castro *et al.* \(1993\)](#) and [Etukudo *et al.* \(1999\)](#) as animals fed low protein and energy diet showed fatty liver or liver steatosis and may be accompanied by fibrosis with significant increase in ALT and AST activities (Table 7).

The level of serum creatinine is known to be elevated linearly with a decline in serum BUN when feed is not available ([Nelson *et al.* 1984](#)). This finding is in agreement with our result as there was a significant increase ($P < 0.05$) in creatinine concentration during the gestation period of the two experimental groups compared to the control. Similar result was achieved by [Valtonen *et al.* \(1982\)](#) and [Wolkers *et al.* \(1994\)](#).

[Manning *et al.* \(1994\)](#) showed that BUN depends on the amount of protein in diet. Our result showed a significant decrease in BUN concentration during pregnancy period accompanied with decrease of feed crude protein in the diet, but this decrease was in fluctuating manner and seemed to behave rather similar to the control.

In the current study, the concentrations of calcium, phosphorus and iron was not affected by low protein diets (Table 8). These results are in agreement with those obtained by [Gangadevi and Kunjikutty \(1984\)](#), [Chiericato *et al.* \(1985\)](#), [Orwoll *et al.* \(1992\)](#) and [Barone *et al.* \(1998\)](#). It is well known that hypocalcaemia occurring in severe protein malnutrition reflected a reduction in the protein bound fraction of serum calcium ([Manning *et al.* 1994](#); [Frenk *et al.* 2000](#)). With reference to previous explanation, our result revealed non-significant changes in calcium, total protein and albumin levels.

Results of plasma insulin concentration (Table 8) revealed that low protein diet (12% CP) showed significant decrease ($P < 0.05$) in insulin concentration through three stages of pregnancy when compared to the control. This result is in agreement with that reported by [Usami *et al.* \(1982\)](#) and [Chew *et al.* \(1984\)](#). The exact cause of decrease insulin output remains to be clearly elucidated, in which carbohydrate intolerance was attributed to insulin lack. Moreover, hepatic dysfunction and decrease glucose disposal consequent to protein deprivation may be the causes of this decrease ([Khardori *et al.* 1980](#)). Malnutrition that leads to low concentration of insulin suggests that a

neural or hormonal system was activated in the early stage of under nutrition which potentiate the decrease in central neural drive to the reproductive axis ([Cameron, 1996](#)). Also, feeding low protein may exercise its effect on the ovary indirectly via IGF-1 ([Monget and Martin, 1997](#)).

Regarding to the result of maternal IGF-1 concentration, the groups fed low protein diet showed non-significant change in plasma IGF-1 concentration. In the light of our information, IGF-1 levels are relatively stable; the stability was due to its constitutive pattern of secretion and its high affinity to binding protein which prolonged the half-life and titrate the supply of this hormone to its receptors ([McCusker and Clemmons, 1992](#); [Baxter, 1993](#)). Our result is consistent with that achieved by [Armstrong *et al.* \(2001\)](#) who noticed that plasma IGF-1 was unaffected by the protein concentration in the diet. Meantime, [Tirapegui *et al.* \(1996\)](#) clarified that serum concentration of IGF-1 was correlated with protein synthesis and content in the diet as well as feed intake. In this respect, there was a linear increase in serum IGF-1 as protein intake increased. In conclusion, short term of dietary protein deficiency had no great effect on some blood parameters (protein profile, AST activity, mineral profile and IGF-1).

Results of reproductive parameters revealed that percentage kindling and fertility had significant decrease ($P < 0.05$) in the two tested groups versus control.

Assessing of rabbit reproductive indices, the results showed that the conception (%) and gestation period made no significant changes between the low protein groups and the control (Table 9). This result was coincided with those reported by [Raharjo *et al.* \(1986\)](#) and [Yamani *et al.* \(1991\)](#).

[Omole \(1982\)](#) found that gestation period of does fed 18% CP diet averaged 31.2 day while high values recorded 33.7 day when dietary CP reduced to 10%. However, at 1st generation low protein level recorded adverse effect on kindling%, fertility% and prolificacy%, while an improvement was found in 2nd one. With reference to [Anthony *et al.* \(1986\)](#) and [Badaway *et al.* \(2002\)](#), low dietary protein levels were insufficient to maintain normal reproductive performance. In this respect, there were significant positive relationships between embryo quality, feeding level and maternal blood glucose concentration at the time of ovulation ([McKelvey and Robinson, 1988](#)). Generally, protein deficiency diet may have adverse effect on some reproductive patterns even when high FI was noticed (kindling, fertility and prolificacy percentage).

Regarding some productive parameters of kids at 7th day of age (Table 10), the results of either litter size or bunny weight showed significant decrease ($P < 0.05$) for both 14% and 12% CP fed groups; meantime, there was no significant effect of generation on litter size ($P > 0.5$) but bunny weight was decreased significantly ($P < 0.05$) in the 2nd generation

Table 8 Minerals profile of New Zealand female rabbits fed on different dietary protein levels during various stages of pregnancy*

| parameters | Calcium (mg/dL) | | | Ior. Phosphorus (mg/dL) | | | Iron (mg/L) | | |
|------------------------------------|-----------------|------------|------------|-------------------------|-----------|-----------|-------------|-----------|-----------|
| | 16% | 14% | 12% | 16% | 14% | 12% | 16% | 14% | 12% |
| 1 st stage of pregnancy | 11.36±0.49 | 10.70±0.70 | 10.89±1.01 | 4.09±0.87 | 4.64±0.48 | 4.52±0.5 | 2.61±0.71 | 2.22±0.84 | 2.05±0.60 |
| 2 nd stage of pregnancy | 11.23±0.51 | 10.60±0.33 | 10.73±0.51 | 4.51±0.56 | 4.19±0.52 | 4.42±0.84 | 2.34±0.39 | 2.32±0.48 | 2.20±0.77 |
| 3 rd stage of pregnancy | 10.56±0.95 | 10.52±0.49 | 10.87±0.63 | 3.96±0.55 | 4.36±0.70 | 4.39±0.69 | 2.32±0.69 | 2.32±0.19 | 2.03±0.76 |
| Overall mean | 11.01±0.73 | 10.64±0.53 | 10.83±0.72 | 4.18±0.70 | 4.39±0.59 | 4.44±0.68 | 2.42±0.60 | 2.28±0.59 | 2.12±0.70 |

Values represent Means±SD 16%, 14%, 12% are the protein levels (Group fed on 16% protein level is considered as control).

*The means within the same row for the same item that have at least one common letter, do not have significant difference (P>0.05).

Table 9 Some hormonal assays of New Zealand female rabbits fed on different dietary protein levels during various stages of pregnancy*

| parameters | Insulin (µIU/mL) | | | IGF-1 (mg/mL) | | |
|------------------------------------|-------------------------|-------------------------|-------------------------|---------------|--------------|--------------|
| | 16% | 14% | 12% | 16% | 14% | 12% |
| 1 st stage of pregnancy | 20.00±3.26 ^a | 19.00±5.38 ^a | 11.54±4.72 ^b | 294.60±27.06 | 294.20±29.57 | 333.40±30.13 |
| 2 nd stage of pregnancy | 18.32±3.34 ^a | 18.10±4.82 ^a | 11.55±4.00 ^b | 294.00±59.35 | 290.00±51.06 | 352.00±45.56 |
| 3 rd stage of pregnancy | 15.00±2.21 ^a | 17.45±2.00 ^a | 9.06±2.21 ^b | 421.60±52.82 | 353.20±21.32 | 402.00±50.21 |
| Overall mean | 17.80±2.73 ^a | 18.99±4.57 ^a | 10.71±3.98 ^b | 336.72±76.65 | 319.13±51.91 | 355.84±47.85 |

Values represent Means±SD 16%, 14%, 12% are the protein levels (Group fed on 16% protein level is considered as control).

*The means within the same row for the same item that have at least one common letter, do not have significant difference (P>0.05).

compared to that of the 1st one. Also, both kidney and liver weights of kids followed the same trend for bunny weight.

The same result was achieved by Ibrahim (1985) and Yamani *et al.* (1991) who reported that litter size at birth was increased in non significant manner from 6.28 kids at 1st generation to 6.59 kids at 3rd parity. Also, the results showed that both low dietary protein levels and generation significantly affected (P<0.05) the bunny, kidney and liver weights at 7th day of age in groups fed 14% and 12% CP. Similar results were reported in rabbits by Rashwan (1990), in rats by Sharasgard *et al.* (2001) and Joshi *et al.* (2003). In our results, some studies explained that maternal hypoglycaemia following malnutrition accounts for the reduced glycogen concentration and consequently lowering serum glucose level in their offspring. In this respect, long term maternal hypoglycaemia reduced fetal glucose supply, eventually resulted in fetal growth retardation (Grup-Puso *et al.* 1981; Edward and McMillen, 2002). In general, kids born to dams fed low protein diet showed a negative effect on their litter size, bunny, liver and kidney weights. It must be considered on CP levels in diet formulated according to the National Research Council of the rabbit requirement.

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