

Health status and blood parameters of weaner rabbits fed diets containing varying dietary fibre and digestible energy levels

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Abstract

The maintenance of a good health status in rabbits is hinged upon appropriate balance between dietary fibre and energy level. A 70-day feeding trial was conducted to study the health status and blood parameters of weaner rabbits fed diets containing varying fibre and digestible energy (DE) using a total of ninety 4-weeks-old rabbits. There were 9 dietary treatments laid out in a 3 × 3 factorial arrangement of 3 levels of dietary fibre [low (249-258 g/kg NDF and 149-157 g/kg ADF), optimum (349-381 g/kg NDF and 188-193 g/kg ADF) and high (430-456 g/kg NDF and 249-253 g/kg)] and digestible energy levels [low (8-8.5 MJ/kg), optimum (10.5-11 MJ/kg) and high (12-12.30 MJ/kg)]. Each of the diet was fed to 10 rabbits individually caged in a completely randomized design. Polynomial contrasts were done for linear and quadratic effects. Results indicated that rabbits fed low fibre diets showed high incidence of transitory diarrhoea, total morbidity and symptoms of other sickness. Interaction effect (fibre × digestible energy) indicated that irrespective of the digestible energy level of the diet, mortality, tendency to go off feed and incidence of transitory diarrhoea increased as dietary fibre level increased. Rabbits fed low fibre diets had the least packed cell volume (36.00%) and highest serum uric acid concentration (51.22 mg/dl). Rabbits fed diet containing high fibre + optimum DE recorded the highest packed cell volume (44.00%) and serum glutamate oxaloacetate transaminase (29.00 IU/L). Highest haemoglobin (16.00 g/dl), total serum protein (66.00 g/L) and serum cholesterol concentration (165.00 mg/dl) were recorded with rabbits fed diet containing optimum fibre + optimum DE. It was concluded that feeding low fibre diets increased the susceptibility of rabbits to transitory diarrhoea, morbidity and tendency to go off feed. High fibre diets in rabbit nutrition thus favoured improved health status.

Keywords: Health status, Blood parameters, Weaner rabbits, Dietary fibre, Digestible energy

1.0 Introduction

Fibrous feedstuffs and agro-industrial by-products have been used extensively in rabbit nutrition by small scale farmers (Garcia *et al.*, 1999). However, under commercial production of rabbits, finished or concentrate feeding are usually preferred. Rabbits raised solely on concentrate diets (typically low in fibre but high in energy) were reported to have incidence of non-specific enteritis, loss of appetite (Bennegadia *et al.*, 2001) and sometimes diarrhoea (Gidenne *et al.*, 2000). On the other hand, excessive intake of fibrous feedstuffs has been implicated in health problems such as impaired digestion and caecal impaction (Pinheiro and Gidenne, 1999). The maintenance of a good health status in rabbits is therefore hinged upon appropriate balance between dietary fibre and energy level.

Variations in dietary components have measurable effects on blood parameters of animals (Church *et al.*, 1984). Blood examination also played a vital role in assessing the nutritional (Odunsi, 1991), physiological and pathological status of an animal (Schalm *et al.*, 1975). Blood examination is also used to monitor and evaluate disease prognosis of animals (Karesh and Cook, 1995). Normal range of blood parameters in healthy growing rabbits have been reported in literature (Ewuola and Egbunike, 2008). This research work was conducted to study the health status and determine the blood parameters of weaner rabbits fed diets containing varying dietary fibre and digestible energy.

2.0 Materials and Methods

2.1 Experimental design and diets

Nine experimental diets with three levels of dietary fibre (low, optimum and high), and in each case with three

levels of digestible energy (low, medium and high DE), were formulated in a 3×3 factorial arrangement (Table 1). Levels of dietary fibre used were low (249 – 258 g/kg NDF and 149-157 g/kg ADF), optimum (349 – 381 g/kg NDF and 188-193 g/kg ADF) and high (430 – 456 g/kg NDF and 249-253 g/kg ADF) following previous reports (Gidenne *et al.*, 2004; Chao and Li, 2008; Pinheiro *et al.*, 2009). Digestible energy levels used were low (8 – 8.5 MJ/kg), optimum (10.5 – 11 MJ/kg) and high (12 – 12.30 MJ/kg), respectively (INRA, 1989; Lebas, 1991). The diets were given in mash form and *ad libitum* throughout the trial.

2.2 Animal and health status

A total of 90 four-week old mixed-breed rabbits (New Zealand white \times Chinchilla) were used for this trial. Rabbits were housed individually in cage units (each measuring 40 mm \times 50 mm \times 45 mm). The feeding trial lasted 70 days. Rabbits were randomly allotted to dietary treatments such that there were 10 rabbits assigned to a treatment. Health status was monitored. Indices of health status used included mortality and morbidity (which might include diarrhoea, presence of mucus in excreta samples, low or no feed intake and other disease conditions) expressed as percentage.

2.3 Collection of blood samples

At the end of the feeding trial, five rabbits were selected at random from each treatment and used for blood sample collection. The rabbits were bled through the ear vein and the blood collected into vacutainer tubes for each animal. Two sets of blood samples (2.5 ml each) were collected from each rabbit into separate ethylene diamine tetra acetate (EDTA) bottles and heparinised tubes.

2.4 Haematological indices and serum biochemistry

Haemoglobin concentration (Hb) was measured in fresh EDTA anti-coagulant samples using the Sahl's (acid haematin) method (Benjamin, 1978). The packed cell volume (PCV) and red blood cell (RBC) were determined using the improved Neubauer haemocytometer as described by Baker and Silverton (1985). White blood cell (WBC) was measured using the blood smears (Tavares-Dias *et al.*, 2008).

Total serum protein (Kohn and Allen, 1995), serum albumin (Peter *et al.*, 1982), serum creatinine (Bousnes and Taussky, 1945) and serum uric acid (Wootton, 1964) were determined according to standard procedures. Serum glutamate pyruvate transaminase and serum glutamate oxalo-transaminase were analysed using commercially available diagnostic kits (Randox® Test Kits). Alkaline phosphatase (ALP) activity was measured colorimetrically with absorbance measurement at 405 nm using specific commercial clinical diagnostics kits (ELItech, France). The serum cholesterol was estimated using the commercial diagnostic kits (Qualigens India. Pvt. Ltd., Catalogue number 72201-04).

2.5 Statistical Analysis

Data obtained were laid out in a 3×3 factorial arrangement of treatments having three dietary fibre and three digestible energy levels. Data obtained were analysed by the general linear model of SPSS (1997). Analysis was done to determine separately the main effects of dietary fibre, digestible energy levels and their interaction. Polynomial contrast (linear and quadratic) was applied to determine the main effect of dietary fibre and digestible energy levels. A probability of $P < 0.05$ was considered to be statistically significant.

3.0 Results

3.1 Health status

Main effect of varying fibre and digestible energy on health status of growing rabbits is as showed in Table 2. Mortality of rabbits and tendency to go off feed reduced (L.Q. $P < 0.05$) with increasing level of dietary fibre. Increased (L.Q. $P < 0.05$) incidence of transitory diarrhoea and other signs of sickness were noticed with rabbits fed with low fibre diet. Rabbits fed high DE diets recorded the least mortality (1.07%) and tendency to go off feed (2.22%).

Interaction effect of DE and fibre level as shown in Table 3 revealed that rabbits fed low fibre diets irrespective of the DE level resulted in increased mortality, incidence of transitory diarrhoea, tendency to go off feed and highest total morbidity. Highest transitory diarrhoea (15.4%) and total morbidity (30.80%) were noticed with rabbits fed low fibre + low energy diets.

3.2 Haematological indices and serum metabolites

Main effect of varying dietary fibre and digestible energy on haematological indices and serum metabolites of

rabbits is shown in Table 4. Rabbits fed diets containing optimum fibre had the highest PCV (42.00%) and WBC ($7.63 \times 10^3/L$) count. Digestible energy level of the diet showed no influence ($P > 0.05$) on Hb, RBC and WBC values. Rabbit fed with optimum DE level recorded the highest PCV (43.33%). Rabbit fed with low fibre diets had the lowest total serum protein (50.67 g/L) and serum globulin (10.67 g/L). Highest serum uric acid concentration was obtained with rabbits fed with low fibre diets. Rabbits fed with optimum fibre recorded the highest cholesterol concentration (115.3 mg/dl).

Interaction effect of DE and fibre level on haematological indices and serum metabolites of rabbits is shown in Table 5. Rabbits fed with high fibre + optimum DE diet had the highest PCV (44.00%) while rabbits fed with low fibre + low DE diet recorded the least PCV (33.00%) and Hb (11.00 g/dl). Rabbits fed with optimum fibre + optimum DE had the highest total serum protein (66.00 g/L) and serum cholesterol (165.00 mg/dl).

4.0 Discussion

High mortality obtained with rabbits fed low fibre diets agreed with previous findings that a fibre below 300 g/kg NDF in rabbits increases mortality (Nicodemus *et al.*, 2004). High incidence of transitory diarrhoea and total morbidity obtained in this study with rabbits fed low fibre + low DE diets could be implicative of digestive disturbances in the gut (Bennegadia *et al.*, 2001). This agreed with Bennegadi-Laurent *et al.* (2004) who reported that dietary fibre deficiency favored the occurrence of digestive problems in the growing rabbit. Similar health problems have been reported in rabbits fed with low fibre diets (Gidenne *et al.*, 2000; Bennagadia *et al.*, 2001). Dietary fibre regulates intestinal transit, gut microflora, intestinal mucosa integrity and digestive health of rabbits (Fortun-Lamothe and Boullier, 2007). The findings of this study agreed with Gidenne and Licois (2005) who reported that immunity of the growing rabbit to a specific enteropathy was improved by a high fibre intake.

The Hb and PCV values obtained in the current study lied within the normal range (9-15g/dL and 35-45%) for healthy growing rabbits (Anon 1980; Fraser and Mays, 1986). This implied that the dietary treatment fed was capable of supporting high oxygen carrying capacity of the blood. High dietary fibre intake favoured increased PCV while rabbits fed with low fibre + low DE showed low PCV and Hb concentration. Anaemia has been diagnosed when there is a fall in PCV values of the blood (Fraser and Mays, 1986). Low fibre intake in this study therefore increased the susceptibility of the rabbits to anaemia.

Rabbit fed with optimum fibre diets irrespective of the level of digestible energy resulted in white blood cell values which lied within the normal range of $6 \times 10^3/L$ - $13 \times 10^3/L$ reported by Fraser and Mays (1986). This is implicative of well-developed immune system with such number of immune cells capable of performing their phagocytic action and proffer good health. The RBC values obtained in the current study showed adequacy of dietary protein fed to the rabbits since their values fell within the normal range of $5 \times 10^3/L$ - $8 \times 10^6/L$ reported by Anon (1980). Low values of RBC have been implicated in situations of inadequate dietary protein (Harper, 1971).

The total serum protein values obtained for rabbits fed diet containing optimum fibre + optimum DE, optimum fibre + high DE and high fibre + optimum DE showed values within the normal range (58-67.5 g/L) reported in literatures (Anon, 1980; Onifade, 1993). Lower serum protein values below normal recommendations were reported to be indications of hypoproteinemia (Altman, 1979) and alteration in protein metabolism (Iyayi and Tewe, 1998). The concentration of total serum protein at any given time is a function of the nutritional status, water balance and state of health of the animal (Jain, 1986). Serum globulin concentration of the rabbits also followed similar trend with total serum protein values. Values obtained for serum albumin in the current study lied within the normal range reported for healthy rabbits (Anon, 1980).

Rabbits fed diets containing low fibre + optimum DE, optimum fibre + optimum DE, optimum fibre + high DE, optimum fibre + optimum DE and high fibre + optimum DE showed high serum cholesterol concentration values than the normal range reported by Anon (1982). This implied good nutritional status and health. Low serum cholesterol levels have been reported under conditions of protein and energy malnutrition (Madden and Whipple 1940). Animal fed poor plane of nutrition metabolize their fat and free fatty acids to meet their energy requirements resulting in high turnover of cholesterol and a resultant fall on plasma cholesterol levels. Serum enzyme values obtained in this study also lied within the normal range (Anon, 1980). The fact that serum creatinine concentration were not influenced by dietary treatment showed that there was no muscle wastages as creatinine levels have been directly related to muscle volume and activity (Rajman *et al.*, 2006).

5.0 Conclusion

The result of this study showed that feeding of low fibre diets to rabbits irrespective of DE level resulted in increased incidence of transitory diarrhoea and high morbidity. High fibre diets in rabbit production favoured improved health status with no deleterious effect on the haematological indices and serum biochemistry.

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Table 1: Gross composition of experimental diets

| Fibre level | Low | | | Optimum | | | High | | |
|--|--------|---------|--------|---------|---------|--------|--------|---------|--------|
| | Low | Optimum | High | Low | Optimum | High | Low | Optimum | High |
| Digestible energy level | | | | | | | | | |
| Maize | 225 | 190 | 130 | 235 | 195 | 135 | 204 | 164 | 124 |
| Maize starch | 90 | 110 | 145 | 90 | 115 | 140 | 80 | 100 | 120 |
| Vegetable oil | 5 | 20 | 45 | 5 | 20 | 55 | 10 | 30 | 50 |
| wheat offal | 230 | 230 | 230 | 140 | 140 | 140 | 60 | 60 | 60 |
| Rice bran | 90 | 90 | 90 | 130 | 130 | 130 | 195 | 195 | 195 |
| Rice husk | 60 | 60 | 60 | 100 | 100 | 100 | 151 | 151 | 151 |
| Fixed ingredient ^a | 300 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | 300 |
| Composition (in g/kg) | | | | | | | | | |
| Dry matter | 901.4 | 910.4 | 901.1 | 909.2 | 899.7 | 908.8 | 906.2 | 900.9 | 899.7 |
| Organic matter | 898.8 | 897.0 | 898.5 | 901.1 | 890.2 | 889.5 | 901.8 | 892.2 | 900.5 |
| NDF | 257.71 | 252.11 | 249.70 | 380.11 | 370.79 | 350.22 | 445.70 | 440.41 | 431.24 |
| ADF | 157.00 | 154.20 | 149.70 | 192.40 | 189.61 | 188.00 | 252.10 | 250.09 | 249.44 |
| Gross energy (MJ/Kg) | 12.03 | 16.09 | 17.62 | 12.67 | 16.95 | 19.13 | 14.19 | 18.56 | 21.29 |
| Digestible energy ^b (MJ/Kg) | 8.17 | 10.97 | 12.11 | 8.06 | 10.84 | 12.27 | 8.01 | 10.52 | 12.09 |

^a Contained : 250 g/kg soyabean meal (440 g/kg crude protein), 25.0 g/kg bone meal, 15.0 g/kg oyster shell, 2.0 g/kg lysine- HCL, 2.0 g/kg DL-methionine, 3.0 g/kg salt (NaCl) and 3.0 g/kg vitamin/mineral premix (providing the following per kg of diet 0.04g manganese, 0.034 g zinc, 0.023 g iron, 0.0026 g copper, 2.48 mg retinol, 0.003 mg cholecalciferol, 5.55 mg riboflavin, 0.70 mg thiamin, 0.70 mg pyridoxine, 2.80 mg niacin, 0.35mg calcium pantothenate and 0.70 mg cyanocobalamin)

^bCalculated using deBlas et al. (1992) (DE=GE × (0.867-0.0012ADF))

Table 2: Main effect of varying dietary fibre and digestible energy on health status of rabbits

| Parameters | Fibre levels | | | SEM | *Probability | | Digestible energy levels | | | SEM | *Probability | |
|----------------------|--------------------|-------------------|-------------------|------|--------------|---|--------------------------|-------------------|--------------------|------|--------------|----|
| | Low | Optimum | High | | L | Q | Low | Optimum | High | | L | Q |
| Morbidity (%) | | | | | | | | | | | | |
| Off- feed | 9.55 ^a | 2.38 ^b | 0.0 ^c | 0.97 | * | * | 4.95 ^a | 4.76 ^a | 2.22 ^b | 0.99 | * | * |
| Transitory | 14.34 ^a | 2.38 ^b | 2.22 ^b | 2.03 | * | * | 7.51 ^a | 4.76 ^c | 6.67 ^b | 0.96 | * | NS |
| Diarrhoea | | | | | | | | | | | | |
| Others | 4.79 ^a | 0.0 ^c | 2.22 ^b | 0.99 | * | * | 2.57 ^b | 0.0 ^c | 4.45 ^a | 0.19 | * | * |
| Total | 28.68 ^a | 4.76 ^b | 4.44 ^b | 4.76 | * | * | 15.03 ^a | 9.52 ^c | 13.34 ^b | 2.66 | * | * |
| Mortality (%) | | | | | | | | | | | | |
| | 6.67 ^a | 3.26 ^b | 1.07 ^c | 0.66 | * | * | 6.67 ^a | 3.26 ^b | 1.07 ^c | 0.46 | * | * |

NS: Not significant,

*Probability for Linear (L) and Quadratic (Q) trend,

*P<0.05

Table 3: Interaction effect of varying dietary fibre and digestible energy on health status of rabbits

| Fibre level | Low | | | Optimum | | | High | | | SEM | *Probability | |
|----------------------|--------------------|--------------------|--------------------|--------------------|-------------------|------------------|------------------|------------------|--------------------|------|--------------|----|
| | Low | Optimum | High | Low | Optimum | High | Low | Optimum | High | | L | Q |
| Morbidity(%) | | | | | | | | | | | | |
| Off-feed | 7.7 ^b | 14.28 ^a | 6.67 ^c | 7.14 ^c | 0.0 ^d | 0.0 ^d | 0.0 ^d | 0.0 ^d | 0.0 ^d | 1.09 | * | * |
| Transitory diarrhoea | 15.4 ^a | 14.28 ^b | 13.33 ^c | 7.14 ^d | 0.0 ^e | 0.0 ^e | 0.0 ^e | 0.0 ^e | 6.67 ^d | 1.16 | * | * |
| Others | 7.7 ^a | 0.0 ^c | 6.67 ^b | 0.0 ^c | 0.0 ^c | 0.0 ^c | 0.0 ^c | 0.0 ^c | 6.67 ^b | 0.16 | * | * |
| Total | 30.80 ^a | 28.60 ^a | 26.67 ^b | 14.28 ^c | 0.0 ^d | 0.0 ^d | 0.0 ^d | 0.0 ^d | 13.34 ^c | 3.44 | ** | ** |
| Mortality(%) | | | | | | | | | | | | |
| | 13.33 ^a | 6.67 ^b | 0.0 ^d | 6.67 ^b | 3.10 ^c | 0.0 ^d | 0.0 ^d | 0.0 ^d | 3.22 ^c | 0.96 | * | * |

NS: Not significant, *Probability for Linear (L) and Quadratic (Q) trend, *P<0.05, **P<0.01

Table 4: Main effect of fibre and digestible energy on haematological indices and serum biochemistry of rabbits

| Parameters | Fibre levels | | | SEM | *Probability | | Digestible energy levels | | | SEM | *Probability | |
|---|--------------------|--------------------|--------------------|-------|--------------|----|--------------------------|---------------------|--------------------|-------|--------------|----|
| | Low | Optimum | High | | L | Q | Low | Optimum | High | | L | Q |
| Haematological indices | | | | | | | | | | | | |
| Packed cell volume (%) | 36.00 ^c | 42.00 ^a | 40.33 ^b | 1.66 | * | * | 37.00 ^b | 43.33 ^a | 35.10 ^b | 6.01 | * | * |
| Haemoglobin (g/dl) | 12.00 | 14.00 | 13.47 | 0.67 | NS | NS | 12.33 | 14.47 | 12.67 | 1.51 | NS | NS |
| Red blood cell (x 10 ⁶ /L) | 5.47 | 6.40 | 6.13 | 0.24 | NS | NS | 5.63 | 6.60 | 5.76 | 0.22 | NS | NS |
| White blood cell (x 10 ³ /L) | 5.60 ^b | 7.63 ^a | 5.43 ^b | 0.32 | NS | * | 6.37 | 6.27 | 6.03 | 0.25 | NS | NS |
| Serum biochemistry | | | | | | | | | | | | |
| Total protein (g/L) | 50.67 ^c | 61.00 ^a | 53.33 ^b | 2.64 | * | * | 51.33 ^c | 58.00 ^a | 55.67 ^b | 2.29 | * | * |
| Albumin(g/L) | 41.11 | 43.00 | 39.67 | 2.38 | NS | NS | 42.00 | 42.33 | 39.44 | 2.98 | NS | NS |
| Globulin(g/L) | 10.67 ^c | 18.00 ^a | 13.67 ^b | 2.81 | * | * | 9.33 ^c | 15.67 ^b | 17.33 ^a | 1.90 | ** | ** |
| SGOT(IU/L) | 21.33 | 23.33 | 24.67 | 2.72 | NS | NS | 21.33 ^b | 25.67 ^a | 22.23 ^b | 2.97 | NS | * |
| SGPT(IU/L) | 51.00 | 50.00 | 54.33 | 5.44 | NS | NS | 52.67 | 51.33 | 51.33 | 3.51 | NS | NS |
| Alkaline phosphatase (IU/L) | 12.22 | 13.33 | 14.33 | 2.96 | NS | NS | 11.00 ^b | 14.33 ^a | 15.00 ^a | 3.82 | * | NS |
| Serum uric acid (mg/dl) | 51.22 ^a | 44.89 ^b | 45.00 ^b | 4.03 | * | * | 44.22 ^c | 50.22 ^a | 46.67 ^b | 1.77 | * | * |
| Creatinine (mg/dl) | 1.10 | 1.03 | 1.10 | 0.07 | NS | NS | 1.00 | 1.13 | 1.17 | 0.06 | NS | NS |
| Cholesterol(mg/dl) | 90.0 ^b | 115.3 ^a | 92.7 ^b | 11.10 | * | * | 88.00 ^b | 141.33 ^a | 77.67 ^b | 22.56 | ** | ** |

NS: Not significant, *Probability for Linear (L) and Quadratic (Q) trend, *P<0.05, **P<0.01

Table 5: Interaction effect of varying dietary fibre and digestible energy on haematological indices and serum biochemistry of rabbits

| Fibre levels | Low | | | Optimum | | | High | | | SEM | *Probability | |
|---|--------------------|---------------------|--------------------|--------------------|---------------------|---------------------|---------------------|---------------------|--------------------|------|--------------|----|
| | Low | Optimum | High | Low | Optimum | High | Low | Optimum | High | | L | Q |
| Haematological indices | | | | | | | | | | | | |
| Packed cell volume (%) | 33.00 ^e | 38.10 ^b | 37.00 ^b | 37.00 ^b | 40.00 ^b | 41.00 ^b | 41.00 ^b | 44.00 ^a | 36.00 ^b | 2.73 | * | * |
| Haemoglobin (g/dl) | 11.00 ^d | 12.70 ^c | 12.30 ^c | 12.30 ^c | 16.00 ^a | 13.70 ^b | 13.70 ^b | 14.70 ^b | 12.00 ^c | 1.51 | * | * |
| Red blood cell (x 10 ¹² /L) | 5.00 | 5.80 | 5.60 | 5.70 | 7.30 | 6.20 | 6.20 | 6.70 | 5.50 | 0.26 | NS | NS |
| White blood cell (x 10 ⁹ /L) | 5.60 | 6.20 | 5.00 | 7.20 | 6.60 | 8.90 | 6.30 | 5.80 | 4.20 | 0.12 | NS | NS |
| <i>Serum biochemistry</i> | | | | | | | | | | | | |
| Total protein (g/L) | 50.00 ^f | 50.00 ^f | 52.00 ^a | 55.00 ^d | 66.00 ^a | 62.00 ^b | 49.00 ^f | 58.00 ^e | 53.00 ^a | 1.55 | * | * |
| Albumin (g/L) | 40.00 ^d | 45.00 ^b | 38.33 ^a | 48.00 ^a | 42.00 ^c | 39.00 ^a | 38.00 ^a | 40.00 ^d | 41.00 ^d | 2.40 | * | * |
| Globulin (g/L) | 10.00 ^d | 5.00 ^a | 14.00 ^c | 7.00 ^a | 24.00 ^a | 23.00 ^a | 11.00 ^d | 18.00 ^b | 12.00 ^d | 1.78 | ** | ** |
| SGOT (IU/L) | 20.00 ^d | 25.00 ^b | 19.00 ^d | 26.00 ^b | 23.00 ^c | 21.00 ^d | 18.00 ^d | 29.00 ^a | 27.00 ^a | 1.55 | * | * |
| SGPT (IU/L) | 55.00 ^a | 50.00 ^c | 48.00 ^c | 52.00 ^b | 49.00 ^c | 49.00 ^c | 51.00 ^b | 55.00 ^a | 57.00 ^a | 2.73 | * | NS |
| Alkaline phosphatase (IU/L) | 10.00 ^c | 15.00 ^a | 13.00 ^b | 10.00 ^c | 12.00 ^b | 18.00 ^a | 13.00 ^b | 16.00 ^a | 14.00 ^b | 1.15 | * | * |
| Serum uric acid (mg/dl) | 48.00 ^b | 54.00 ^a | 51.00 ^a | 41.00 ^d | 44.67 ^c | 49.00 ^b | 43.00 ^c | 52.00 ^a | 40.00 ^d | 2.45 | * | * |
| Creatinine (mg/dl) | 1.00 | 1.20 | 1.10 | 0.90 | 1.00 | 1.20 | 1.10 | 1.20 | 1.00 | 0.12 | NS | NS |
| Cholesterol (mg/dl) | 84.00 ^a | 135.00 ^b | 78.00 ^a | 76.00 ^a | 165.00 ^a | 105.00 ^d | 104.00 ^d | 124.00 ^c | 50.00 ^f | 3.57 | ** | ** |

NS: Not significant,

*Probability for Linear (L) and Quadratic (Q) trend,

*P<0.05,

**P<0.01

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