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Physiological response of rabbits to organic selenium: Serum metabolites, liver and kidney function tests and hematological indices

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ABSTRACT

An experiment was carried out to evaluate the serum total protein, glucose, liver, kidney and haematological response to varied levels of exogenous L-Selenomethionine (L-SeMet). Twenty four male rabbit bucks, about 10 months old were randomly allotted to four treatments in a completely randomized design. Blood samples were collected at day 21 and day 42 from the bucks and processed for total protein, glucose, haematocrit, haemoglobin, erythrocytes, leukocytes, and leukocyte differential count determination. Serum total protein was significantly ($p < 0.05$) higher in rabbits administered 0.3 mgkg-1 and 0.4 mgkg-1 at day 21 than those fed 0.2 mgkg-1. However, Glucose, alanine aminotransferase, spartate aminotransferase, and alkaline phosphatase were not significantly influenced by L-SeMet administration at both days 21 and 42. Blood urea was significantly ($p < 0.05$) lower in bucks administered 0.4 mgkg-1 L-SeMet compared to the control. Creatinine and sodium levels in the bucks were also not significantly influenced by L-SeMet. This suggested that exogenous supplementation of L-SeMet did not have adverse effect on the physiological processes of the rabbit bucks. Increased serum total protein observed in the experimental animals could be attributed to L-SeMet administration to the rabbit bucks which probably enhanced dietary protein utilization and/or protein synthesis.

Keywords: Selenium, L-Selenomethionine; haematology; serum biochemistry; rabbit bucks
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Introduction

Selenium (Se) has been categorized as an essential trace element as it is involved in a myriad of biochemical processes (Burk *et al*., 2003). It is an indispensable component of selenoporoteins (Kryukov *et al*., 2003) which play important roles in many biological functions such as antioxidant defence, formation of thyroid hormones, DNA synthesis, fertility and reproduction (Youcef *et al*., 2013). Se has also been established to be involved in enhancing immunity and also anti-cancer activities in humans and animals (Rayman, 2005; Cao *et al*., 2014), keshan disease and coronary heart disease in humans (Fairweather-Trait *et al.*, 2011; Rayman 2012; Youcef *et al*., 2013), white muscle disease in calves and in lambs, hepatosis dietetica in pigs, exudative diathesis in poultry, degeneration of the pancreas in poultry, necrosis of the liver, and mulberry heart disease in humans and animals (Gupta, 2007; Soetan *et al*.,

2010). Selenosis on the other hand results in depression, weakness, emaciation, anaemia, hair loss, anorexia, weight loss, lameness and death (O'Toole & Raisbeck, 1995; Underwood & Suttle, 1999; Raisbeck, 2000). Peculiar to horses, cattle and pigs are the interruption of the development of the coronary band, deformities in the hoof, and separation and sloughing of the hoof wall (Gupta, 2007). Other clinical signs include poor hatchability, embryonic death and deformities in farm animals (Gupta, 2007).

The soil selenium (Se) concentration in the world vary greatly, with there being consequentially a direct relationship between the Se amounts found in soil, and the corresponding concentration of Se in crops used for animal diets. Efforts have been made to provide alternative means by which selenium can be made available to livestock. Inorganic Se sources are generally cheap but highly toxic, with organic sources preferred since they are less toxic, better absorbed and retained in tissues, and more efficiently utilized in the body (Kim & Mahan, 2001a, b, c; Zhan *et al.*, 2007). Se exists in the form of selenomethionine (Whanger, 2002) in plants and it is generally referred to as the organic form. This has led to the development and introduction of various sources of organic Se such as Se-enriched yeast, Se-proteinate and Se-amino acid to the animal feed industries (Jang *et al*., 2010). Industrially manufactured forms of organic selenomethionine has received "generally recognized as safe" (GRAS) approval from the Food and Drug Administration (FDA) of the US Department of Agriculture for use in livestock feed. Se-enriched yeast is one such product to have attracted attention.

Several studies have found out that Se-enriched yeast improved growth performance, glutathione peroxidase (GSH-Px) Se-tissue concentration, and carcass characteristics of broilers and pigs (Jacyno *et al.* 2002; Wang & Xu, 2008; Upton *et al.*, 2008; Speight *et al.*, 2012). Peretz *et al.* (1991) in a separate study observed that Se-enriched yeast resulted in immunological response in elderly humans. Given that selenomethionine has been established to be the major form of organic Se in Se-enriched yeast (Kelly & Power, 1995; EFSA, 2009), it was hypothesized that pure selenomethionine could perform better than Se-enriched yeast.

The majority of studies on the bioavailability of selenium from L-selenomethionine (L-SeMet) in rats have been carried out using Se-enriched yeast rather than L-SeMet (EFSA, 2009). This experiment was therefore carried out to assess the haematological and serum biochemical response of adult rabbit bucks to varied levels of oral L-SeMet administration.

Materials and Methods

Source

For this study, an organic source of selenium was obtained. L-Selenomethionine (Thorne Research USA) containing 200µg Se per capsule was used for the experiment.

The research design of the study

The experiment was carried out at the Rabbitry Research Unit of the Teaching and Research farm, University of Ibadan, Ibadan, Nigeria. A total of twenty-four (24) bucks (2300 ± 8.33g) were used for the experiment. Four (4) mixed breeds of rabbit were used for the study. The experiment was set up using a completely randomized design model having four treatments with each treatment having six replicates. The bucks were about 10 months old at the commencement of the experiment and were housed in individual metal cages. The animals were randomly allotted to treatments and the test ingredient was administered to them at varied levels.

Treatment one (T1) was the control and had no exogenous selenium supplementation. Treatments two (T2), three (T3), and four (T4) had selenium supplemented to the animals at 0.2 mgkg-1, 0.3 mgkg-1, and 0.4 mgkg-1 body weights respectively. The same basal feed containing 17.78% crude protein, 8.21% crude fibre and 2525.2 kcal/kg digestible energy and water were provided for the animals. The required dosage was dissolved in 2 ml water and then drenched to each animal orally in the morning, before feeding at 48-hour intervals for a period of six (6) weeks.

Sampling procedure

At days 21 and 42, blood samples were collected from the bucks via jugular venipuncture. The blood samples were collected using a sterile 5ml syringe (Hyproject IV 0.8x40 mm 21G x 11/2″). 2 ml of the blood collected was dispensed into 5 ml lithium heparinized bottles (Medi-Scan non-vacuum PP tube), whilst another 3 ml was dispensed into 5 ml sterile bottles (GD medical non-vacuum sterile IVD PP tube) for each blood sample collected from all the rabbit bucks. Lithium heparin prevented coagulation of blood which was used for haematological assessment whilst the blood from the plain sample bottles had no anticoagulant and was centrifuged (Bosch 90-2 12-place interlock centrifuge) at 3,500 rpm for 15 minutes to separate sera from the whole blood for analyses.

Data collection and analysis

For total protein assay, 20 µl of the sera samples were collected into test tubes. Total protein assay reagent – TP 245 (Randox Laboratories Ltd. United Kingdom) was collected using a 1 ml graduated micropipette and thereafter dispensed into the test tubes containing the sera samples. The test tubes were then left to incubate for 10 minutes. The solutions were then transferred into cuvettes and the absorbance of each read using a spectrophotometer (Jenway 6305 Single beam UV/Visible Spectrophotometer) at a wavelength of 540 nanometer (nm).

To determine serum glucose concentration, 10 µl of each serum sample was taken into test tubes for all the serum samples. Glucose assay reagent – Randox Gluc-CAP (Randox Laboratories Ltd. United Kingdom) was dispensed at a volume of 1 ml into the test tubes. The solution was left to incubate for 10 minutes. Afterwards, the solution was transferred into cuvettes and the absorbance of the solution was read using the same spectrophotometer at a wavelength of 500 nm.

Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were assayed to assess the toxicity of the test ingredient in the experimental animals. Commercial reagents – Randox AL100, Randox AS101 and Randox AP542/AP307 (Randox Laboratories Ltd. United Kingdom) were used to assay for the enzymes ALT, AST and ALP respectively using the procedural steps outlined by the manufacturers. All haematological assessment were carried out as described by MAFF (1984), and Ewuola & Egbunike (2008). Serum albumin, urea, creatinine and sodium were assayed using respective commercial kits and procedures were carried out as outlined by the manufacturers.

All data collected were subjected to a one-way statistical analysis of variance of the Statistical Analysis System (SAS) version 9.3 (SAS, 2011). The means of the treatments were separated using the Fisher's Least Significant Difference (α0.05) procedure of the same software.

Results and Discussion

Haematological indices

The haematological values of the rabbit bucks administered varied levels of L-Selenomethionine at days 21 and 42 are shown in Tables 1 and 2 respectively. The means observed for all haematological parameters (haematocrit, haemoglobin, erythrocytes, leucocytes, lymphocytes, neutrophils, monocytes, eosinophils, and platelet cells) for all the experimental bucks were not significantly different at day 21. It was however observed at day 42 that leucocytes, neutrophils and lymphocytes count were significantly ($p < 0.05$) different among the treatments. The leucocytes count of the bucks on T3 was significantly ($p < 0.05$) higher (5.63±0.95 x 103/mm3) than those on T1 (4.34±0.76 x 103/mm3).

However, there was no significant difference in the leucocytes count of the bucks on T3 and T4. The lymphocytes count of the bucks on T3 was significantly ($p < 0.05$) lower (60.67±5.61 %) compared to those of the bucks on T2 (68.50±5.58 %). There was also no significant difference in the leucocyte count of the bucks on T1 (65.67±5.82 %), T2 (68.50±5.58 %) and T4 (66.50±5.89 %). The neutrophils count of the bucks on T3 was significantly ($p < 0.05$) higher (34.33±6.22 %) than the bucks on T2 (26.00±6.23 %). However, there was no significant difference in the neutrophils count of the bucks on T1 (28.83±4.83 %) and T4 (27.83±4.26 %).

Serum total protein and glucose

The effect of exogenous L-Selenomethionine supplementation on the serum glucose and total protein profile of the rabbit bucks at days 21 and 42 are presented in Table 3. At day 21, serum glucose concentration was not significantly different among the treatments. Serum total protein concentration was significantly different amongst the treatments. Serum total protein of the bucks on T4 was significantly ($p < 0.05$) higher (7.14±0.65 g/dL) than bucks on T1 (5.41±0.50 g/dL) and T2 (5.71±0.29 g/dL). At day 42 however, there was no significant difference among the treatments for all the parameters assessed.

Liver function test

The liver function indices of the bucks administered oral L-SeMet at days 21 and 42 are shown in Table 4. At day 21, there was no significant difference observed in the means of the ALT, AST, and ALP activity in all the experimental rabbit bucks. At day 42, alanine amino transferase (ALT) was observed to show no significant difference among the treatments. However, alkaline phosphatase (ALP) activity in the bucks on T2 was significantly ($p < 0.05$) lower (17.17±8.06 IU/L) than the bucks on T4 (35.33±20.05 IU/L), but it was not significantly different from the bucks on T1 (22.00±2.12 IU/L) and T3 (21.33±13.29 IU/L).

Serum aspartate amino transferase (AST) level was also significantly different among the treatments. The bucks on T1 had significantly ($p < 0.05$) higher (93.60±8.32 IU/L) AST activity compared to the bucks on T2 (39.50±30.72 IU/L). However, there was no significant difference between the AST level of the bucks on T3 (54.50±33.13 IU/L) and the bucks on T4 (56.83±37.72 IU/L).

Kidney function test

The effect of the exogenous L-Selenomethionine on the kidney function parameters are presented in Table 5. At day 21, serum urea of the bucks on T1 (1.02±0.37 mmol/l) was significantly ($p < 0.05$) higher compared to that of the animals on T2 (0.53±0.38 mmol/l) and T4 (0.53±0.38 mmol/l). However, there was no significant difference between the

concentration of urea in the serum of bucks on T2, T3 and T4. Serum creatinine concentration was not significantly different in the rabbit bucks across the treatments. Serum sodium level was not significantly different among the treatments at day 21. However at day 42, it was observed that serum urea was significantly ($p < 0.05$) different among the treatments. Serum urea was significantly ($p < 0.05$) lower in bucks on T3 (4.82±0.74 mmol/L) and T4 (6.19±1.07 mmol/L) than the bucks on T2 (7.14±1.78 mmol/L).

However, there was no significant difference in the serum urea level in the bucks on T3, T4, and T1. Also, no significant difference was observed in the urea level of the bucks on T4 (6.19±1.07 mmol/L) and the bucks on T2. Serum creatinine was significantly ($p < 0.05$) higher (1.15±0.45 mg/dL) in the rabbit bucks on T2 than T3 (0.67±0.12 mg/dL). However, no significant difference was observed in the creatinine level in the bucks on T4 and T1. Serum sodium level was not significantly different among the treatments.

TABLE 1

Haematological indices of rabbit bucks administered supplemental levels of L-Selenomethionine at day 21

Parameters	*T1* *(0mg/kg)*	*T2* *(0.2mg/kg)*	*T3* *(0.3mg/kg)*	*T4* *(0.4mg/kg)*
Haematocrit (%)	36.83±5.85	30.50±12.80	39.00±2.00	35.00±11.92
Haemoglobin (%)	12.48±2.24	10.25±4.68	13.27±0.78	11.97±4.39
Erythrocytes (x106 /mm3)	6.20±1.11	5.3±1.88	6.48±0.52	6.09±1.95
Leucocytes (10^3/mm^3)	4.91±3.56	4.11±1.32	3.87±1.38	4.22±7.71
Lymphocytes (%)	68.33±9.40	61.83±8.95	66.33±6.56	67.17±7.57
Neutrophils (%)	26.67±8.43	32.83±9.91	28.83±6.77	29.00±7.43
Monocytes (%)	2.17±1.17	2.83±1.17	2.50±1.05	2.00±0.89
Eosinophils (%)	2.83±0.75	2.50±1.38	2.33±1.21	1.83±0.98
Platelets (x 10^3/µL)	81.33±48.64	69.50±26.55	67.50±21.93	66.50±11.33

TABLE 2

Haematological response of rabbit bucks administered exogenous L-Selenomethionine supplementation at day42

Parameters	*T1* *(0mg/kg)*	*T2* *(0.2mg/kg)*	*T3* *(0.3mg/kg)*	*T4* *(0.4mg/kg)*
Haematocrit (%)	34.83±3.54	37.00±4.34	36.50±3.62	37.67±2.42
Haemoglobin (%)	12.00±1.28	12.50±1.32	12.22±1.17	12.62±0.88
Erythrocytes (x106 /mm3)	5.75±0.71	6.22±0.71	6.04±0.57	6.47±0.55
Leucocytes (10^3/mm^3)	4.34±0.76^{b}	5.11±1.05^{ab}	5.63±0.95^{a}	5.24±0.82^{ab}
Platelets (x 10^3/µL)	99.00±8.44	97.50±44.50	83.83±32.00	88.50±8.34

Lymphocytes (%)	65.67±5.82^{ab}	68.50±5.58^{a}	60.67±5.61^{b}	66.50±5.89^{ab}
Neutrophils (%)	28.83±4.83^{ab}	26.00±6.23^{b}	34.33±6.22^{a}	27.83±4.26^{ab}
Monocytes (%)	3.33±1.37	2.83±1.47	2.67±0.82	3.50±1.22
Eosinophils (%)	2.17±0.75	2.67±1.37	2.33±1.37	2.17±1.47

a b - Means along the same row with different superscripts are significantly ($p < 0.05$) different.

TABLE 3

Serum glucose and total protein concentration of rabbit bucks administered exogenous L-Selenomethionine at days 21 and 42

Parameters	*T1 (0mg/kg)*	*T2 (0.2mg/kg)*	*T3 (0.3mg/kg)*	*T4 (0.4mg/kg)*
Day 21				
Glucose (mg/dL)	112.04 ±6.30	115.67 ±8.05	119.3 ±7.26	114.46 ±7.24
Total Protein (g/dL)	5.41±0.50^{b}	5.71±0.29^{b}	6.92±0.58^{a}	7.14±0.65^{a}
Day 42				
Total protein (g/dL)	6.58±0.43	6.68±0.2	6.78±0.58	6.62±0.33
Albumin (g/dL)	3.46±0.43	3.62±0.25	3.47±0.72	3.38±0.44
Globulin (g/dL)	2.92±0.48	3.07±0.14	3.00±0.45	3.23±0.18
Albumin-Globulin ratio	1.20±0.35	1.15±0.10	1.15±0.41	0.98±0.18
Glucose (mg/dL)	90.6±16.09	89.67±8.14	95.33±21.42	94.00±12.84

a b - Means along the same row with different superscripts are significantly ($p < 0.05$) different.

TABLE 4

Liver function indices of rabbit bucks administered supplemental levels of L-Seleno methionine at days 21 and 42

Parameters	*T1 (0mg/kg)*	*T2 (0.2mg/kg)*	*T3 (0.3mg/kg)*	*T4 (0.4mg/kg)*
Day 21				
Aspartate aminotransferase (IU/L)	241.67±35.75	253.67±33.41	230.00±25.6	114.46 ±7.24
Alanine aminotransferase (IU/L)	36.33±11.41	39.33±8.71	35.50±13.84	36.33±12.93
Alkaline phosphatase (IU/L)	297.00±123.41	279.17±99.92	252.00±133.26	334.83±51.43
Day 42				
Alanine Aminotransferase (IU/L)	95.80±69.16	61.83±19.67	98.50±48.89	97.83±36.91
Alkaline Phosphatase (IU/L)	22.00±2.12^{ab}	17.17±8.06^{b}	21.33±13.29^{ab}	35.33±20.05^{a}
Aspartate Aminotransferase (IU/L)	93.60±8.32^{a}	39.50±30.72^{b}	54.50±33.13^{ab}	56.83±37.72^{ab}

a b - Means along the same row with different superscripts are significantly ($p < 0.05$) different.

TABLE 5
Kidney function indices of rabbit bucks administered supplemental levels of L-Selenomethionine at days 21 and 42

Parameters	*T1* *(0mg/kg)*	*T2* *(0.2mg/kg)*	*T3* *(0.3mg/kg)*	*T4* *(0.4mg/kg)*
Day 21				
Urea (mmol/L)	1.02±0.37[a]	0.53±0.38[b]	0.62±0.39[ab]	0.53±0.38[b]
Creatinine (mg/dL)	0.82±0.18	0.70±0.26	0.70±0.19	0.70±0.15
Sodium (mEq/L)	512.83±64.27	447.67±72.57	474.33±65.89	442.5±77.62
Day 42				
Urea (mmol/L)	5.21±0.82[b]	7.14±1.78[a]	4.82±0.74[b]	6.19±1.07[ab]
Creatinine (mg/dL)	0.70±0.07[b]	1.15±0.45[a]	0.67±0.12[b]	0.95±0.37[ab]
Sodium (mEq/L)	134.60±4.16	134.50±8.94	131.50±4.09	129.00±5.83

a b - Means along the same row with different superscripts are significantly ($p < 0.05$) different.

All the haematological parameters measured in the present experiment were within the physiological ranges reported for normal rabbits (Jenkins, 1993; Hillyer, 1994). This suggests that all the experimental bucks, irrespective of the level L-Selenomethionine supplementation had haematopoiesis taking place at normal rates in their body systems and in adequate form. It was observed that selenium supplementation up to 0.4 mgkg-1 did not adversely affect the utilization of iron especially in the L-SeMet treated groups and the haematological parameters.

Serum glucose concentration was observed not to be significantly influenced by L-SeMet for the animals that were provided oral administration of L-SeMet compared to the control bucks. This finding supports the works of Sugden *et al*. (1978) and Becker *et al*. (1996) who reported no significant influence in the serum glucose concentration of ewes and diabetic rats supplemented with selenium through feed and drinking water respectively. It has been reported that the inorganic form of selenium (selenite) influences serum glucose concentration in diabetic rats by, firstly, reducing the upraised serum glucose level, and secondly, reducing alteration in the expression of abnormally expressed glycolytic and gluconeogenic marker enzymes in rats (Stapleton *et al.*, 1998; Becker *et al*., 1996). This is as a result of the insulinomimetic properties of selenate (Stapleton *et al*., 1997; Hei *et al*., 1998).

This suggests that L-SeMet supplementation did not influence glycolytic and gluconeogenetic activities in the blood stream of the rabbit bucks, which indicates that they were non-diabetic and that carbohydrate metabolism was taking place at normal physiological rates. However, Bunk & Combs (1980) reported an increase in the plasma glucose concentration in chicks provided oral administration of 0.205mg/kg sodium selenite. ElSammani & ElSheikh (2013) also reported that acute intraperitoneal injection of sodium selenite to healthy rats slightly increased the serum glucose level at different time intervals post-injection in a dose-dependent manner.

Serum total protein concentration was significantly influenced by L-SeMet administration in the treated bucks compared to the control group. This suggests that organic selenium had more pronounced effect on serum total protein since it is preferentially utilized by the body compared to methionine. Sunde *et al.* (1997), Kim & Mahan (2003), and EFSA (2009) reported that selenomethionine could be incorporated into proteins at a rate similar to methionine in the body system. Combs & Combs (1986) reported that organic Se supplemented to broiler breeders and layers was actively absorbed and can be directly incorporated into the body proteins. This supported the findings of Kamel (2012) that observed significant differences in the serum total protein of rabbit bucks that were on selenium and folic acid supplementation compared to the control. Attia & Kamel (2011) in a separate study observed that rabbit bucks fed diets supplemented with different concentrations of soybean lecithin had significantly different serum total protein values. Increase in the total protein induced by L-SeMet may have probably enhanced dietary protein utilization and/or protein synthesis in the animal

Liver function test revealed that serum enzymes examined were not significantly influenced by Se supplementation. This suggests that L-SeMet did not cause liver damage or dysfunction (Singh *et al.*, 2011) in all groups of bucks. Although AST is not absolutely liver-specific as it is also found in other organs like heart, muscle, brain and kidney. Injury to any of these tissues can cause an elevated blood level (Nathwani *et al.*, 2005). It also helps in detecting hepatocellular necrosis but is considered a less specific biomarker enzyme for hepatocellular injury (Ozer *et al*., 2008), due the fact that it can also signify abnormalities in heart, muscle, brain or kidney (Dufour *et al*., 2000). This was however not observed in this study.

Conclusion and Recommendation

In summary, this study has revealed that oral supplementation of selenium in the organic form of L-Selenomethionine up to 0.4 $mgkg^{-1}$ to rabbit bucks for six weeks influenced serum total protein and did not cause organ toxicity, or any physiological disorder in the blood profile of the animal.

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