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Age – related changes in serum biochemical parameters in the domestic rabbit (*Oryctolagus cuniculus*)

Adebisi, K. A.
Department of Animal Science, University of Ibadan, Ibadan.
Corresponding author: karamatsanusi@yahoo.co.uk

Abstract

The influence of age on serum biochemical components in the domestic rabbit was investigated. The serum total protein (g/dL), albumin (g/dL), globulin (g/dL), creatinine (mg/dL), urea mg/dL, cholesterol (mg/dL), Alkaline phosphatase (ALP; iu/l), Aspartate aminotranferase (AST; iu/l) and Alanine amimotransferase (ALT; iu/l) were assessed in 24 male rabbits at 7, 14 and 21 weeks of age to represent weaners, growers and pubertal stages. Data were analysed using descriptive statistics and analysis of variance at $\alpha_{0.05}$*. Results of serum biochemistry revealed a steady significant increase in total protein (4.2±0.3; 5.3±0.5; 6.9±0.2), albumin (2.2±0.7; 2.8±0.4; 4.0±0.2), globulin (1.9±0.3; 2.5±0.4, 2.9±0.2), cholesterol (49.5±1.1; 56.4±4.2; 62.4±5.4) and creatinine (0.8±0.1; 1.2±0.1; 1.9±0.3) at 7, 14 and 21 weeks respectively. Serum urea levels were similar across the ages. The values recorded for ALP and AST at 7 weeks (39.6±7.5; 101.1±6.7) and 14 weeks (38.7±6.4; 104.5±8.7) were significantly higher than the 21 weeks (33.5±2.7; 95.8±5.9) while ALT at 7 weeks (27.33±5.6) was significantly lower than 14 (38.2±3.9) and 21 weeks (37.4±3.0). The age of the rabbits had a significant effect on their serum biochemical parameters. They should therefore be grouped according to age when setting serum reference values for them.*

Keywords: Age of rabbit, Serum Biochemical Values, Serum Reference Values.

Introduction

The domestic rabbit is used extensively in medical and biological research because they are mammals, small in size and have relatively short gestation period and generation interval. They can also be kept for meat, wool and as a pet. Rabbits have received a considerable research attention in Nigeria. This stems from the current trend in the country to exploit some unpopular but potential meat resource to complement the conventional ones like cattle, sheep, poultry or pigs. These studies are usually aimed at how best to produce numerous rabbits, within the shortest possible time at a relatively low cost without compromising health and performance. When such studies are carried out, usually, rabbits of any age, sex or physiological status may be used and the blood profile (haematology or serum biochemistry) is usually one of the tools used to monitor the study. This creates a need to establish reference values for the blood profile to serve as a guide in interpreting results from such research. In establishing such values, it is important to bear in mind that several factors such as analytical methods, age of animal, environmental factors or seasonal influence may affect results apart from the physiological status of the animal (Mitruka and Rawnsley, 1977). These therefore necessitate the establishment of reference values for our local use and consider how they vary with age. For technical reasons, many biochemical measurements are more conveniently made on serum rather than plasma but the concentrations of most analytes are effectively the same in both fluids (Marshall, 2000). A lot of studies have been carried out on the blood profile of various domestic animals, but there is dearth of information on the reference haematological and serum biochemical values for rabbits in the warm humid

environment (Chineke *et al.*, 2002) and more especially, considering the influence of age. Ozegbe *et al.* (2000)[1], Ozegbe *et al.* (2000)[2], Awojobi and Opiah (2002) have documented changes in plasma or serum profile at some physiological stages for sows, cows and doe rabbits respectively. Tambuwal *et al.* (2002) have reported some serum biochemical values for goats considering age and sex. For rabbits, Chineke *et al.* (2002) considered some breeds and crosses while Iwuji *et al.* (2017) also reported serum biochemical values for rabbits at different locations. The objective of this study was therefore to assess the influence of age at an actively growing phase of male rabbits on some serum biochemical components.

Materials and methods

The study was carried out at the Teaching and Research Farm of the University of Ibadan. A total of 24 New Zealand x Chinchilla crossbred male rabbits were used for the study. The rabbits were purchased from a breeder farm at 5 weeks of age immediately after weaning. On arrival, the rabbits were given vitamins and necessary prophylaxis and allowed to equilibrate for about two weeks afterwards to provide time for homeostatic return to physiological normality and to stabilize their nutritional and environmental conditions. Rabbits were checked regularly for signs of illness to ensure only healthy ones were used and housed individually in cages. Pelleted concentrate rations containing 19% crude protein, 10% crude fibre and 2,480Kcal digestible energy/Kg feed was provided ad-lib along with *Tridax procumbens* as forage. Fresh clean water was also supplied ad-lib. Blood samples for serum biochemistry were taken at intervals of seven weeks. The first was at seven weeks of age, second at 14 weeks and third at 21 weeks representing the weaners, growers and pubertal stages respectively. Blood sample taken from each rabbit was analysed separately and taken as a replicate. The experimental design was a completely randomized design and analysis of variance was done to check for significant difference of each of the blood parameters across the ages.

Blood sample collection and analysis

The rabbits were starved for about twelve hours prior to collection of blood samples to minimize the effect of feed on the concentration of the various blood constituents (Mitruka and Rawnsley, 1977). Blood was sampled from the marginal vein of the ear. About 5mL of blood from individual rabbits was collected into separate labelled sterile tubes. Serum was separated about an hour after clotting by centrifuging for about 30 minutes. The serum was drawn off by pipette and immediately frozen until analysis was carried out. The serum constituents analysed for were total protein, albumin, globulin, Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), creatinine, urea and cholesterol. Total protein was determined by Biuret reaction, albumin by bromocresol green reaction and globulin by subtracting albumin from total protein as described by Toro and Ackermann (1979). Urea was determined by the use of enzyme urease and cholesterol by direct serum method as described by Burtis and Ashwood (1996). The enzymes and creatinine were determined indirectly and reaction monitored spectrophotometrically at fixed time intervals (Burtis and Ashwood, 1996). All the parameters were analysed using Randox Reagent kit (from Randox Laboratories Ltd.). The data obtained were subjected to analysis of variance of SAS (1999) and means were separated using Duncan multiple range test.

Results

The mean serum biochemical values for the rabbits are presented in Table 1. The results indicated a consistent significant (P<0.05) increase in mean values for total protein, albumin, globulin, cholesterol and creatinine from 7 to 21 weeks. Values recorded for urea were similar across the ages. It ranged from 24.26±2.51 (7 weeks) to 26.65±5.06 mg/dL (14 weeks).

Table 1: ***Mean (±SD) serum biochemical values of rabbits as influenced by age***

	Age of Rabbits		
Serum components	7 weeks	14 weeks	21 weeks
Total protein (g/dL)	4.18±0.26[c]	5.32±0.50[b]	6.88±0.23[a]
Albumin (g/dL)	2.19±0.72[c]	2.82±0.36[b]	4.03±0.24[a]
Globulin (g/dL)	1.86±0.33[c]	2.50±0.38[b]	2.86±0.24[a]
Creatinine (mg/dL)	0.79±0.10[c]	1.23±0.10[b]	1.87±0.30[a]
Urea (mg/dL)	24.26±2.51	26.65±5.06	25.21±4.72
Cholesterol (mg/dL)	49.52±1.13[c]	56.44±4.24[b]	62.36±5.41[a]
ALP (iu/L)	39.60±7.54[a]	38.70±6.44[a]	33.50±2.70[b]
AST (iu/L)	101.14±6.66[a]	104.48±8.71[a]	95.81±5.94[b]
ALT (iu/L)	27.33±5.64[b]	38.19±3.91[a]	37.39±2.97[a]

Means across the rows with different superscripts are significantly different (P<0.05), SD- Standard Deviation,
ALP – Alkaline phosphatase; AST- Aspartatae aminotransferase; ALT - Alanine aminotransferase.

The values recorded for ALP and AST at 7 and 14 weeks were significantly (P<0.05) higher than 21weeks while ALT at 7 weeks was significantly (P<0.05) lower than 14 and 21 weeks thus implying ALP and AST decreased with age while ALT increased with age. The range of serum biochemical values recorded at the three stages of growth is presented in Table 2. The range of values recorded at 7 weeks for total protein, albumin and globulin were lower than that reported in literature for those parameters while the range recorded for other parameters at the different age groups were within those reported in literature.

Discussion

The increase in values for total protein, albumin, globulin, cholesterol and creatinine with respect to age is as with reference values for humans in which case values set at different age groups for these parameters increase steadily with increase in age (Burtis and Ashwood, 1996). Ignjatovic *et al.* (2011) reported qualitative and quantitative changes in plasma proteins with age in humans. They concluded that the age-related changes are not a function of sexual maturity because the proteins expressed for gender are different from that expressed for age. Total proteins were also reported to increase with age in age in mice (Frith *et al.*, 1980) and Rabbits (Chineke *et al.*, 2002). The serum total protein increased from mean of 4.5 at 42 days to 6.8 at 91 days, albumin, 3.18 to 3.78 and globulin 1.23 to 2.42 respectively (Chineke *et al.*, 2002). The increase in creatinine supports the fact that creatinine is expected to increase from infancy to puberty, parallel to the development of skeletal muscle as this is the period growth is most prominent. However, in maturity, as reported in healthy rats, it appeared to remain unchanged and not affected by age at that stage (Kitagaki *et al*., 2005).

The increase in cholesterol confirm reports from Marshal (2000) that blood cholesterol increase with age in humans while Frith *et al*. (1980) also reported an increase with age in male mice. However, Yamada *et al.* (2004) reported high serum cholesterol in suckling rabbits when compared to adults. The similarity across the age groups for urea was probably because blood urea levels in healthy animals are more affected

Table 2: Range of serum biochemical values recorded at different ages

Serum components	*Age of rabbits*			[1]Range in literature	[2]Range in literature
	7 weeks	14 weeks	21 weeks		
Totalprotein(g/dL)	3.86 4.58	4.50 – 5.75	6.58 – 7.22	5.00 - 8.00	5.40 – 7.30
Albumin (g/dL)	2.00 – 2.27	2.10 – 3.40	3.58 – 4.59	2.80 - 4.00	2.40 – 4.50
Globulin (g/dL)	1.42 – 2.44	1.94 – 3.09	2.27 – 3.29	2.20 - 4.00	2.90 – 4.90
Creatinine(mg/dL)	0.60 – 0.95	0.80 – 1.40	1.16 – 2.33	-	0.50 – 2.20
Urea (mg/dL)	19.30 – 28.90	20.60 – 36.60	17.50 – 32.80	9.00 - 32.00	10.00 – 33.00
Cholesterol(mg/dL)	47.50 – 51.30	50.30 – 62.00	56.00 – 75.00	20.00 - 83.00	10.00 – 80.00
ALP (iu/L)	31.80 – 50.10	33.00 – 51.00	32.00 – 40.20	10.00 - 90.00	4.00 – 20.00
AST (iu/L)	82.35– 109.00	99.00– 111.40	84.40 – 108.00	42.00 - 98.00	10.00 – 120.00
ALT (iu/L)	20.00 – 30.00	30.20 – 43.00	30.90- 41.00	49.00 - 79.00	10.00 – 45.00

[1]CCAC (1980), [2]Rosenthal (2003)
ALP – Alkaline phosphatase; AST- Aspartatae aminotransferase; ALT- Alanine aminotransferase.

by diet than other factors. Kitagaki *et al*. (2005) reported that blood urea did not seem to be affected by age in normal healthy rats.

The values recorded for ALP, AST and ALT supports reports of Burtis and Ashwood (1996) that the serum activity of most serum enzymes decrease from childhood to puberty in humans but that of ALT may continue to rise at least in men until middle age. Frith *et al*. (1980) also reported a decrease in ALP during the first year of life in male and female guinea pigs while Yamada *et al.* (2004) reported a decrease from 2 to 12 months in Rabbits. Clifford and White (1999) reported that ALP values decreased with increase in body weight in Guinea pigs while AST and ALT were reported to increase from 2 to 6 months and thereafter, remained constant (Yamada *et al*., 2004).

The lower range of values recorded at 7 weeks for total protein, albumin and globulin may partly be as a result of the lower mean values recorded at that stage (Table 1). This further stresses the fact that the parameters may be highly influenced by age and the need to classify these reference values according to age groups. The range recorded for the other parameters were however within that reported in literature.

Conclusion

The study showed that age influenced the serum biochemical components of rabbits and the most important influence that determines the overall effect of age were the degree of sexual maturity, the body weight and the amount of skeletal muscle mass of the animal. Since significant differences were observed for virtually all serum biochemical components assessed in the study at different ages, it will be necessary to group rabbits according to age when setting reference values for them to avoid misguidance in interpreting laboratory results. Results from this study can therefore serve as a contribution to reference serum biochemical values for rabbits.

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