World Rabbit Science

World Rabbit Sci. 2006, 14: 175 - 187

AN INVESTIGATION INTO HAEMATOLOGICAL AND SERUM CHEMISTRY PARAMETERS OF RABBITS IN TRINIDAD

Burnett N., Mathura K., Metivier K.S., Holder R.B., Brown G., Campbell M.

School of Veterinary Medicine, The University of the West Indies, St. Augustine, Trinidad, W.I.

Abstract: Blood samples were collected from a total of 70 rabbits at three locations in Trinidad. The locations were the University Field Station (n=46), the School of Veterinary Medicine (n=11), and the Eastern Caribbean Institute of Agriculture and Forestry (n=13). Complete blood counts and serum chemistry determinations were done for each sample. Values obtained were compared to reference ranges in the literature. The effects of gender, maturity (juveniles *vs.* adults), breed (New Zealand White *vs.* Mixed), and rabbitry on all parameters were examined. Mixed rabbits were crossbreds consisting of at least two breeds: New Zealand White, Californian, Checkered Giant. A comparison was made between values for haemoglobin, packed cell volume, mean corpuscular haemoglobin concentration, and white blood cell count, obtained by manual and automated methods. Most values obtained were within the ranges of those in the literature with the exception of urea (5.5–7.0 mmol/L), albumin (50.56–52.98 g/L) and creatine phosphokinase (CPK) (572.70–821.98 U/L). Albumin and CPK concentrations were higher and urea lower (P<0.05) for the present study. Significant differences (P<0.05) between automated and manual values were found for haemoglobin (Hb), packed cell volume (PCV), and mean corpuscular haemoglobin concentration (MCHC), with values for automated methods being higher for Hb and MCHC and lower for PCV. For the leukon, husbandry practices had an effect on neutrophil, eosinophil, basophil, lymphocyte, and platelet values while maturity influenced neutrophil and lymphocyte counts (P<0.05). The automated white blood cell count (WBC_a) was affected by breed (P<0.05). In the case of the erythron, husbandry practices affected automated PCV and MCHC values, and red blood cell counts (RBC) (P<0.05). Maturity influenced automated Hb and PCV values, and RBC (P<0.05). Mixed breeds had higher automated Hb, PCV and RBC values than New Zealand White rabbits (P<0.05). Male rabbits had higher values than females for manual and automated Hb, manual and automated PCV, and RBC (P<0.05). For serum chemistry, husbandry practices had an effect on potassium (K), phosphorus (P), creatinine, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), amylase and calcium (P<0.05). Phosphorus, AST, cholesterol and glucose were higher for juveniles; while chloride was higher for adult rabbits (P<0.05). Only CPK was affected by breed, with the mixed breed having higher values than New Zealand White rabbits (P<0.05). Males had higher values for potassium, total protein, and albumin, while females had higher values for cholesterol (P<0.05). Haematology and serum chemistry reference intervals obtained in this study may therefore be considered useful baseline values for domestic rabbit populations in the Caribbean.

Key words: Reference values; Haematology; Serum chemistry; Rabbits; Trinidad and Tobago

INTRODUCTION

In Trinidad and other islands of the Caribbean, rabbits have been used as a source of protein for several years. The number of rabbitries in Trinidad has doubled between 1998 and 2003 (Malcolm, pers. commun.). Among the reasons suggested for this growth in popularity of the domestic rabbit are its delicious high protein meat that is low in calories and fat, its prolificacy and short gestation length, the ease with which the animal can be reared, the small investment required to start a rabbitry, the absence of restrictions on eating rabbit meat, and the fact that they can be fed on local forages and by-products that are of no direct use to humans.

Correspondence: M. Campbell, vynmer@yahoo.com
Received *September 2005* - Accepted *November 2005.*

In addition to the use of the domestic rabbit as a source of protein, the animal is a popular companion animal among children and adults, and is also used widely in biomedical research. The most popular breeds in Trinidad are the Californian (CN), the New Zealand White (NZW) and the Checkered Giant (CG).

Concurrent with the increasing popularity in rabbit rearing has been the increasing demand for veterinary services to treat rabbits. It is therefore important to have available, a reliable set of normal haematological and biochemical reference values to aid in the diagnosis and prognosis of rabbit diseases. Most of the normal reference values quoted in the literature were derived from rabbits reared under temperate conditions. Cazabon *et al*. (2000) reported on haematological values in rabbits reared at a single rabbitry in Trinidad. These rabbits were, however, reared under conditions and feeding regimes that are different from those at most of the other rabbitries in Trinidad (Malcolm, *pers commun*.). It was therefore important to sample a wider cross-section of rabbitries in order to obtain more reliable estimates for haematological parameters as well as serum chemistry values.

The objectives of this study were: (i) to examine the effects of gender, breed, age and husbandry practices on haematological and serum chemistry values, (ii) to establish normal haematological and serum chemistry values for rabbits in Trinidad and to compare these with existing reference ranges in the literature, and (iii) to compare the results obtained from manual and automated methods for selected haematological parameters.

MATERIALS AND METHODS

Experimental animals

Experimental procedures were conducted within the guidelines suggested by the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Consortium, 1988).

Seventy (70) apparently healthy rabbits, at three rabbitries in Trinidad, were bled over a period of four months (September to December) in 2002. A physical examination was conducted on each rabbit and those considered unhealthy (that is, those possessing an elevated temperature, ocular or nasal discharge, ear mites or infection, diarrhoea, abnormal lung sounds, or major wounds) were excluded from the study. The three rabbitries were the University Field Station (UFS, n=46), The University of the West Indies; the Eastern Caribbean Institute of Agriculture and Forestry (ECIAF, n=13), Ministry of Agriculture, Land and Marine Resources, Trinidad; and the Animal House of the School of Veterinary Medicine (SVM, n=11), The University of the West Indies.

Management and production practices

At the UFS the animals were housed in wire mesh cages, which were elevated 60 cm above the ground. Muscovy ducks were allowed to roam freely throughout the rabbitry. The animals were fed grass in the morning and a mixture of 50% pig grower (crude protein, 16%; crude fat, 2.5%; crude fibre, 7.0%) and 50% broiler finisher (crude protein, 18.5%; crude fat, 4.0%; crude fibre, 5.0%) in the afternoon (Table 1). Does received 100 g/day, juveniles, 30-80 g/day, and does with kits, 100 g plus 10 g per kit/day. Bucks were fed 80 g/day. Dried coconut was provided *ad libitum* for fibre.

At the ECIAF the animals were housed in cages made of wire mesh and wood, which were elevated 60 cm above the ground. There were also some double-decker cages. The animals were fed broiler finisher (crude protein, 18.5%; crude fat, 4.0%; crude fibre, 5.0%), once per day in the morning. Adults were fed 114 g per animal per day. The amount fed to juveniles was quite variable. Grass and legumes were fed occasionally. At the SVM Animal House the animals were housed in double-decker wire-floored cages. The building was air-conditioned (20 – 24 °C) and rabbits were fed rabbit pellets

ad libitum (crude protein, 17.0%; crude fat, 3.0%; crude fibre, 13.0%; Larro Feeds, Trinidad). Grass was offered three times per week.

Animals at the three rabbitries were classified according to breed (NZW or mixed) and age (juvenile or adult). Rabbits 10 to 24 weeks of age (approx. 1.5 to 2.4 kg live weight) were classified as juveniles and those greater than 24 weeks (approx. 2.5 – 4.0 kg live weight) were considered adults. Pregnant does were not sampled. Rabbits classified as mixed breed were crosses consisting of two or more of the three breeds: NZW, CG, and CN.

Blood collection and processing

The dorsal surface of the pinna was first cleaned using cotton swabs impregnated with 70% isopropanol followed by an application of petroleum jelly. Heat was then applied to the ear using either a brooding lamp or desk lamp at a height of approximately forty-five centimetres above the ear, until the veins were dilated.

Approximately six millilitres of blood were collected from the marginal ear vein of each rabbit, using either an eighteen or nineteen gauge 3.8 cm needle. Each sample was divided into two and placed in a purple (potassium EDTA) and a red (no anticoagulant) top tube. The samples were then placed on ice and submitted to the laboratory where the contents of red top tubes were centrifuged using the

Table 1: Some characteristics of the three rabbitries used in the present study

Characteristics		Rabbitry		
		UFS (n=46)	ECIAF (n=13)	SVM (n=11)
Diet		Broiler finisher[1] in afternoon, Pig grower[2] in afternoon, Grass in morning, Coconut fibre *ad libitum*	Broiler finisher in morning, Grass & legumes occasionally	Rabbit pellets[3] *ad libitum*, Grass offered three times per week
Housing		Wire-mesh cages, 60 cm above ground	Cages made of wire mesh and wood, 60 cm above ground, Some double-decker cages	Double-decker wire-floored cages in air-conditioned building
Breed samples	NZW	39	9	11
	Mixed	7	4	0
Gender samples	Male	30	3	7
	Female	16	10	4
Maturity samples	Juvenile	27	1	0
	Adult	19	12	11

[1]Broiler finisher: crude protein, 18.5%; crude fat, 4.0%; crude fibre, 5.0%; [2]Pig grower: crude protein, 16.0%; crude fat, 2.5%; crude fibre, 7.0%; [3]Rabbit pellets: crude protein, 17.0%; crude fat, 3.0%; crude fibre, 13.0% ; UFS = University Field Station; ECIAF = Eastern Caribbean Institute of Agriculture and Forestry; SVM = School of Veterinary Medicine.

Beckman centrifuge TJ – 6 at 2500 rpm for 10 minutes. The serum was then extracted, placed in separate collecting tubes, refrigerated at 4°C and analyzed within 24 hours. Samples from the purple top tube, after adequate mixing, were used to make smears. Smears were then stained using the method outlined by Benjamin (1978).

Complete blood count determination

Manual determinations in this study were made using the methods outlined by Benjamin (1978). The packed cell volume (PCV_m) was determined using the Autocrit Ultra 3-speed centrifuge (11700 rpm for 5 minutes) and the manual haemoglobin (Hb_m) was determined using the Leica haemoglobinometer. The mean corpuscular haemoglobin concentration ($MCHC_m$) was calculated by dividing the Hb_m (g/L) value by the PCV_m (L/L) value. The Leica Refractometer (TS meter) was used for total protein measurement. Determination of the white blood cell count (WBC_m) was performed using the Hausser Scientific Brightline Haemocytometer, and the differential WBC was determined by manual examination of blood smears. For the automated method, erythron and leukon parameters were established using the Sysmex K4500 Haematology Analyzer (Channel 4). An automated differential WBC was not available.

Serum chemistry

The Menarini Spotlyte Na/K/Cl Analyzer was used to determine the sodium (Na), potassium (K) and chloride (Cl) concentrations. The Menarini Classic Chemistry Analyzer was used to determine the calcium (Ca), phosphorus (P), magnesium (Mg), blood urea, creatinine, total bilirubin, total protein, albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine phosphokinase (CPK), cholesterol, glucose, amylase, and gamma-glutamyl transferase (GGT). The globulin concentration was determined by subtracting the albumin concentration from the total protein concentration.

The ambient temperature and relative humidity during the four-month sampling period, remained relatively constant. Monthly average ambient temperature (max) ranged from 31.2 °C to 32.4 °C, while monthly average relative humidity ranged from 71 % to 77 % (meteorological data, 2002).

Statistical analysis

The effects of breed, maturity, gender, and husbandry practices on response variables were analyzed using analysis of variance (ANOVA). Two-way interaction terms were included in the model. Means for the three farms were separated using the Tukey procedure. The type 1 error was set at $\alpha = 0.05$ and analyses were performed using the general linear model procedure of SAS (1988). Main effects were discussed only when their interaction terms were not significant.

Values for the manual and automated methods were compared using a paired t-test and the data analysed using the means procedure of SAS (1988). The type 1 error was set at $\alpha = 0.05$. Reference values in the literature (Flecknell, 2000) were compared to 95% confidence intervals for parameters in the present study. Only ranges outside of the reference values were considered significantly different.

RESULTS

The erythron

The effects of rabbitry, maturity, breed, and gender on the erythron are presented in Tables 2 and 3. There were significant differences among the three rabbitries in respect of PCV_a (Table 2). Rabbits at the SVM had higher PCV_a values than those of the other two rabbitries ($P<0.05$). The values for $MCHC_a$ were lower for SVM rabbits when compared to those values for the other rabbitries ($P<0.05$). In the case of RBC the only significant difference was between the SVM and ECIAF, the former with higher values. In the case of maturity effects, adult rabbits showed higher values than their juvenile

Table 2: Effects of rabbitry and maturity on erythron values (least squares means and standard errors)

Variable[2]	Rabbitry[1]				Maturity		
	UFS	SVM	ECIAF	*P*-value	Adults	Juveniles	*P*-value
Hb_m (g/L)	107.4±2.4	117.1±3.7	110.5±4.4	0.09	110.8±2.5	112.5±3.3	0.64
Hb_a (g/L)	120.8±2.6	127.6±4.1	119.4±4.9	0.29	127.7±2.8	117.5±3.7	0.01
PCV_m (L/L)	0.360±0.010	0.370±0.010	0.360±0.010	0.45	0.360±0.010	0.360±0.010	0.45
PCV_a (L/L)	$0.340±0.010^b$	$0.390±0.010^a$	$0.330±0.010^b$	0.00	0.360±0.010	0.340±0.010	0.03
$MCHC_m$ (g/L)	301.9±5.1	314.70±0.40	307.3±9.5	0.40	306.6±5.4	309.3±7.1	0.73
$MCHC_a$ (g/L)	$354.6±3.6^a$	$331.0±5.3^b$	$357.9±6.3^a$	0.00	306.6±5.4	309.3±7.1	0.73
RBC (x 10^{12}/L)	$5.40±0.10^{ab}$	$6.00±0.20^a$	$5.30±0.20^b$	0.03	5.80±0.10	5.30± 0.20	0.03
MCV (fl)	63.0±0.6	65.0±1.0	64.0±1.1	0.21	63.4±0.7	64.4±0.9	0.38
MCH (pg)	22.40±0.30	21.50±0.13	23.00±0.60	0.13	21.50±0.50	23.00±0.60	0.74

Means not sharing the same letter were significantly different at $P<0.05$. [1]UFS, University Field Station; SVM, School of Veterinary Medicine; ECIAF, Eastern Caribbean Institute of Agriculture and Forestry. [2]Hb, haemoglobin; PCV, packed cell volume; MCHC, mean corpuscular haemoglobin concentration; RBC, red blood cell count; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; subscript 'a' = automated; subscript 'm' = manual

counterparts for Hb_a, PCV_a and RBC ($P<0.05$). Crossbred rabbits had significantly higher values than NZW rabbits for the Hb_a, PCV_a and RBC (Table 3). Gender differences were noted, with males having higher values than females for Hb_m, Hb_a, PCV_m, PCV_a and RBC ($P<0.05$).

The leukon

The effects of the independent variables on the leukon are shown in Tables 4 and 5. Significant differences were observed among the three rabbitries for segmented neutrophils, lymphocytes, eosinophils, basophils and platelets (Table 4). In the case of segmented neutrophils, rabbits from the ECIAF had higher values than those for the other two rabbitries. Rabbits at the UFS had higher lymphocyte counts than those at the ECIAF ($P<0.05$). Eosinophil counts were significantly higher in the SVM rabbits than in the ECIAF rabbits. In the case of basophils, rabbits at the SVM had higher

Table 3: Effects of breed and gender on erythron values (least squares means and standard errors)

Variable[2]	Breed[1]			Gender		
	NZW	Mixed	*P*-value	Male	Female	*P*-value
Hb_m (g/L)	110.2±2.2	113.1±3.8	0.50	116.7±2.7	106.6±2.9	0.00
Hb_a (g/L)	117.3±2.4	127.9±4.3	0.03	128.4±3.0	116.8±3.2	0.00
PCV_m (L/L)	0.360± 0.010	0.370±0.010	0.49	0.370±0.010	0.350±0.010	0.03
PCV_a (L/L)	0.340± 0.010	0.370±0.010	0.03	0.370±0.010	0.340±0.010	0.00
$MCHC_m$ (g/L)	306.2±4.7	309.7±8.3	0.70	313.9±5.8	302.0±6.2	0.08
$MCHC_a$ (g/L)	345.6±3.1	350.0±5.5	0.47	349.4±3.8	346.3±4.1	0.48
RBC (x 10^{12}/L)	5.20±0.10	5.80±0.20	0.01	5.80±0.10	5.30±0.20	0.00
MCV (fl)	65.0±0.6	63.0±1.0	0.06	63.8±0.7	64.2±0.7	0.57
MCH (pg)	22.50±0.30	22.10±0.50	0.47	22.30±0.40	22.30±0.40	0.99

[1]NZW = New Zealand White; Mixed = crossbreds consisting of at least two breeds: NZW, Californian, Checkered Giant; [2]Hb, haemoglobin; PCV, packed cell volume; MCHC, mean corpuscular haemoglobin; concentration; RBC, red blood cell count; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; subscript 'a' = automated; subscript 'm' = manual.

Table 4: Effects of rabbitry and maturity on leukon values (least squares means and standard errors)

Variable[2]	Rabbitry[1]				Maturity		
	UFS	SVM	ECIAF	*P*-value	Adults	Juveniles	*P*-value
WBC_m (x 10^9/L)	7.90±0.50	7.90±0.80	9.90±0.90	0.09	8.80±0.50	8.30±0.70	0.51
WBC_a (x 10^9/L)	8.50±0.50	7.90±0.80	9.70±0.90	0.06	9.10±0.50	7.90±0.70	0.11
Neutrophils-Segs (x 10^9/L)	3.80±0.40^b	3.80±0.60^b	7.10±0.70^a	0.00	5.70±0.40	4.10±0.60	0.01
Lymphocytes (x 10^9/L)	3.40±0.30^a	2.80±0.40^{ab}	1.70±0.50^b	0.00	2.10±0.30	3.20±0.40	0.01
Eosinophils (x 10^9/L)	0.09±0.02^{ab}	0.14±0.03^a	0.03±0.03^b	0.03	0.07±0.02	0.10±0.03	0.40
Monocytes (x 10^9/L)	0.40±0.10	0.40±0.20	0.30±0.20	0.68	0.40±0.10	0.30±0.10	0.34
Basophils (x 10^9/L)	0.20±0.30^b	0.50±0.10^a	0.02±0.06^b	0.00	0.20±0.04	0.20±0.05	0.81
Total Protein (x 10^9/L)	63.0±1.1^b	66.4±1.6^{ab}	70.0±2.0^a	0.00	66.3±1.1	66.6±1.5	0.87
$Platelets_a$ (x 10^9/L)	313.8±31.5^a	168.3±48.9^c	294.4±33.3^b	0.00	373.2±43.9	339.7±51.1	0.10

Means within a row followed by the same letter are not significantly different (P> 0.05). [1]UFS, University Field Station; SVM, School of Veterinary Medicine; ECIAF, Eastern Caribbean Institute of Agriculture and Forestry. [2]WBC = white blood cell count; subscript 'a' = automated; subscript 'm' = manual

counts than those at the other two rabbitries (P<0.05). Platelet counts were highest in the UFS rabbits, followed by the ECIAF rabbits, with the SVM rabbits showing the lowest counts (P<0.05).

Segmented neutrophils showed higher values for adult rabbits than for juveniles, whereas lymphocyte counts were higher for juveniles than for adults (P<0.05). The only response variable in which there was a breed effect was WBC_a where crossbred rabbits had a higher count than NZW rabbits (Table 5). No gender effects were observed for the leukon in this study.

Serum chemistry

The effects of the independent variables on serum chemistry parameters are presented in Tables 6 and 7. Significant differences were found between rabbitries in the case of K, P, Ca, creatinine, bilirubin, AST, ALT, and amylase (Table 6). In the case of creatinine and AST, differences were

Table 5: Effects of breed and gender on leukon values (least squares means and standard errors)

Variable[2]	Breed[1]			Gender		
	NZW	Mixed	*P*-value	Male	Female	*P*-value
WBC_m (x 10^9/L)	8.00±0.50	9.20±0.80	0.18	8.90±0.60	8.20±0.60	0.32
WBC_a (x 10^9/L)	7.30±0.40	9.60±0.80	0.01	8.60±0.50	8.30±0.60	0.55
Neutrophils-Segs (x 10^9/L)	4.30±0.40	5.60±0.70	0.07	5.00±0.50	4.8±0.50	0.67
Lymphocytes (x 10^9/L)	2.70±0.20	2.60±0.40	0.81	2.80±0.30	2.50±0.30	0.37
Eosinophils (x 10^9/L)	0.07±0.02	0.10±0.03	0.40	0.08±0.02	0.09±0.02	0.82
Monocytes (x 10^9/L)	0.40±0.10	0.40±0.20	0.96	0.40±0.10	0.30±0.10	0.59
Basophils (x 10^9/L)	0.20±0.03	0.20±0.05	0.55	0.30±0.04	0.20±0.04	0.35
Total Protein (x 10^9/L)	65.0±1.0	67.9±1.7	0.12	67.4±1.2	65.4±1.3	0.13
$Platelets_a$ (x 10^9/L)	327.9±28.9	337.9±28.9	0.83	328.9±35.6	328.7±38.1	0.81

[1]NZW = New Zealand White; Mixed = crossbreds consisting of at least two breeds: NZW, Californian, Checkered Giant.
[2]WBC = white blood cell count, subscript 'a' = automated; subscript 'm' = manual

observed between the UFS and the SVM rabbits with the UFS rabbits having higher values for the former and the SVM rabbits having higher values for the latter variable ($P<0.05$). Potassium values were higher for the UFS rabbits when compared to the ECIAF rabbits ($P<0.05$). Phosphorus values were highest for the ECIAF and lowest for the SVM with the UFS values intermediate ($P<0.05$). Amylase values were highest for the SVM rabbits, followed by the ECIAF and the UFS, respectively. Alanine aminotransferase values were lowest for the ECIAF rabbits when compared to the other two rabbitries. Bilirubin values were higher for the UFS compared to the ECIAF and the SVM. Rabbits from the SVM rabbitry were found to have higher serum calcium concentrations than those at the ECIAF. Juveniles were found to have significantly higher serum biochemical values for AST, cholesterol, glucose, and phosphorus. Adults on the other hand had higher values for chloride ($P<0.05$).

The only breed effect on serum chemistry was found for CPK where crossbred rabbits had higher values than their NZW counterparts (Table 7). Males showed significantly higher values for potassium, total protein, and albumin. Females, on the other hand, had higher serum cholesterol values than males ($P<0.05$).

Table 6: Effects of rabbitry and maturity on serum chemistry values (least squares means and standard errors)

Variable[1]	Rabbitry[2]				Maturity		
	UFS	SVM	ECIAF	*P*-value	Adults	Juveniles	*P*-value
Na (mmol/L)	140.5±0.8	141.2±1.2	141.5±1.4	0.79	141.7±0.8	140.5±1.1	0.31
K (mmol/L)	6.00±0.20^{a}	5.40±0.30^{ab}	5.00±0.40^{b}	0.02	5.50±0.20	5.50±0.30	0.94
Cl (mmol/L)	104.9±0.8	103.2±1.3	103.0±1.5	0.34	105.6±0.8	101.8±1.1	0.00
P (mmol/L)	1.60±0.10^{b}	1.20±0.10^{c}	3.50±0.10^{a}	0.00	2.00±0.10	2.20±0.10	0.02
Mg (mmol/L)	0.90±0.03	0.90±0.10	0.90±0.05	0.30	0.80±0.04	0.80±0.05	0.81
Ca (mmol/L)	3.70±0.10^{ab}	3.90±0.10^{a}	3.50±0.10^{b}	0.01	3.60±0.10	3.80±0.10	0.16
Urea (mmol/L)	4.9±0.9	5.50± 0.80	7.2±1.1	0.13	6.3±0.7	5.4±1.0	0.45
Creatinine (µmol/L)	162.7±8.5^{a}	117.3±12.6^{b}	156.5±15.0^{ab}	0.01	154.3±8.7	136.6±11.5	0.16
Bilirubin (µmol/L)	6.3±1.5^{a}	6.1±2.4^{b}	6.0±2.9^{b}	0.00	10.7±1.6	8.2±2.1	0.29
Total protein (g/L)	70.5±1.4	70.9±2.2	69.7±2.6	0.95	70.4±1.5	70.3±2.0	0.96
Albumin (g/L)	52.5±0.9	54.7±1.5	50.6±1.7	0.15	52.9±1.0	52.3±1.3	0.68
Globulin (g/L)	18.0±1.1	16.3±1.7	19.1±2.0	0.51	17.6±1.2	18.0±1.5	0.82
ALP (U/L)	107.7±19.8	148.2±30.7	164.7±36.5	0.26	116.0±20.9	164.4±27.5	0.11
AST (U/L)	35.1±5.3^{b}	69.4±9.0^{a}	52.3±9.7^{ab}	0.01	44.2±5.6	60.4±7.3	0.04
ALT (U/L)	92.2±7.9^{a}	105.9±12.7^{a}	50.0±14.5^{b}	0.01	79.3±8.3	86.0±10.9	0.58
CPK (U/L)	708±95	1020±147	1049±74	0.08	1006±100	845±132	0.27
Cholesterol (mmol/L)	2.10±0.20	2.00±0.30	1.70±0.40	0.65	1.60±0.20	2.30±0.30	0.03
Glucose (mmol/L)	7.30±0.20	6.60±0.30	6.90±0.30	0.10	6.60±0.20	7.20±0.20	0.03
Amylase (U/L)	354.0±26.9^{c}	694.9±42.2^{a}	510.3±49.5^{b}	0.00	512.4±38.4	527.1±37.5	0.72
GGT (U/L)	0.40±0.70	3.30±1.1	0.00±1.20	0.06	1.10±0.70	1.30±1.0	0.85

Means within a row followed by the same letter are not significantly different (P> 0.05) [1] ALP=alkaline phosphatase, AST=aspartate aminotransferase, ALT=alanine aminotransferase , CPK=creatine phosphokinase, GGT=gamma-glutamyl transferase . [2] UFS, University Field Station; SVM, School of Veterinary Medicine; ECIAF, Eastern Caribbean Institute of Agriculture and Forestry

Table 7: Effects of breed and gender on serum chemistry values (least squares means and standard errors)

Variable[1]	Breed[2]			Gender		
	NZW	Mixed	*P*-value	Male	Female	*P*-value
Na (mmol/L)	140.0±0.7	142.3±1.2	0.08	142.0±0.9	140.1±0.9	0.07
K (mmol/L)	5.20±0.20	5.80±0.30	0.09	5.80±0.20	5.20±0.20	0.02
Cl (mmol/L)	103.8±0.7	103.6±1.3	0.89	104.3±0.9	103.1±1.0	0.24
P (mmol/L)	2.10±0.10	2.10±0.10	0.82	2.00±0.10	2.20±0.10	0.08
Mg (mmol/L)	0.80±0.03	0.90±0.10	0.65	0.80±0.04	0.80±0.04	1.00
Ca (mmol/L)	3.70±0.05	3.70±0.09	0.48	3.70±0.07	3.70±0.06	0.85
Urea (mmol/L)	6.30±0.40	5.40±1.20	0.46	6.00±0.80	5.70±0.80	0.77
Creatinine (µmol/L)	134.8±7.4	156.1±13.6	0.15	148.0±9.4	143.0±9.8	0.64
Bilirubin (µmol/L)	8.9±1.4	10.0±2.5	0.69	10.9±1.7	8.0±1.9	0.14
Total protein (g/L)	68.3±1.3	72.5±2.3	0.10	72.4±1.6	68.3±1.7	0.03
Albumin (g/L)	51.0±0.9	54.1±1.5	0.07	54.1±1.1	51.1±1.2	0.02
Globulin (g/L)	17.3±1.0	18.3±1.8	0.59	18.4±1.2	17.2±1.3	0.39
ALP (U/L)	111.1±18.1	69.3±32.1	0.10	123.9±22.3	156.5±23.9	0.20
AST (U/L)	52.1±5.1	52.4±8.5	0.96	51.6±6.0	53.0±6.4	0.84
ALT (U/L)	86.6±7.3	78.8±12.7	0.58	83.2±9.0	82.2±9.5	0.93
CPK (U/L)	713±87	1138±153	0.01	1017±107	835±114	0.14
Cholesterol (mmol/L)	1.80±0.20	2.10±0.30	0.42	1.60±0.20	2.30±0.20	0.02
Glucose (mmol/L)	6.70±0.20	7.20±0.30	0.09	6.70±0.20	7.10±0.20	0.07
Amylase (U/L)	492.4±25.5	547.0±43.2	0.26	506.0±30.6	533.5±32.8	0.44
GGT (U/L)	1.80±0.60	0.70±1.10	0.37	1.00±0.80	1.43±0.80	0.63

[1]ALP=alkaline phosphatase, AST=aspartate aminotransferase, ALT=alanine aminotransferase, CPK=creatine phosphokinase, GGT=gamma-glutamyl transferase. [2]NZW = New Zealand White; Mixed = crossbreds consisting of at least two breeds: NZW, Californian, Checkered Giant

Comparison of ranges in present study with established reference ranges

The 95% CI for most parameters studied fell within the normal ranges found in the literature (Tables 8, 9, and 10). Urea levels were found to be lower than those found in the literature (Table 10). On the other hand albumin and CPK levels were higher than the values quoted in the literature (Table 10).

Comparison of manual and automated values

Results for the comparison of manual and automated values are presented in Table 11. Manual values were significantly higher for PCV, while automated values were higher for Hb and MCHC. No difference was observed between automated and manual values of WBC ($P>0.05$).

DISCUSSION

The effects of gender, breed, age and husbandry practices on blood parameters

The erythron.

Rabbits at the SVM tended to have higher values for a number of erythron parameters. Some of these differences were significant (RBC and PCV_a), while others were numerical only. However, the

Table 8: Comparison of normal erythron values from literature with 95% confidence interval (CI) for the variables in the present study

Variable[3]	Values from literature[1]	95% CI for present study	Comments
Hb (g/L)	100 - 175	106 - 112	NS[2]
PCV (L/L)	0.34 - 0.50	0.36 - 0.36	NS
MCHC (g/L)	280 - 360	298 - 310	NS
RBC ($x10^{12}$/L)	4.7 - 7.2	5.2 - 5.5	NS
MCV (fl)	50 - 75	63 - 65	NS
MCH (pg)	16 - 23	22 - 23	NS
Platelets ($x10^9$/L)	200 - 1000	262 - 351	NS

[1]Flecknell (2000). [2]NS, not significant at $\alpha = 0.05$. [3]Hb, haemoglobin; PCV, packed cell volume; MCHC, mean corpuscular haemoglobin concentration; RBC, red blood cell count; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin

differences may be related to management practices. The SVM was found to use anthelmintics more often than the other rabbitries. A high parasitic load may result in lower RBC and PCV values as a result of chronic blood loss. None of the rabbitries routinely screened for internal parasites. However, records from the UFS confirmed that coccidiosis had been diagnosed there on past occasions. Notwithstanding the absence of coprological studies, the routine use of anthelmintics at the SVM, may explain the higher values for PCV_a at this rabbitry. The $MCHC_a$ being a calculated value, would tend to increase as PCV_a decreases. The higher $MCHC_a$ values were observed at the rabbitries where the PCV_a values were lower relative to their Hb_a values (UFS and ECIAF).

The effect of maturity on erythron values was significant for Hb_a, PCV_a and RBC. The lack of a significant effect of maturity on manual erythron parameters corroborates the findings of Cazabon *et al*. (2000). Adults had higher values for Hb_a, PCV_a and RBC than their juvenile counterparts. Possible explanations may be the lower body water percentage in adults compared to juveniles, and the greater need for a higher oxygen carrying capacity in adults.

Breed did not have an effect on the manual erythron parameters of Hb and PCV; whereas gender did influence them, with males having the higher values for both variables. Androgens may increase erythropoietin production, while oestrogen may decrease it (Kerr, 2002). Cazabon *et al*. (2000) found no effect of breed or gender on manual erythron parameters in their study. In the case of automated erythron values in the present study, both gender and breed had significant effects with males and mixed breeds having higher values for Hb_a, PCV_a and RBC than females and NZW.

Table 9: Comparison of normal leukon values from literature with 95% confidence interval (CI) for the variables in the present study

Variable[3]	Values from literature[1]	95% CI for present study	Comments
WBC ($x\ 10^9$/L)	5 - 12	7.24 - 8.48	NS[2]
Neutrophils-Segs	1.5 - 4.73	3.44 - 4.66	NS
Lymphocytes ($x10^9$/L)	2 - 20	2.59 - 3.33	NS
Eosinophils ($x10^9$/L)	0 - 7.0	0.06 - 0.10	NS
Monocytes ($x10^9$/L)	0 - 1.8	0.31 - 0.53	NS
Basophils ($x10^9$/L)	0 - 8.4	0.16 - 0.27	NS
Protein (g/L)	49 - 71	62.36 - 65.15	NS

[1] Flecknell (2000). [2] NS, not significant at $\alpha = 0.05$. [3] WBC = white blood cell count

Table 10: Comparison of normal serum chemistry values from literature with 95% confidence interval (CI) for the variables in the present study

Variable[1]	Values from literature[2]	95% CI for present study	Comments
Na (mmol/L)	130 - 155	139- 141	NS[3]
K (mmol/L)	3.3 - 5.7	5.3 - 5.9	NS
Cl (mmol/L)	92 - 120	104 - 106	NS
P (mmol/L)	0.9 - 2.4	1.6- 2.0	NS
Mg (mmol/L)	0.8 - 1.2	0.8 - 0.90	NS
Ca (mmol/L)	2.2 - 4.6	3.6 - 3.8	NS
Urea (mmol/L)	9.3 - 25.5	5.5 - 7.0	*
Creatinine (µmol/L)	70 - 150	137 - 158	NS
Bilirubin (µmol/L)	4.3 - 12.8	6.0 - 10.1	NS
Total protein (g/L)	28 - 100	67- 71	NS
Albumin (g/L)	33 - 50	51 - 53	*
Globulin (g/L)	15 - 27	16 - 19	NS
ALP (U/L)	100 - 400	74 - 123	NS
AST (U/L)	33 - 99	35 - 48	NS
ALT (U/L)	55 - 260	80 - 100	NS
CPK (U/L)	140 - 372	573 - 822	*
Cholesterol (mmol/L)	0.1 - 2.0	1.6 - 2.1	NS
Glucose (mmol/L)	4.2 - 8.3	6.6 - 7.1	NS
Amylase (U/L)	200 - 500	369 - 460	NS
GGT (U/L)	0 - 5	0.36 - 2.06	NS

[1]ALP=alkaline phosphatase, AST=aspartate aminotransferase, ALT=alanine aminotransferase, CPK=creatine phosphokinase, GGT=gamma-glutamyl transferase. [2]Flecknell (2000). [3]NS, not significant at $\alpha = 0.05$. *, significant at $\alpha = 0.05$

The leukon.

There were differences among the three rabbitries for neutrophil, lymphocyte, eosinophil and basophil counts. The highest neutrophil count was observed at the ECIAF; while the highest basophil count was at the SVM. The high neutrophil count at the ECIAF did not appear to be associated with any on-going disease process since no adverse effect on productivity had been recorded at this rabbitry. There was also no increase in the number of band neutrophils or lymphocytes.

Table 11: Comparison of manual and automated counts for Hb, PCV, MCHC and WBC

	Method		
Variable[1]	Manual	Automated	*P*-value
Hb (g/L)	108.9±3.0	119.8±3.6	0.0001
PCV (L/L)	0.360± 0.001	0.340± 0.001	0.0004
MCHC (g/L)	304.2± 6.1	350.1±4.5	0.0001
WBC (x 10^9/L)	7.90± 0.60	7.80± 0.60	0.7724

[1] Hb, haemoglobin; PCV, packed cell volume; MCHC, mean corpuscular haemoglobin concentration; WBC, white blood cell count

The higher eosinophil count observed at the SVM relative to the ECIAF and the higher basophil count at the former rabbitry may be related to a greater exposure to allergens at the SVM. Rabbits at the SVM are housed in an enclosed air-conditioned building with other animal species such as guinea pigs, laboratory rats and mice. Allergens may therefore accumulate in the building and predispose to hypersensitivity reactions. The only apparent explanation for the higher lymphocyte count at the UFS relative to the ECIAF was the chronic exposure to antigens associated with the ducks that were allowed to roam freely throughout the rabbitry. The differences do not appear to be of any clinical importance based on reference ranges.

The lower platelet count observed for the SVM may have been due to poor sampling technique in the earlier stages of the study. The first batch of blood samples was collected at the SVM and there may have been more clot formation than in successive samples.

The plasma protein level for the ECIAF was the only one outside of the 95% confidence interval for this blood parameter in the present study. The higher value obtained may have been a result of dehydration since a number of the nipple drinkers at this rabbitry were malfunctioning. There is, however, a greater degree of subjectivity associated with plasma protein concentrations determined by the refractometry method when compared to the biuret method. Protein levels determined by the latter method showed no difference among rabbitries. No obvious explanation existed for the higher neutrophil counts and lower lymphocyte counts in adult rabbits.

A number of findings for the effect of independent variables on the leukon are at variance with those in the study done by Cazabon *et al*. (2000). The latter researchers reported a breed effect for lymphocyte count and WBC_m. They also found a gender effect on monocyte and basophil counts. There was no gender effect on leukon parameters in the present study. A maturity effect was also reported for monocyte count in that study. These differences may be due, in part, to the fact that the earlier study focussed on only one of the three rabbitries used in the present study.

Blood chemistry.
There were a number of differences among the rabbitries for several blood chemistry parameters. These differences may have been related to differences in husbandry practices; thus the higher K value for the UFS may have been associated with the feeding of coconut fibre which has a high percentage of K (Child, 1974).

Higher levels of AST and glucose in juveniles may be due to the effect of handling. Juveniles are not handled as frequently as adults and may respond with higher levels of these two analytes than adults. Handling alone has been shown to cause increase in blood glucose (Knudtzon, 1988), and physical exertion or tissue damage during blood collection will elevate AST levels (Harcourt-Brown, 2002). The higher protein and albumin levels in males may be explained by the anabolic effect of androgens; while the higher cholesterol values in females may be related to the tendency to feed slightly larger quantities to adult females relative to their male counterparts just prior to breeding. Hypercholesterolaemia in domestic rabbits has been reported to be more readily induced by dietary manipulation (Kaneko *et al.*, 1997). The finding of higher cholesterol levels in females is corroborated by Loeb and Quimby (1999).

Comparison of 95% confidence intervals for parameters studied with existing reference ranges
The 95% confidence intervals for all the haematological parameters studied and for most serum chemistry parameters, were within the reference ranges stated in the literature. The confidence intervals for the present study were narrower than the reference ranges and in many cases were at either the upper or lower end of the reference range. While this observation may not be of major clinical importance, it may be useful in evaluating productivity at various rabbitries. By way of an example,

the reference range for potassium is 3.3 to 5.7 mmol/L. The 95% confidence interval for potassium in the present study is 5.34 to 5.86 mmol/L. The two intervals overlap, but the interval for the present study is clearly at the higher end of the reference values currently used in Trinidad. If rabbits with potassium values in the interval 5.34 to 5.86 mmol/L were to consistently outperform those in the interval between 3.3 and 5.34 mmol/L, then this observation has relevance for the local producer; despite the finding that, at present, rabbits in both categories are considered to be clinically normal.

The three analytes whose 95% confidence intervals were found to differ from the reference ranges in the literature were CPK, urea and albumin. The lower blood urea levels observed in the present study may, in part, be due to increased water intake and increased frequency of urination under the higher environmental temperatures in subtropical Trinidad. While the protein composition of the diet may influence the blood urea level, it is worth noting that crude protein content of the diets used at the three rabbitries was higher than the 14-15% recommended by Gendron (2000) and the 12-16% recommended by Lowe (1998). Other reasons for lower blood urea levels, such as impaired liver function and use of anabolic steroids (Benson and Paul-Murphy, 1999) were also ruled out. Creatine phosphokinase levels may have increased as a result of increased muscle activity from struggling in rabbits that are not frequently handled. Haemolysis can also result in falsely elevated CPK levels (Sonntag, 1986; Latimer *et al*., 2003). The higher albumin concentration observed in the local rabbits may have been associated with lower total body water content (Harcourt-Brown, 2002).

Comparison of results from automated and manual methods

The finding of differences between automated and manually obtained values for Hb, PCV, and MCHC would suggest that veterinary laboratories in developing countries, where resources are often limited and automation is not readily available, should have available two sets of reference values. Further, machines used for the generation of automated values breakdown from time to time and considerable periods can elapse before repairs are effected. A set of manually generated reference values should be used for comparisons if laboratory personnel have to resort to manual determination of haematological values. The differences observed between the two methods may be related to the different approaches used to calculate the respective variables. The automated method relies on the machine; while the manual approach relies more on the technician/researcher conducting the test.

CONCLUSION

The results of this study provide an alternative set of reference values that can be used in the clinical evaluation of local rabbits. The ranges are narrower, suggesting that the estimates are more precise and perhaps of greater value for monitoring herd health at local rabbitries. Further, the sampling of private rabbitries, when owners are more amenable to the idea of on-farm studies, would enhance the validity of estimates obtained for the various blood parameters.

Given the small number of rabbits available at two of the rabbitries, and the possible confounding of the effects of independent variables, the effects on blood parameters reported in the present study are best regarded as suggestive rather than conclusive.

Acknowledgements: The authors wish to thank Ms. Annette Williams and Mr. Lester Gilkes for their assistance with the laboratory aspect of the study. Special thanks also to Dr. Rastogi and Mr. Blackman for allowing us to sample the rabbits at the UFS and the ECIAF. The assistance of the technical staff at the latter institutions is also greatly appreciated. The authors also wish to extend their gratitude to Dr. Vicki Aimey for her invaluable comments and suggestions.

REFERENCES

Benjamin M., 1978. Outline of Veterinary Clinical Pathology, 3rd ed. *Iowa: Iowa State University Press.*

Benson K.G., Paul-Murphy J., 1999. Clinical Pathology of the Domestic Rabbit. *Vet. Clin. N. Am.: Exotic Anim. Pract*., 2, 539-552.

Cazabon E.P.I., Rastogi R.K., Laukner F.B., Ali B.A., 2000. Some haematological values in rabbits from subtropical Trinidad, West Indies. *World Rabbit Sci., 8, 63-65*.

Child R., 1974. Coconuts, 2nd ed. *London: Longman Group.*

Consortium, 1988. Guide for the care and use of agricultural animals in agricultural research and teaching. *Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching*, *Illinois, U.S.A.*

Flecknell P., 2000. Manual of Rabbit Medicine and Surgery. *Gloucester: British Small Animal Veterinary Association.*

Gendron K., 2000. The Rabbit Handbook, *Hong Kong: Barron's Educational Series, Inc.*

Harcourt-Brown F., 2002. Textbook of Rabbit Medicine, *Oxford: Butterworth: Heinemann.*

Kaneko J.J., Harvey W., Bruss M.L., 1997. Clinical Biochemistry of Domestic Animals, 5th ed. *London: Academic Press.*

Kerr M., 2002. Veterinary Laboratory Medicine: Clinical Biochemistry and Haematology, 2nd Ed. *Oxford: Blackwell Publications*.

Knudtzon J., 1988. Plasma levels of glucagon, insulin, glucose and free fatty acids in rabbits during laboratory handling procedures. *Z Versuchstierk*, 26, *123-133*.

Latimer K.S., Mahaffey E.A. Prasse K.W., 2003. Duncan and Prasse's Veterinary Laboratory Medicine, Clinical Pathology, 4th ed. *Iowa: Iowa State University Press*.

Loeb W.F. Quimby F.W., 1999. The Clinical Chemistry of Laboratory Animals, 2nd ed. *Philadelphia*: *Taylor and Francis.*

Lowe J.A., 1998. Pet Rabbit Feeding and Nutrition. In: *de BLAS, C., WISEMAN, J. (ed). The nutrition of the rabbit. CABI Publishing*.

Meteorological data, 2002. *Department of Soil Science, The University of the West Indies, Trinidad.*

Sas, 1988. sas/stat^{O} User's Guide (Release 6.03). *SAS Inst. Inc.*, *Cary, NC, U.S.A.*

Sonntag O., 1986. Haemolysis as an interference factor in clinical chemistry. *J. Clin. Chem. and Clin. Biochem*., 24 (2) *127-139*.