



## Clinical assessment of acid-base balance in Netherland Dwarf rabbit

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### Abstract

Pet rabbits have increased their popularity in a lot of countries. However, most of the laboratory profiles in rabbit medicine come from the observations made in rabbit as biomodels or meat production. So that further researches are necessary to obtain reference values for hematology and biochemical profiles in pet rabbits and the different breeds, especially, in relation to acid-base balance. The aim of this report was to offer the mean values of the main parameters connected with acid-base profile in Netherland Dwarf breed. Thirty-five healthy rabbits (15 males and 20 females) were studied. Venous blood sample from lateral saphenous vein was analyzed to measure: haematocrit, haemoglobin, blood urea nitrogen, glucose, blood pH, partial pressure of CO<sub>2</sub> (pCO<sub>2</sub>), total CO<sub>2</sub>, ions bicarbonate, chloride, sodium, potassium, base excess and anion Gap. Results showed a shorter range than those reported by different researchers. Moreover, differences between genders were showed in pCO<sub>2</sub>, its values were higher in males. It may be associated with a greater cellular metabolism. Values obtained in this research should be taken into account by veterinary clinicians for this breed in their clinical assessments. Besides, these values provide new results in parameters with few reference values.

**Keywords:** pet rabbit, blood sample, portable analyzer, acid-base profile, reference range.

### Avaliação clínica do equilíbrio ácido-básico em coelhos Anã Holandês

#### Resumo

A popularidade de coelhos como animais de estimação aumentou em muitos países. No entanto, a maioria dos perfis de laboratório em medicina de coelhos advém das observações de biomodelos animais ou da produção de carne. Assim, são necessárias pesquisas adicionais para obter valores de referência para hematologia e perfis bioquímicos em coelhos de estimação, e das diferentes raças, especialmente, em relação ao equilíbrio ácido-base. O objetivo deste relatório foi oferecer os valores médios dos principais parâmetros ligados ao perfil ácido-base na raça Anã Holandês. Trinta e cinco coelhos saudáveis (15 machos e 20 fêmeas) foram estudados. A amostra de sangue venoso da veia safena lateral foi analisada para mensuração: hematócrito, hemoglobina, nitrogênio ureico sanguíneo, glicose, pH sanguíneo, pressão parcial de CO<sub>2</sub> (pCO<sub>2</sub>), CO<sub>2</sub> total, íons bicarbonato, cloreto, sódio, potássio, excesso de base e ânion Gap. Os resultados apresentaram um intervalo menor do que aqueles relatados por diferentes pesquisadores. Além disso, as diferenças entre os gêneros foram mostradas na pCO<sub>2</sub>, seus valores foram maiores no sexo masculino. Pode estar associado a um maior metabolismo celular. Os valores obtidos nesta pesquisa devem ser levados em consideração pelos clínicos veterinários para esta raça em suas avaliações clínicas. Além disso, esses valores fornecem novos resultados em parâmetros com poucos valores de referência.

**Palavras-chave:** coelho de estimação, amostra de sangue, analisador portátil, perfil ácido-base, intervalo de referência.

#### 1. Introduction

Rabbit ownership has increased significantly over the past decade. This pet has been bred in captivity for years and is ideal for a variety of pet owners because of their small space requirements, quiet nature, ease of handling and relatively simple husbandry requirements. For this reason, it has been observed an increase in visits to veterinary clinics. However, most of the laboratory profiles in rabbit medicine come from the observations made in

rabbit as experimental biomodels for toxicological and physiological studies (Hewitt et al., 1989; Suckow and Douglas, 1996; Archetti et al., 2008). But the current pet rabbits that arrive to veterinary clinics show differences from those, especially in their environment, nutritional conditions or breeds. For this reason, is necessary to establish proper metabolic profiles, depending on the breed of rabbit.

When clinicians use biochemical profile in conjunction with the history, physical examination, and even other laboratory tests, the chemistry panel may be useful for the establishment of initial baseline analytes for a patient, formulating a rule out list, confirming a diagnosis and/or determining the prognosis (Russell and Roussel, 2007). Reference values showed in books are useful for many hematologic analytes, although their use is limited for interpretation of serum chemistries. For example, many published reference intervals found in textbooks that are established for rabbits often do not distinguish between purpose (production, biomodels in research) or between gender, age or physiological stage (Hewitt et al., 1989; Suckow and Douglas, 1996; Melillo, 2007; Archetti et al., 2008; Jenkins, 2008).

The acid-base balance define the basic principles of the internal balance, necessary to maintain the structure and function of proteins essential for normal progression of metabolic events. Most enzymatic reactions have a narrowly defined range of pH optimum, and changes in hydrogen ion concentration have direct effects on the rates of reaction and, thus, resulting in many basic biological processes (Kaneko et al., 2008). According to Ardiaca et al. (2013), the use of point-of-care blood gas analyzers allows rapid turnaround time of results, improvement of diagnostics, and immediate therapeutic management with potential improvement in outcome. Moreover, stress is reduced by this way because prolonged stress such as transportation or unfamiliar noises are removed (Melillo, 2007; Jenkins, 2008).

To the best of the author's knowledge, there are no reports about biochemical and hematological profiles of the most common bunny pets. In this scenario, the aim of this report is to offer the mean values of the main parameters closely connected with this profile in a very common bunny such as the Netherland Dwarf breed and to assess changes between genders.

## 2. Material and Methods

### 2.1. Animals and housing

Thirty-five healthy rabbits (Netherland Dwarf breed) were used for the study with an average age of 11.5 months old (ranged from 8-16 months) and mean weight 1.18 kg ( $\pm 0.17$ ). Animals were enrolled with owner consent and were divided by sex: male (n=15) and female (n=20). All rabbits were handled in our Department in a quiet and safe place.

Animals were housed in a proper rabbit hutch outside provided with a large enough living area and a secure shelter. Each animal's health condition was assessed by a complete physical examination according to the recommendations made by Varga (2014). All rabbits received the same kind of diet, consisted in concentrate (same pellet brand) and hay. Sanitary management was uniform among animals, with all animals being helminth free and immunized periodically.

### 2.2. Blood sampling

Venous blood samples were collected via the lateral saphenous vein (Melillo, 2007) using a 1-mL syringe and a 25-G needle. The procedure was done without

chemical restraint using a hand-held portable analyzer (i-STAT® 1 Analyzer, East Windsor, NJ, USA) and i-STAT EC8+® cartridges, that have been used in previous studies in rabbits (Ardiaca et al., 2013; Bonvehi et al., 2014; Varga, 2014). The stress effect was minimized using wrap rabbits in a towel such as was described by Varga (2014).

Measured parameters were haematocrit, haemoglobin, blood urea nitrogen (BUN), glucose, blood pH, partial pressure of CO<sub>2</sub> (pCO<sub>2</sub>), total CO<sub>2</sub> (TCO<sub>2</sub>), ions bicarbonate (HCO<sub>3</sub><sup>-</sup>), chloride, sodium, potassium, base excess (BE<sub>ecf</sub>) and anion Gap (AnGap). Less than one minute elapsed between sampling and analysis.

### 2.3. Statistical analyses

All data obtained were analysed using the statistical program SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Data were tested for normal distribution using the Shapiro-Wilk test. All normalized data were subjected to a Student's t test considering sex (male and female) as fixed factors and blood parameters as dependent variables. Those variables not normally distributed were analysed using nonparametric test (the Mann-Whitney U test). Statistical differences were considered significant at  $P < 0.05$ .

## 3. Results

In our study, significant differences were not found in the acid-base parameters between genders (Table 1 and 2), with the exception of pCO<sub>2</sub>. PCO<sub>2</sub> values in males (46.2 mm Hg) were higher than values in females (41.85 mm Hg).

Regarding electrolyte (Table 1) and haematological values (haematocrit and haemoglobin; Table 2), these parameters did not show difference between sex and their values were practically identical. Moreover, BUN showed a similar range between male (6.39-7.32 mmol/L) and female (6.53-7.54 mmol/L) and a width range was obtained in the glucose values in both sexes. Despite of this fact, no significant differences were observed in those two parameters.

## 4. Discussion

The acid-base parameters (pH, TCO<sub>2</sub>, pCO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, BE<sub>ecf</sub>, AnGap) and electrolytes analysed in our study remained within physiological ranges given by several authors for other breeds of rabbit (Suckow and Douglas, 1996; Flecknell, 2002; Ardiaca et al., 2013; Williams et al., 2014; Leineweber et al., 2018), except data reported by Suckow and Douglas (1996) for pCO<sub>2</sub> values. Our study has shown pCO<sub>2</sub> values higher than their study. However, range reference reported by Suckow and Douglas (1996) was referred to laboratory animals. On other hand, Ardiaca et al. (2013) have studied pet rabbits with a similar range in relation to our results. Moreover, other acid-base parameters such as pH or HCO<sub>3</sub><sup>-</sup> were within the normal range as we have cited before.

Acid-base parameters should be analysed together because, for example, neutral pH could be shown in a concurrent respiratory alkalosis and metabolic acidosis

**Table 1.** Venous blood acid-base balance and electrolyte profile of Netherland Dwarf rabbit.

Parameter	Gender	95% RI	$\bar{x}$	SD	Range	P-value
pH	Male	7.32-7.41 <sup>†</sup>	7.36	0.07	7.28 -7.44	n.s.
	Female	7.39-7.42 <sup>†</sup>	7.40	0.04	7.35 - 7.46	
HCO <sub>3</sub> (mmol/L)	Male	24.85-26.31 <sup>†</sup>	25.58	1.03	23.90 - 26.90	n.s.
	Female	23.61-26.37 <sup>†</sup>	24.99	2.94	20.90 - 30.30	
BEecf (mmol/L)	Male	-2.00-(2.00) <sup>‡</sup>	0	1.49	-2.00-(2.00)	n.s.
	Female	-5.00-(6.00) <sup>‡</sup>	0	3.27	-5.00-(6.00)	
TCO <sub>2</sub> (mmol/L)	Male	25.00-27.00 <sup>‡</sup>	27.00	0.84	25.00-27.00	n.s.
	Female	22.00-32.00 <sup>‡</sup>	26.00	3.02	22.00-32.00	
PCO <sub>2</sub> (mm Hg)	Male	37.60-54.80 <sup>*b</sup>	46.20	6.00	37.60-54.80	0.022
	Female	34.60-45.30 <sup>*‡</sup>	41.85	3.41	34.60-45.30	
AnGap (mmol/L)	Male	15.00-19.00 <sup>‡</sup>	16.00	1.55	15.00-19.00	n.s.
	Female	15.00-20.00 <sup>‡</sup>	18.00	2.11	15.00-20.00	
K (mmol/L)	Male	4.05-4.31 <sup>†</sup>	4.18	0.18	4.00-4.50	n.s.
	Female	4.05-4.35 <sup>†</sup>	4.20	0.32	3.60-4.60	
Na (mmol/L)	Male	141.00-145.00 <sup>‡</sup>	143.00	1.40	141.00-145.00	n.s.
	Female	141.00-144.00 <sup>‡</sup>	143.00	0.92	141.00-144.00	
Cl (mmol/L)	Male	102.00-105.00 <sup>‡</sup>	104.00	1.15	102.00-105.00	n.s.
	Female	99.00-108.00 <sup>‡</sup>	104.00	2.63	99.00-108.00	

RI, Reference Interval;  $\bar{x}$ , mean; SD, standard deviation; BEecf, base excess extracellular fluid; TCO<sub>2</sub>, total CO<sub>2</sub>; pCO<sub>2</sub>, partial pressure of CO<sub>2</sub>; AnGap, anion Gap; n.s., not significant; \*P < 0.05; <sup>†</sup>parametric robust method; <sup>‡</sup>nonparametric percentile method.

**Table 2.** Haematological and biochemical values in venous blood of Netherland Dwarf rabbit.

Parameter	Gender	95% RI	$\bar{x}$	SD	Range	P-value
Ht (%)	Male	34.00-43.00 <sup>‡</sup>	35.50	3.43	34.00-43.00	n.s.
	Female	33.00-38.00 <sup>‡</sup>	36.00	1.91	33.00-38.00	
Hb (g/dL)	Male	11.60-14.60 <sup>‡</sup>	12.2	1.16	11.60-14.60	n.s.
	Female	11.20-12.90 <sup>‡</sup>	12.05	0.64	11.20-12.90	
BUN (mmol/L)	Male	6.39-7.32 <sup>†</sup>	6.85	0.65	5.71-7.50	n.s.
	Female	6.53-7.54 <sup>†</sup>	7.03	1.07	5.35-8.92	
Glu (mmol/L)	Male	6.38-8.80 <sup>‡</sup>	7.48	0.89	6.38-8.80	n.s.
	Female	5.89-10.07 <sup>‡</sup>	7.04	1.33	5.89-10.07	

RI, Reference Interval;  $\bar{x}$ , mean; SD, standard deviation; Ht, haematocrit; Hb, haemoglobin; Glu, glucose; BUN, blood urea nitrogen; n.s., not significant; <sup>†</sup>parametric robust method; <sup>‡</sup>nonparametric percentile method.

(Ardiaca et al., 2013). PH levels are associated with HCO<sub>3</sub> and pCO<sub>2</sub> serum values. PCO<sub>2</sub> is linked to the respiratory function. Moreover, CO<sub>2</sub> is produced by cellular metabolism. The HCO<sub>3</sub>, together with BEecf, usually indicate metabolic imbalances (Kaneko et al., 2008; Irizarry and Reiss, 2009; Ardiaca et al., 2013).

In our study, significant differences were not found in the acid-base parameters between genders, with the exception of pCO<sub>2</sub>. PCO<sub>2</sub> values in males (46.2 mm Hg) were higher than values in females (41.85 mm Hg). This difference could be associated with a greater cellular metabolism in males, which raises the amount of CO<sub>2</sub> in blood (Kaneko et al., 2008; Ardiaca et al., 2013); in this sense, Freitas et al. (2010) suggest that the energy metabolism of insectivorous bats may be affected by sex. Moreover, other studies have showed or suggested that sex can influence many factors related to a species (Terrien et al., 2011; Friedrich et al., 2013; Sandoval Salinas et al., 2017).

Regarding blood electrolyte values (Table 1), Özkan et al. (2012) did not observe statistical differences for electrolyte between male and female New Zealand rabbits, except serum potassium levels. In our study, no statistical difference was observed. When blood electrolyte imbalances appear, acid-base alterations should be kept in mind because rabbits have a complex gastrointestinal physiology and a limited kidney function (Melillo, 2007; Varga, 2014). For example, higher potassium levels due to metabolic acidosis or alkalosis, can either increase or reduce the exchange of potassium ions across the cell membrane (Melillo, 2007); or low chloride serum concentration, which increases HCO<sub>3</sub>; but only one-third of hypochloremic rabbits showed this compensation. Ardiaca et al. (2013) suggest that it may be due to the concurrent hyponatremia in this species.

Results of haematological values (Table 2) were within the range of normal values defined for these parameters by previous studies in other breeds (Hewitt et al., 1989;

Suckow and Douglas, 1996; Benson and Paul-Murphy, 1999; Flecknell, 2002; Melillo, 2007; Jenkins, 2008; Putwain, 2010; Özkan et al., 2012; Ardiaca et al., 2013; Bonvehi et al., 2014; Varga, 2014; Moore et al., 2015; Leineweber et al., 2018) and similar to values obtained by Šimek et al. (2017) in Netherland Dwarf rabbits females (35-39%). Melillo (2007) and Varga (2014) consider that the normal range of haematocrit in pet rabbit has values from 30 to 40% and those values higher than 45% may indicate dehydration due to gastrointestinal stasis, trichobezoar or malocclusion. Haematological results obtained in our research were higher than 30% and lower than 45%. Besides, haemoglobin concentrations were in the range from 11.20 to 14.60 g/dL and they were within the values showed by Šimek et al. (2017) in Netherland Dwarf rabbits females. Some studies have shown that erythrocytes, haematological and haemoglobin values in male rabbits were higher than in female (Özkan et al., 2012; Moore et al., 2015). However, our results were not statistically significant differences.

Blood urea nitrogen concentrations (Table 2) did not differ from the previously reported reference values in other rabbit breeds (Hewitt et al., 1989; Suckow and Douglas, 1996; Jenkins, 2008; Özkan et al., 2012; Ardiaca et al., 2013; Bonvehi et al., 2014; Leineweber et al., 2018). In those studies, range of urea blood values is very wide because physiological factors, such as dietary protein concentrations, liver function or natural circadian rhythms, can affect its values (Melillo, 2007). Our results showed a shorter range between sexes (Table 2). The same kind of diet could be shortening its variability. No statistically significant differences were observed between males and females. Özkan et al. (2012) did not find differences either in New Zealand rabbits.

In our study, median glucose values remained within physiological ranges given by several authors (Hewitt et al., 1989; Suckow and Douglas, 1996; Flecknell, 2002; Melillo, 2007; Jenkins, 2008; Özkan et al., 2012; Ardiaca et al., 2013; Bonvehi et al., 2014; Varga, 2014). However, our range of glucose levels was higher than some reference ranges reported by different authors (Suckow and Douglas, 1996; Flecknell, 2002; Melillo, 2007; Varga, 2014). It is difficult to obtain a fasting sample in rabbits because they do caecotrophy (caecotrophs provides a source of glucose) and volatile fatty acids, that are produced by their cecal flora, are continually absorbed as primary energy source (Melillo, 2007). It has been reported that blood glucose values may be increased up to 145 mg/dL (8.05 mmol/L) 3 hours postfeeding (Jenkins, 2008). This could explain our results. Besides, other causes of hyperglycaemia may be due to gut stasis, high painful conditions or acute intestinal blockage by a foreign body. Moreover, hyperglycaemia may occur in diabetes mellitus, in addition there will be glucosuria, polydipsia and polyphagia. However, diabetes mellitus has not been described in pet rabbits and hyperglycaemia was in the range of 30 – 33.4 mmol/L in laboratory rabbits (Varga, 2014).

In conclusion, results obtained in this research may be useful as a reference range in veterinary clinics because there is not enough information regarding normal ranges in this species as a pet and, more concretely, in Netherland Dwarf rabbits. On other hand, further researchers should be performed to know the cut-off between normality and disease in pet rabbits. Moreover, to know if the great amount of CO<sub>2</sub> in blood in males Netherland Dwarf rabbits are due to a greater cellular metabolism.

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