

# Hematological Reference Intervals of Milk-fed Calves during the First Four Weeks after Arrival to the Farm

Monica Maria Baquero Parra<sup>1</sup> / Felipe Antonio García Amórtegui<sup>2</sup> / Dumar A. Jaramillo-Hernández<sup>3</sup>

## Abstract

This investigation aimed to establish the hematological reference intervals of milk-fed calves during the first four weeks after arrival to the farm. Eighty Holstein dairy neonates (2 to 7 days of age) were used. All calves in the study were fed standard milk replacer twice daily. The blood samples to establish the hematological reference intervals in milk-fed calves were taken 24 hours after arrival and on days 7, 14, 21, and 28 (five samples in 4 weeks). A complete blood count and total serum protein measurement were performed; the calves were weighed 24 h and on day 28 after arrival. Parameters expressed in minimum and maximum ranges were obtained, as follows: WBC:  $3.5\text{--}53.6 \times 10^9/\text{L}$ , segmented neutrophils:  $46.6\text{--}0.43 \times 10^9/\text{L}$ , lymphocytes:  $13.4\text{--}1.1 \times 10^9/\text{L}$ , monocytes:  $2.7\text{--}0.2 \times 10^9/\text{L}$ , eosinophils:  $0\text{--}0.8 \times 10^9/\text{L}$ , basophils:  $0\text{--}0.5 \times 10^9/\text{L}$ , RBC:  $5.2\text{--}12.1 \times 10^{12}/\text{L}$ ; HGB:  $61\text{--}153 \text{ g/L}$ , HCT:  $0.18\text{--}0.45 \text{ L/L}$ , MCV:  $24\text{--}47 \text{ fL}$ , MCH:  $8\text{--}15 \text{ pg}$ , and MCHC:  $298\text{--}354 \text{ g/L}$ . The total serum protein for these five sampling times was  $\bar{x}$  5.11, 4.76, 4.63, 4.65, and 5.14 g/100 mL. Weight gain of  $\bar{x}$  51.82 kg (minimum 31.8 and maximum 72.6 kg) was recorded. Given the non-existence of reference blood parameters in milk-fed calves, this study provides the basis to support initial reference parameters during the first 4 weeks of arrival in production systems for animals between 30 and 37 days of age.

**Keywords:** Erythrogram; internal medicine; laboratory analysis; thrombogram.


## Intervalos hematológicos de referencia de terneros alimentados con leche durante las primeras cuatro semanas de llegada al sistema de producción bovino

## Resumen

El objetivo de esta investigación fue establecer los intervalos de referencia hematológicos de los terneros alimentados con lactorreemplazador durante las primeras cuatro semanas de llegada al sistema de producción. Se utilizaron 80 neonatos Holstein lecheros (de 2 a 7 días de edad). Todos los terneros fueron alimentados con sustituto de leche propicia para este sistema de producción, dos veces al día. Las muestras de sangre para establecer los intervalos de referencia hematológicos en estos terneros se tomaron 24 horas después del arribo, a los 7 días, 14 días, 21 días y 28 días (5 muestras en 4 semanas). Se realizó un hemograma completo y la medición de proteínas séricas totales, además, los terneros se


1 DVM. MSc. PhD. G&B Animal Research Consulting, Cambridge, Canada.

✉ [mmbaquero@live.com](mailto:mmbaquero@live.com)

 <https://orcid.org/0000-0001-6048-1940>


2 DVM. G&B Animal Research Consulting, Cambridge, Canada.

✉ [pipega11@hotmail.com](mailto:pipega11@hotmail.com)

 <https://orcid.org/0000-0002-1466-0603>

3 MVZ. Esp. MSc. PhD. Escuela de Ciencias Animales, Facultad de Ciencias Agropecuarias y Recursos Naturales, Universidad de Los Llanos, Villavicencio, Meta, Colombia.

✉ [dumar.jaramillo@unillanos.edu.co](mailto:dumar.jaramillo@unillanos.edu.co)

 <https://orcid.org/0000-0003-1377-1747>

**To reference this article:** Baquero Parra MB, García Amórtegui FA, Jaramillo-Hernández DA. Hematological reference intervals of milk-fed calves during the first four weeks after arrival to the farm. *Rev Med Vet.* 2025;(50): E5129. <https://doi.org/10.19052/mv.vol1.iss50.5129>



pesaron a las 24 h y al día 28 después de su llegada. Se obtuvieron parámetros expresados en rangos mínimos y máximos de WBC  $3,5-53,6 \times 10^9/L$ ; neutrófilos segmentados  $46,6-0,43 \times 10^9/L$ , linfocitos  $13,4-1,1 \times 10^9/L$ ; monocitos  $2,7-0,2 \times 10^9/L$ ; eosinófilos  $0-0,8 \times 10^9/L$ ; basófilos  $0-0,5 \times 10^9/L$ , RBC  $5,2-12,1 \times 10^{12}/L$ ; HGB 61-153 g/L; HTC 0,18-0,45 L/L, MCV 24-47 fL, MCH 8-15 pg; y MCHC 298-354 g/L. Para estos cinco tiempos de muestreo, la proteína sérica total fue  $\bar{x}$  5,11 g/100 mL, 4,76 g/100 mL, 4,63 g/100 mL, 4,65 g/100 mL y 5,14 g/100 mL. Se registró un aumento de peso  $\bar{x}$  51,82 kg (mínimo 31.8 y máximo 72.6 kg). Dada la inexistencia de parámetros sanguíneos de referencia en este tipo de terneros, este estudio proporciona las bases para sustentar parámetros de referencia iniciales durante las primeras 4 semanas de llegada a los sistemas de producción (animales entre 30 y 37 días de edad).

**Palabras clave:** análisis de laboratorio; eritrograma; medicina interna; trombograma.

## INTRODUCTION

Despite the availability of sexed semen, the majority of pregnancies are the result of conventional semen, with a significant number of male calves being born in the dairy industry (1). About half of Canada's nearly 1 million calves are estimated to produce male calves (2). The veal industry remains an important outlet for these male calves. In Canada, calves are predominantly grown in Ontario and Quebec (2, 3).

Although many calves enter into these industries, there is very little data on blood reference intervals for this group of animals. They are managed differently than calves raised for the beef and dairy industries, especially in the milk-fed veal industry (4). Calves in the milk-fed veal industry are fed a predominantly milk diet, with very little forage provided. This contrasts with calves raised for the dairy and grain-fed veal industries, where they are eventually weaned to a solid-based diet (5).

Typically, bovine neonatal diarrhea is one of the most important enteric pathologies of morbidity and mortality in calves under 28 days of age (6–9). In this pathology, damage to the villi of the intestinal mucosa caused by various microorganisms (e.g., *Cryptosporidium*) results in severe inflammatory processes (10), triggering severe states of dehydration, hypoglycemia, and hypothermia in affected calves (11).

On the other hand, *Mycoplasma bovis* is the most prevalent pathogen in pulmonary consolidations in milk-fed calves, which can cause severe respiratory disease, bringing with it changes in the acid-base balance and acute inflammatory processes in the lungs, among other signs (12). It is in these phenomena where the knowledge of hematic and biochemical reference intervals of milk-fed calves can provide precise information to classify the severity of the clinical picture (e.g., the severity of dehydration), considering that the clinical signs and behavioral alterations do not always represent the actual severity of the pathological process in bovines (13). In the case of pneumonia by *M. bovis* (14), the lesion can only be detected postmortem. Therefore, treatment should be based on clinical and paraclinical evidence to avoid the eventual death of the newborn (15).

Recently, (16) demonstrated that the decrease in milk consumption is the first indication of behavioral alteration in the calf before showing clinical signs of diarrhea; if we add to this finding early changes in the reference intervals of the leukogram and total serum protein, immediate primary pharmacological therapies could be established before aggravating the clinical signs of this pathology. An example of this possibility is the report by (17), where hematocrit (HCT)  $>46\%$  was associated with dehydration in cattle; in turn, (18) indicated that the HCT could be an important indicator in considering dehydration in newborn calves. It is vital to identify this clinical condition early and establish adequate treatment

since severe dehydration is a predictor of mortality in calves (19).

Due to the levels of disease and antimicrobial use in the veal industry, high levels of antimicrobial resistance in commensals and pathogens have been identified. Action and alternative strategies must be sought to ensure prudent antimicrobial use (20). Complete blood counts could justify the use of antimicrobials or help choose the most appropriate antimicrobials to be used. This investigation aimed to establish the hematological reference intervals of milk-fed calves during the first four weeks upon arrival to the farm through paraclinical parameters such as erythrogram, thrombogram, and total serum proteins in addition to the weight of clinically healthy milk-fed calves.

## MATERIALS AND METHODS

### Animals and management

This study used 80 neonatal Holstein dairy bull calves sourced from various local producers and sale barns drawn from southern and southwestern Ontario. Colostrum intake and farm management after birth were unknown. They arrived at the farm by standard livestock trailer. Calves were individually housed in pens within a room, and the rooms themselves were separated by physical concrete barriers so that ventilation, manure, or feed systems were not in contact. All calves in the study were fed standard milk replacer twice daily at the well-established rate according to the farm's feeding program and were allowed access to water *ad libitum*. Each calf was clinically examined before sampling to determine its subjective health status. The daily animal health check was performed over four weeks. Only clinically healthy calves were included in the study. The authors used the guide of the Canadian Council on Animal Care (CCAC) regarding the care and use of farm animals in research, teaching, and testing (21). All the animals linked to this study were sheltered under the premises of the Code of Practice for the Care and Handling of Veal Cattle, proposed by (22).

### Blood samples

Blood samples were collected via jugular venipuncture via 13- × 75-mm Vacutainer tubes (BD Biosciences) with and without EDTA 24 h after arrival and at 7, 14, 21, and 28 d. They were stored at 4 °C and promptly transferred to the laboratory.

### Laboratory analysis

**Complete blood count.** Samples were submitted to the Animal Health Laboratory (University of Guelph, Guelph, Ontario), and hematological analysis was performed on an Advia 120 Hematology Analyzer (Bayer Corporation, Newbury, Berks, United Kingdom) using standard protocols. Hematological variables obtained corresponded to leukogram: leukocyte count (WBC), segmented neutrophil, lymphocyte number, monocyte number, eosinophil number, basophil number; erythrogram: erythrocyte count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular erythrocyte volume (MCV), mean corpuscular erythrocyte hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW); and thrombogram: platelet count (PLTS), and mean platelet volume (MPV).

**Total serum protein.** Blood collected in Vacutainer tubes without EDTA was centrifuged at 1000  $\times$  g for 10 min at room temperature to separate serum. Total serum protein (TSP) was quantified using a handheld refractometer.

### Weight

Each calf's weight was recorded 24 h and on day 28 after arrival, thus establishing its average daily gain (ADG).

### Bovine hematological reference intervals

Hematological reference intervals for milk-fed veal calves do not exist. Reference intervals are summarized in Table 1 to analyze the hematological values obtained. These reference intervals are for the ADVIA 120

hematology analyzer from 99 clinically healthy cows, 50 % during their first lactation, all milking for 30–150 days, from 10 farms in Ontario, Canada (23).

Table 1. Bovine hematology reference intervals

Parameter	Unit	Reference interval
Erythrocytes	$10^{12}/L$	4.9–7.5
Hematocrit	L/L	0.21–0.30
Hemoglobin	g/L	84–120
Mean corpuscular volume	fL	36–50
Mean corpuscular hemoglobin	Pg	14–19
Mean corpuscular hemoglobin concentration	g/L	380–430
Red cell distribution width	%	16–20
Reticulocytes	$10^9/L$	0
Leukocytes	$10^9/L$	5.1–13.3
Segmented neutrophils	$10^9/L$	1.7–6.0
Band neutrophils	$10^9/L$	0–0.2
Lymphocytes	$10^9/L$	1.8–8.1
Monocytes	$10^9/L$	0.1–0.7
Eosinophils	$10^9/L$	0.1–1.2
Basophils	$10^9/L$	0–0.2
Platelets	$10^9/L$	160–650
Mean platelet volume	fL	4.6–7.4

## Statistical analysis

Descriptive statistics of each variable were analyzed using SAS 9.4 software (SAS Institute, Cary, NC, USA) and GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA, USA). The data obtained for each variable in the five measurements were expressed in minimum and maximum ranges, as well as average ( $\bar{x}$ ) and standard deviation ( $\pm$ ). The comparison of reference ranges (22) with the data obtained was carried out through Student's t-test, assuming a  $p < 0.05$  and a confidence interval of 95 %.

## RESULTS

Figures 1, 2, and 3 show the results obtained in the leukogram, erythrogram, and thrombogram, respectively, of the milk-fed veal calves in each sample taken on days 1,

7, 14, 21, and 28 post-arrival at the production unit. The gray bands in each figure refer to the intervals for the species proposed by (23). Likewise, in Supplementary Material 1, Table S1 combines the descriptive analysis of the variables obtained in the leukogram, blood count, and thrombogram.

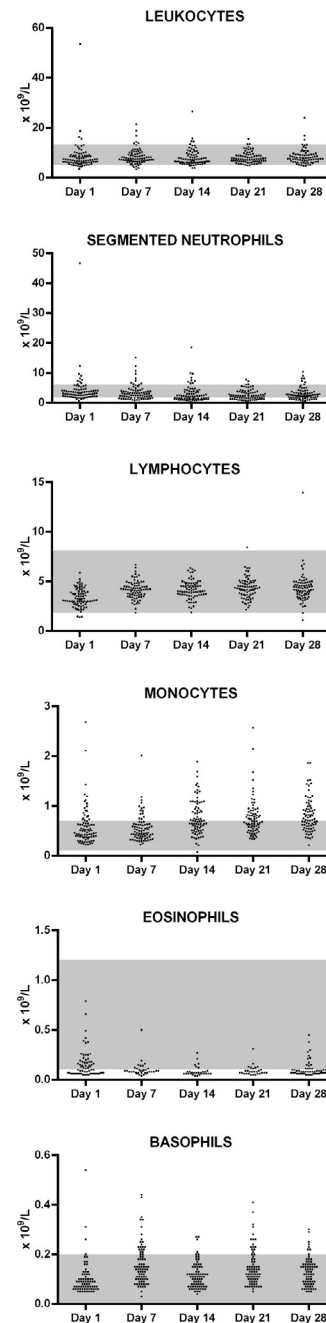


Figure 1. Leukogram in milk-fed veal calves.

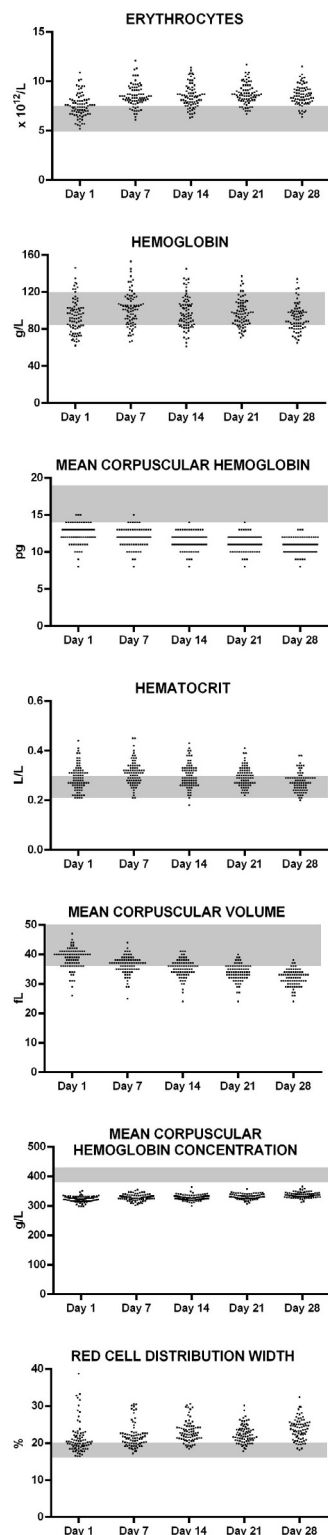


Figure 2. Erythrogram in milk-fed veal calves.

\* Significant difference with gray bands at all times, Student's t-test,  $p < 0.05$ .

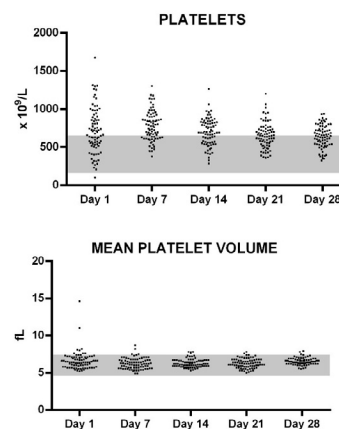


Figure 3. Thrombogram in milk-fed veal calves.

TSP was quantified using a refractometer on days 1, 7, 14, 21, and 28. The protein concentration in blood was on average ( $\bar{x}$ ) 5.11, 4.76, 4.63, 4.65, and 5.14 g/100 mL, respectively. There was a significant decrease in TSP from day one to day 21 (Student's t-test,  $p < 0.0001$ ), and on day 28, the concentration increased to the starting concentration ( $p = 0.6668$ ). Figure 4 shows the concentration of TSP. Likewise, in Supplementary Material 1, Table S2 shows the descriptive analysis of the TSP concentration obtained at the different measurement moments.

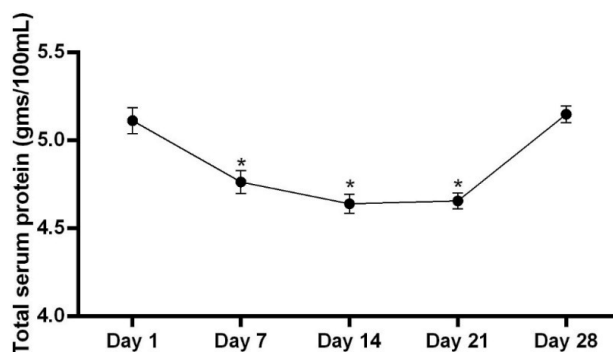


Figure 4. Total serum protein in milk-fed veal calves.

\* Significant difference with first sampling time, Student's t-test,  $p < 0.0001$ .

Each calf was weighed 24 hours and 28 days after arrival to the barn. The average initial weight was 94.71 kg, with a minimum of 80 kg and a maximum of 105 kg. After four weeks, the average weight was 146.53 kg, with a minimum of 123.4 kg and a maximum of 172.6 kg (Figure 5).



The average weight gain was 51.82 kg, with a minimum of 31.8 and a maximum of 72.6 kg. Supplementary Material 1, Table S3, shows the descriptive analysis of calves' initial and final weight measurements.

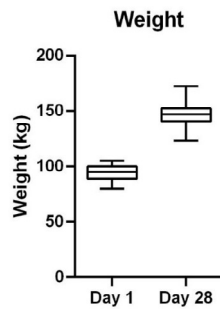


Figure 5. Weight of milk-fed veal calves 24 h and 28 days after arrival to the barn.

## DISCUSSION

The milk-fed veal industry uses calves, preferably males, fed with milk replacers with a low concentration of the mineral iron (compared to the iron content of natural milk) to obtain meat with a particular color (pale pink) due to its low concentration of myoglobin (5). According to (24), these calves must receive increasing amounts of fibrous feed from 50 to 250 g/animal/day up to 20 weeks of age. In this industry, the constant prophylactic and metaphylactic use of oral antibiotics during production time is characteristic (25–27). Even so, this industry has stable markets in European and North American countries (4, 5).

Before arriving at their production unit, these animals suffer a series of stressful events associated with their sale, predisposing them to disease, such as transport, the association between unknown groups of animals, and various age groups, among others (28). Thus, (29) recently found that 40% of these animals arrived with diarrhea, 35% dehydrated, and 25% with omphalophlebitis at their production unit. Likewise, high-incidence diseases drastically affect the productive indicators of these production systems, such as respiratory and enteric pathologies (14, 27). Added to the aggravating factor,

approximately 43% of these animals have presented with passive immunity transfer failure (30), where this inadequate concentration of immunoglobulins is associated with respiratory disease, mainly (31). For these reasons, the highest mortality rate of these animals is in the first three weeks of arrival at their production unit (2), which can fluctuate between 4% and 8% (2, 32).

Knowledge of physiological parameters (ranges) of biological constants in milk-fed calves supports clinical-productive decision-making in cattle production systems in the face of these pathologies (19, 33, 34). Taking biological samples, analyzing them, and interpreting them regarding normal physiological mechanisms will allow significant information in disease management (35, 36). It is indispensable to have specific reference intervals for each age group and management particularities within husbandry production systems (36, 37), such as milk-fed calves.

In our study, the WBC fluctuated between the minimum and maximum ranges from 3.5 to 53.6 ( $\bar{x}$  8.3  $\pm$  5.8), 3.3 to 21.4 ( $\bar{x}$  8.4  $\pm$  3.2), 3.9 to 26.5 ( $\bar{x}$  8  $\pm$  3.2), 4.9 to 15.5 ( $\bar{x}$  7.9  $\pm$  2.2), and 4.8 to 24 ( $\bar{x}$  8.4  $\pm$  3) cells  $\times 10^9/L$ , from the first to the fifth measurement performed, respectively (Figure 1 and Table S1, leukocytes). The WBC in bovine newborns is usually closely associated with adult bovines' physiological ranges or with a slight tendency to be higher, which are regulated after the first week of life (38, 39).

In our study the differential leukocyte count, with respect to the minimum and maximum ranges ( $\bar{x}$ ,  $\pm$ ), was as follows: segmented neutrophils: 0.63 to 46.6 ( $\bar{x}$  4.2  $\pm$  5.2), 0.82 to 15.2 ( $\bar{x}$  3.5  $\pm$  2.6), 0.43 to 18.6 ( $\bar{x}$  2.9  $\pm$  2.8), 0.54 to 7.8 ( $\bar{x}$  2.7  $\pm$  1.6), and 0.53 to 10.3 ( $\bar{x}$  3  $\pm$  2) cells  $\times 10^9/L$ ; lymphocytes: 1.4 to 5.9 ( $\bar{x}$  3.3  $\pm$  0.9), 1.8 to 6.7 ( $\bar{x}$  4.2  $\pm$  0.9), 1.9 to 6.4 ( $\bar{x}$  4.2  $\pm$  0.9), 2.1 to 8.4 ( $\bar{x}$  4.3  $\pm$  1.1), and 1.1 to 13.4 ( $\bar{x}$  4.4  $\pm$  1.5) cells  $\times 10^9/L$ ; monocytes: 0.2 to 2.7 ( $\bar{x}$  0.6  $\pm$  0.4), 0.2 to 2 ( $\bar{x}$  0.6  $\pm$  0.3), 0 to 1.9 ( $\bar{x}$  0.8  $\pm$  0.4), 0.3 to 2.6 ( $\bar{x}$  0.8  $\pm$  0.4), and 0.2 to 1.9 ( $\bar{x}$  0.8  $\pm$  0.3) cells  $\times 10^9/L$ ; eosinophils: 0.5 to 0.8 ( $\bar{x}$  0.1  $\pm$  0.1), 0 to 0.3 ( $\bar{x}$  0.04  $\pm$  0.07), 0 to 0.3 ( $\bar{x}$  0.03  $\pm$  0.05), and 0 to 0.5 ( $\bar{x}$  0.04  $\pm$  0.05) cells  $\times 10^9/L$ ;

basophils: 0 to 0.5 ( $\bar{x}$  0.1  $\pm$  0.07), 0 to 0.4 ( $\bar{x}$  0.2  $\pm$  0.08), 0 to 0.3 ( $\bar{x}$  0.1  $\pm$  0.05), 0 to 0.4 ( $\bar{x}$  0.1  $\pm$  0.07), and 0 to 0.3 ( $\bar{x}$  0.1  $\pm$  0.06) cells  $\times 10^9/L$  (Figure 1, Table S1). According to the measurements taken in the five working moments, respectively, they tend to be within the accepted ranges for adult bovines (39, 40). As observed in Figure 1, the data obtained are closely related to those published by (23), taken as a reference for this study (Table 1). Although the cortisol hormone could alter the physiological ranges of these leukogram variables, both in adult and neonate bovines, (41) demonstrated that cortisol is found in similar concentrations between adult and neonate bovines between 11 and 20 days of age.

Moreover, it is important to mention that the RCB in calves physiologically tends to decrease during the first two weeks of life (42); iron supplementation in the first month of life can prevent this reduction (43). Studies on newborn calves have found a historical trend associated with their development: a decrease in HTC, HGB, MCV, MCH, and MCHC and increased RBC after the first two weeks of life (39, 40, 44).

This behavior in the erythrogram variables of the animals included in this study shows this same trend (Figure 2, Table S1), where RBC was 5.2 to 10.9 ( $\bar{x}$  7.5  $\pm$  1.1), 6.1 to 12.1 ( $\bar{x}$  8.5  $\pm$  1.1), 6.3 to 11.4 ( $\bar{x}$  8.5  $\pm$  1.1), 6.7 to 11.7 ( $\bar{x}$  8.7  $\pm$  1), and 6.4 to 11.5 ( $\bar{x}$  8.6  $\pm$  1) cells  $\times 10^{12}/L$ ; HGB was 62 to 146 ( $\bar{x}$  93.7  $\pm$  18), 66 to 143 ( $\bar{x}$  101.8  $\pm$  18.1), 61 to 145 ( $\bar{x}$  97.8  $\pm$  17.6), 71 to 137 ( $\bar{x}$  97.3  $\pm$  14.5), and 65 to 134 ( $\bar{x}$  92.2  $\pm$  14.3) g/L; HTC was 0.21 to 0.44 ( $\bar{x}$  0.29  $\pm$  0.05), 0.21 to 0.45 ( $\bar{x}$  0.3  $\pm$  0.04), 0.18 to 0.43 ( $\bar{x}$  0.3  $\pm$  0.05), 0.22 to 0.41 ( $\bar{x}$  0.3  $\pm$  0.04), and 0.20 to 0.38 ( $\bar{x}$  0.27  $\pm$  0.04) L/L; and MCV was 26 to 47 ( $\bar{x}$  38.5  $\pm$  3.6), 25 to 44 ( $\bar{x}$  36.3  $\pm$  3.1), 24 to 41 ( $\bar{x}$  35.2  $\pm$  3.1), 24 to 40 ( $\bar{x}$  33.8  $\pm$  2.9), and 24 to 38 ( $\bar{x}$  32  $\pm$  2.7) fL, from the first to the fifth sample, respectively.

Other studies have shown that the reference ranges for RBC and HTC in calves up to 3 months of age from dairy production systems are closely related to the same ranges for adult cattle (39, 40). Contrary to this, (45) published that the hematic parameters of newborns of origin from

dairy production systems should be apart from the ranges for adult bovines. According to breed (aptitude) and sex (females have higher concentrations of RBC), there are substantial differences between these physiological referents.

In the erythrogram variables MCH and MCHC of our study, when compared with the values taken as reference for this study (Table 1), a significant statistical difference was obtained (Student's t-test,  $p < 0.05$ ), with MCH at 8 to 15 ( $\bar{x}$  12.5  $\pm$  1.3), 8 to 15 ( $\bar{x}$  12  $\pm$  1.3), 8 to 14 ( $\bar{x}$  11.5  $\pm$  1.1), 8 to 14 ( $\bar{x}$  11.2  $\pm$  1.1), and 8 to 13 ( $\bar{x}$  10.9  $\pm$  1) pg; and MCHC at 298 to 351 ( $\bar{x}$  323  $\pm$  9.9), 302 to 354 ( $\bar{x}$  328  $\pm$  11.2), 300 to 363 ( $\bar{x}$  327  $\pm$  9.7), 307 to 357 ( $\bar{x}$  331  $\pm$  9.2), and 312 to 365 ( $\bar{x}$  336  $\pm$  9.6) g/L (Table S1).

This difference could be explained by the fact that low concentrations of iron in milk-fed calves' diet, where milk replacers typically provide  $\leq 20$  mg iron/kg, bring about the subclinical manifestation of iron deficiency anemia (hypochromic) in these animals (46). However, low dietary iron concentrations may be associated with important changes in MCV (47). Commonly, MCV exhibits a gradual reduction related to the change from fetal HGB to A HGB, where calf erythrocytes continue to decrease in size throughout their first 3 to 4 months of life (41).

HGB in milk-fed calves is regulated in Europe through (48), where hemoglobin levels must be  $\geq 4.5$  mmol/L (7.3 g/dL). In Canada, (22) established that calves with HGB levels  $\leq 7.7$  g/dL (4.8 mmol/l) show signs of hypochromic anemia. These parameters allow adjusting the concentration of iron in the diet to prevent this pathology and safeguard animal welfare parameters.

The thrombogram associated with the PLT and MPV variables did not have significant differences concerning the values in Table 1 (Figure 3), where the minimum and maximum ranges ( $\bar{x} \pm$ ) fluctuated throughout the five measurements: 101–1675 ( $\bar{x}$  714  $\pm$  301), 378–1,302 ( $\bar{x}$  778  $\pm$  196), 284–1,264 ( $\bar{x}$  692  $\pm$  178), 360–1201 ( $\bar{x}$  650  $\pm$  170), and 322–937 ( $\bar{x}$  637  $\pm$  147) PLT  $\times 10^9/L$  (Table S1), respectively. In this regard, (39, 40, 44) show

that the PLT count in calves is regularly similar to the range proposed for adult bovines or with a tendency to rise in the first 6 days of life.

The TSP provides high information on acute and chronic inflammatory processes and, in turn, reflects nutritional considerations and metabolic processes (digestion-absorption) (38). In our study, a significant decrease in the concentration of TSP was found when we compared day 1 with days 7, 14, and 21 (Student's T-test,  $p < 0.0001$ , Figure 4) to stabilize on day 28 of the sampling. Even so, these TSP fluctuations were similar to approximate values for those reported in Italy, where 128 calves were used, with a TSP of  $5.87 \pm 1.1$  g/100 mL on the day of arrival at the production unit (30 days of age) and  $5.65 \pm 0.65$  g/L on day 60 after arrival (90 days of age) (49).

The TSP is directly correlated with the transfer of passive immunity in calves. The high possibility of failure to transfer passive immunity in milk-fed calves should be the cause of their clinical investigation upon arrival at the farm (50, 51). (52) defined an immunoglobulin (Ig) class G (G) IgG level  $< 10.0$  mg/mL of blood serum as a failure of passive transfer of antibodies in calves from dairy systems. Diet-induced iron deficiency in these animals could also be associated with reduced cell-mediated immune response, predisposing them to a higher probability of infectious disease (53).

Another vital biological variable to consider in the health status of milk-fed calves is weight gain since weight loss in milk-fed calves is associated with various pathologies afflicting them (14). Early on, (54) presented results on the disease risks in these animals, finding that weight loss is a high possibility associated with diarrhea, mainly. In the clinical phase of diarrhea, a dairy calf can lose up to  $0.051$  kg/day (55). A single episode of respiratory disease in milk-fed calves can lead to up to  $8.2$  kg of weight loss in carcasses (14). (56) demonstrated weight loss associated with respiratory disease at  $0.07$  to  $0.28$  kg/day, depending on the pharmacological treatment established for the animals.

In our study, the calves obtained an ADG during the 28 days of analysis of variables in a range of  $31.8$  to  $72.6$  kg ( $1.14$  to  $2.6$  kg/day; Figure 5). This ADG margin in these calves is higher than that reported by (19) in milk-fed veal calves, which obtained  $ADG \bar{x} 1.13 \pm 0.21$  kg/d with a range of  $0.28$  to  $1.82$  kg/d, possibly because their study linked multiple calves ( $n = 4.825$ ) in different seasons of the year (from November 2015 to September 2016). For their part, (14) reported that calves with some pathology incorporated into their study obtained an ADG of  $1.47 \pm 0.26$  kg/d, and clinically healthy calves registered an ADG of  $1.51 \pm 0.29$  kg/d, similar to data reported here.

Lastly, in previous works, (49) presented hemogasometry data of milk-fed calves during their first 3 months of life, where the blood pH fluctuated between  $7.41$  and  $7.44$ ,  $HCO_3$  between  $30.28$  and  $34.62$  mmol/L, and extracellular fluid base excess between  $5.9$  and  $10.67$  mmol/L, among other variables that would help to study the acid-base status in these animals. It is worth clarifying that the high presence of soluble carbohydrates in the diet, lactose  $> 5.0\%$ , in milk replacers could be associated with pathological changes in these biological variables, leading to processes of metabolic acidosis in calves (57). This same study found that most of the blood hemocytometric and biochemical parameters analyzed could be considered normal according to the physiological ranges presented in the literature for newborn and young calves (39, 40, 58).

## CONCLUSION

Given the non-existence of reference blood parameters in milk-fed veal calves, this study provides the bases to support initial reference parameters during the first 4 weeks of arrival in production systems (animals between 30 and 37 days of age) for leukogram (WBC, segmented neutrophil, lymphocyte, monocyte, eosinophil, and basophil number); erythrogram (RBC, HGB, HCT, MCV, MCH, MCHC, and RDW); thrombogram (PLTS and MPV); and TSP.



## REFERENCES

- De Vries A, Overton M, Fetrow J, Leslie K, Eicker S, Rogers G. Exploring the Impact of Sexed Semen on the Structure of the Dairy Industry. *J Dairy Sci.* 2008;91(2):847-856. <https://doi.org/10.3168/jds.2007-0536>
- Winder CB, Kelton DF, Duffield TF. Mortality risk factors for calves entering a multi-location white veal farm in Ontario, Canada. *J Dairy Sci.* 2016;99(12):10174-10181. <https://doi.org/10.3168/jds.2016-11345>
- Statistics Canada. Table 32-10-0130-01. Number of cattle, by class and farm type (x1000). 2023. <https://doi.org/10.25318/3210013001-eng>
- Pardon BB, Catry R, Boone H, Theys K, De Bleecker J, Dewulf P. Characteristics and challenges of the modern Belgian veal. *Vlaams Diergeneesk. Tijdschr.* 2014;83: 155-163. <https://doi.org/10.21825/vdt.v83i4.16641>
- Sans P, De Fontguyon G. Veal calf industry economics. *Rev Med Vet-Toulouse.* 2009;160(8-9):420-424. Available from: <http://www.revmedvet.com/artdes-fr.php?id=1741>
- Gulliksen SM, Lie KI, Loken T, Osteras O. Calf mortality in Norwegian dairy herds. *J Dairy Sci.* 2004;92(6): 2782-2795. <https://doi.org/10.3168/jds.2008-1807>
- United States Department of Agriculture (USDA). Dairy 2007, heifer calf health and management practices on U.S. dairy operations. USDA – Animal and Plant Health Inspection Service – Veterinary Services, Center for Epidemiology and Animal Health, Fort Collins, CO. 2010. Available from: [https://www.aphis.usda.gov/animal\\_health/nahms/dairy/downloads/dairy07/Dairy07\\_ir\\_CalfHealth\\_1.pdf](https://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy07/Dairy07_ir_CalfHealth_1.pdf)
- Cho YI, Yoon KJ. An overview of calf diarrhea-Infectious etiology, diagnosis, and intervention. *J Vet Sci.* 2014;15(1):1-17. <https://doi.org/10.4142/jvs.2014.15.1.1>
- Meganck V, Hoflack S, Piepers V, Opsomer G. Evaluation of a protocol to reduce the incidence of neonatal calf diarrhea on dairy herds. *Prev Vet Med.* 2015;118(1): 64-70. <https://doi.org/10.1016/j.prevetmed.2014.11.007>
- Todd CG, Millman ST, McKnight DR, Duffield TF, Leslie KE. Nonsteroidal anti-inflammatory drug therapy for neonatal calf diarrhea complex: Effects on calf performance. *J Anim Sci.* 2010;88(6):2019-2028. <https://doi.org/10.2527/jas.2009-2340>
- Schroeder ME, Bounpheng MA, Rodgers S, Baker RJ, Black W, Naikare H, et al. Development and performance evaluation of calf diarrhoea pathogen nucleic acid purification and detection workflow. *J Vet Diagn Invest.* 2012;24(5):945-953. <https://doi.org/10.1177/1040638712456976>
- White BJ, Anderson DE, Renter DG, Larson RL, Mosier DA, Kelly LL, et al. Clinical, behavioral, and pulmonary changes in calves following inoculation with *Mycoplasma bovis*. *Am J Vet Res.* 2012;73(4):490-497. <https://doi.org/10.2460/ajvr.73.4.490>
- Weary DM, Huzzey JM, von Keyserlingk MAG. Board-invited review: Using behavior to predict and identify ill health in animals. *J Anim Sci.* 2009;87(2):770-777. <https://doi.org/10.2527/jas.2008-1297>
- Pardon B, Hostens M, Duchateau L, Dewulf J, De Bleecker K, Deprez P. Impact of respiratory disease, diarrhea, otitis and arthritis on mortality and carcass traits in white veal calves. *BMC Vet Res.* 2013;9(1):79. <https://doi.org/10.1186/1746-6148-9-79>
- de Graaf DC, Vanopdenbosch E, Ortega-Mora LM, Abbassi H, Peeters JE. A review of the importance of cryptosporidiosis in farm animals. *Int J.Parasitol.* 1999;29(8):1269-1287. [https://doi.org/10.1016/S0020-7519\(99\)00076-4](https://doi.org/10.1016/S0020-7519(99)00076-4)
- Lowe GL, Sutherland MA, Waas JR, Schaefer AL, Cox NR, Stewart M. Physiological and behavioral responses as indicators for early disease detection in dairy calves. *J Dairy Sci.* 2019;102(6):5389-5402. <https://doi.org/10.3168/jds.2018-15701>
- Kahn CM, Line S, Aiello SE. The Merck veterinary manual. 10th ed. NJ: Merck and Co., Whitehouse Station; 2010.
- Constable PD, Walker PG, Morin DE, Foreman JH. Clinical and laboratory assessment of hydration status of neonatal calves with diarrhea. *J Am Vet Med Assoc.* 1998;212(7):991-996. PMID: 9540870
- Renaud DL, Duffield TF, LeBlanc SJ, Ferguson S, Haley DB, Kelton DF. Risk factors associated with mortality at a milk-fed veal calf facility: A prospective cohort study. *J Dairy Sci.* 2018;101(3):26592668. <https://doi.org/10.3168/jds.2017-13581>
- EMA/EFSA. EMA and EFSA Joint Scientific Opinion on measures to reduce the need to use antimicrobial

- agents in animal husbandry in the European Union, and the resulting impacts on food safety (RONAFA). 2016. Available from: <https://www.efsa.europa.eu/en/efsa-journal/pub/4666>
21. De Passillé AM, Haley D, Rodas-González A, Borderas F. Code of Practice for the Care and Handling of veal Cattle: Review of Scientific Research on Priority Issues. National Farm Animal Care Council (NFACC), Canada. 2016. Available from: <https://www.nfacc.ca/codes-of-practice/veal-cattle>
22. Veal Cattle Code of Practice Scientific Committee (VC-CPSC). Code of Practice for the Care and Handling of Veal Cattle: Review of Scientific Research on Priority Issues. Lacombe, AB: National Farm Animal Care Council. 2016. Available from: [http://www.nfacc.ca/resources/codes-of-practice/veal-cattle/veal\\_cattle\\_SCreport\\_2016.pdf](http://www.nfacc.ca/resources/codes-of-practice/veal-cattle/veal_cattle_SCreport_2016.pdf)
23. Roland L, Drillich M, Iwersen M. Hematology as a diagnostic tool in bovine medicine. *J Vet Diagn Invest*. 2014;26(5): 592-598. <https://doi.org/10.1177/1040638714546490>
24. Council Directive 97/2/EC of 20 January 1997 amending Directive 91/629/EEC laying down minimum standards for the protection of calves. 1997. Available from: <http://data.europa.eu/eli/dir/1997/2/oj>
25. Berge ACB, Atwill AR, Sischo WM. Assessing antibiotic resistance in fecal *Escherichia coli* in young calves using cluster analysis techniques. *Prev Vet Med*. 2003;61(2):91-102. [https://doi.org/10.1016/S0167-5877\(03\)00191-0](https://doi.org/10.1016/S0167-5877(03)00191-0)
26. Brscic M, Leruste H, Heutinck LF, Bokkers EA, Wolthuis-Fillerup M, Stockhofe N, et al. Prevalence of respiratory disorders in veal calves and potential risk factors. *J Dairy Sci*. 2012;95(5):2753-2764. <https://doi.org/10.3168/jds.2011-4699>
27. Pardon B, De Bleecker K, Hostens M, Callens J, Dewulf J, Deprez P. Longitudinal study on morbidity and mortality in white veal calves in Belgium. *BMC Vet Res*. 2012;8(1):26. <https://doi.org/10.1186/1746-6148-8-26>
28. Taylor JD, Fulton RW, Lehenbauer TW, Step DL, Confer AW. The epidemiology of bovine respiratory disease: What is the evidence for predisposing factors. *Can Vet J*. 2010;51(10):1095-1102. PMID: PMC2942046
29. Pempek J, Trearchis D, Masterson M, Habing G, Proudfoot K. Veal calf health on the day of arrival at growers in Ohio. *J Anim Sci*. 2017;95(9):3863-3872. <https://doi.org/10.2527/jas2017.1642>
30. Wilson LL, Smith JL, Smith DL, Swanson DL, Drake TR, Wolfgang DR, et al. Characteristics of veal calves upon arrival, at 28 and 84 days, and at end of the production cycle. *J. Dairy Sci*. 2000;83(4):843-854. <https://doi.org/10.3168/jds>
31. Pardon B, Alliet J, Boone R, Roelandt S, Valgaeren B, Deprez P. Prediction of respiratory disease and diarrhea in veal calves based on immunoglobulin levels and the serostatus for respiratory pathogens measured at arrival. *Prev Vet Med*. 2015;120(2):169-176. <https://doi.org/10.1016/j.prevetmed.2015.04.009>
32. Bähler C, Steiner A, Luginbühl A, Ewy A, Posthaus H, Strabel D, et al. Risk factors for death and unwanted early slaughter in Swiss veal calves kept at a specific animal welfare standard. *Res Vet Sci*. 2012;92:162-168. <https://doi.org/10.1016/j.rvsc.2010.10.009>
33. Thompson PN, Stone A, Schultheiss WA. Use of treatment records and lung lesion scoring to estimate the effect of respiratory disease on growth during early and late finishing periods in South African feedlot cattle. *J Anim Sci*. 2006;84(2):488-498. <https://doi.org/10.2527/2006.842488x>
34. Stanton AL, Kelton DF, Leblanc SJ, Millman ST, Wormuth J, Dingwell RT, et al. The effect of treatment with long-acting antibiotic at postweaning movement on respiratory disease and on growth in commercial dairy calves. *J Dairy Sci*. 2010;93(2):574-581. <https://doi.org/10.3168/jds.2009-2414>
35. Thrall MA. *Veterinary Hematology and Clinical Chemistry*. Baltimore: Lippincott Williams and Wilkins; 2004. pp. 45-46.
36. Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton RM, Barnhart KF, et al. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. *Vet Clin Pathol*. 2012;41:441-453. <https://doi.org/10.1111/vcp.12006>
37. Meyer DJ, Harvey JW. *Veterinary Laboratory Medicine: Interpretation and Diagnosis*, Third ed., St. Louis: Saunders; 2004. 5 p.
38. Zanker IA, Hammon HM, Blum JW. Delayed feeding of first colostrums: are there prolonged effects on

- haematological, metabolic and endocrine parameters and on growth performance in calves? *J Anim Physiol Anim Nutr (Berl)*. 2001;85(3-4):53-66. <https://doi.org/10.1046/j.1439-0396.2001.00296.x>
39. Mohri M, Sharifi K, Eidi S. Hematology and serum biochemistry of Holstein dairy calves: Age related changes and comparison with blood composition in adults. *Res in Vet Sci*. 2007;83(1):30-39. <https://doi.org/10.1016/j.rvsc.2006.10.017>
  40. Knowles TG, Edwards JE, Bazeley KJ, Brown SN, Butterworth A, Warriss PD. Changes in the blood biochemical and haematological profile of neonatal calves with age. *Vet Rec*. 2000;147(21):593-598. <https://doi.org/10.1136/vr.147.21.593>
  41. Jain NC. Schalm's Veterinary haematology, 4th ed., Philadelphia: Lea and Febiger; 1986.
  42. Tennant B, Harrold D, Reina-Guerra M, Kendrick JW, Laben RC. Hematology of the neonatal calf: erythrocyte and leukocyte values of normal calves. *Cornell Vet*. 1974;64(4):516-532. PMID: 4473317
  43. Mohri M, Sarrafzadeh F, Seifi HA, Farzaneh N. Effects of oral iron supplementation on some haematological parameters and iron biochemistry in neonatal dairy calves. *Comp Clin Path*. 2004;13:39-42. <https://doi.org/10.1007/s00580-004-0523-5>
  44. Egli CP, Blum JW. Clinical, haematological, metabolic and endocrine traits during the first three months of life of suckling Simentaler calves held in a cow-calf operation. *J Vet Med A*. 1998;45(2):99-118. <https://doi.org/10.1111/j.1439-0442.1998.tb00806.x>
  45. Panousis N, Siachos N, Kitkas G, Kalaitzakis E, Kritsepi-Konstantinou M, Valergakis GE. Hematology reference intervals for neonatal Holstein calves. *Res Vet Sci*. 2018;118:1-10. <https://doi.org/10.1016/j.rvsc.2018.01.002>
  46. Lindt F, Blum J. Occurrence of iron deficiency in growing cattle. *J Vet Med A*. 1994;41(3):237-246. <https://doi.org/10.1111/j.1439-0442.1994.tb00090.x>
  47. Katunguka-Rwakishaya E, Larkin H, Kelly WR. Blood values of neonatal calves, and blood values and live-weight gains of calves fed on different levels of milk replacer. *Br Vet J*. 1987;143(2):184-90. [https://doi.org/10.1016/0007-1935\(87\)90010-8](https://doi.org/10.1016/0007-1935(87)90010-8)
  48. Council Directive 2008/119/EC of 18 December 2008 laying down minimum standards for the protection of calves. 2008. Available from: <http://data.europa.eu/eli/dir/2008/119/oj>
  49. Giambelluca S, Flore E, Sadocco A, Ganesella M, Vazzana I, Orefice T, et al. Evaluation of venous blood gas levels, blood chemistry and haemocytometric parameters in milk fed veal calves at different periods of livestock cycle. *Pol J Vet Sci*. 2016;19(4):745-52. <https://doi.org/10.1515/pjvs-2016-0094>
  50. Wilson LL, Egan CL, Drake TR. Blood, growth, and other characteristics of special-fed, veal calves in private cooperation herds. *J Dairy Sci*. 1994;77(8):2477-2485. [https://doi.org/10.3168/jds.S0022-0302\(94\)77189-7](https://doi.org/10.3168/jds.S0022-0302(94)77189-7)
  51. Stull CL, McDonough SP. Multidisciplinary approach to evaluating welfare of veal calves in commercial facilities. *J Anim Sci*. 1994;72(9):2518-2524. <https://doi.org/10.2527/1994.7292518x>
  52. United States Department of Agriculture (USDA). 2007. Dairy 2007, Part I: Reference of dairy cattle health and management practices in the United States, 2007. No. N480.1007. USDA – Animal and Plant Health Inspection Service – Veterinary Services, Center for Epidemiology and Animal Health, Fort Collins, CO. Available from: [https://www.aphis.usda.gov/animal\\_health/nahms/dairy/downloads/dairy07/Dairy07\\_dr\\_PartI\\_1.pdf](https://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy07/Dairy07_dr_PartI_1.pdf)
  53. Gygas M, Hirni H, Zwahlen R, Lazary S, Blum JW. Immune functions of veal calves fed low amounts of iron. *J Vet Med A*. 1993;40(5):345-358. <https://doi.org/10.1111/j.1439-0442.1993.tb00638.x>
  54. Postema HJ, Mol J. Risk of disease in veal calves: relationships between colostrum-management, serum immunoglobulin levels and risk of disease. *J Vet Med A*. 1984;31(10):751-762. <https://doi.org/10.1111/j.1439-0442.1984.tb01334.x>
  55. Donovan GA, Dohoo IR, Montgomery DM, Bennett FL. Calf and disease factors affecting growth in female Holstein calves in Florida. USA. *Prev Vet Med*. 1998;33(1-4):1-10. [https://doi.org/10.1016/S0167-5877\(97\)00059-7](https://doi.org/10.1016/S0167-5877(97)00059-7)
  56. Schneider MJ, Tait RG Jr, Busby WD, Reecy JM. An evaluation of bovine respiratory disease complex in feedlot cattle: Impact on performance and carcass traits using treatment records and lung lesion scores. *J Anim Sci*. 2009;87(5):1821-1827. <https://doi.org/10.2527/jas.2008-1283>

57. Dirksen G, Grunder HD, Stober M. Medicina interna e chirurgia del bovino. 4th ed., Milan: Le point veterinaire Italie srl; 2004.
58. Hugi D, Blum JW. Changes of blood metabolites and hormones in breeding calves associated with weaning. J Vet Med A. 1997;44(2):99-108. <https://doi.org/10.1111/j.1439-0442.1997.tb01091.x>