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New Zealand White does' milking methodology and milk composition in Brazil

Abstract - The objective of this work was to develop a milking methodology and to determine the nutrients of New Zealand White does' milk. Two experiments were carried out. The first one used five multiparous does and five milk collections – 1st, 7th, 14th, 21st and 28th day. For milking, an electric pump used chiefly for water bottle carboy was adapted and manual massages in all pairs of teats were as well necessary. The second one used twenty-three does at peak of lactation. In both experiments, bromatological analyses were carried out. During lactation, the average composition of rabbit milk in terms of total solids, ash, fat, protein, and energy was 28.80%, 1.92%, 14.44%, 10.77%, and 1,694.22 kcal L^{-1}, respectively. Energy presented a decreasing linear effect along the lactation curve. Ash level presented a quadratic effect. All macrominerals presented a linear effect. Compared to other milks, rabbit milk was three to four times richer in fat and protein, twice to four times higher in energy, and twice lower in lactose. The milking methodology developed is effective. New Zealand White does' milk is rich in protein, fat, and energy compared to goat and cow's milk.

Index terms: milking, nutrients, milk production.

Metodologia de ordenha e composição do leite de coelhas Nova Zelândia no Brasil

Resumo - O objetivo deste trabalho foi desenvolver uma metodologia de ordenha e determinar os nutrientes no leite de coelhas Nova Zelândia brancas. Dois experimentos foram realizados. O primeiro utilizou cinco fêmeas multíparas e cinco coletas de leite – 1º, 7º, 14º, 21º e 28º dia. Para a ordenha, uma bomba elétrica usada principalmente para galão d'água foi adaptada e massagens manuais em todos os pares de tetos também foram necessárias. O segundo experimento utilizou 23 fêmeas no pico da lactação. Em ambos os experimentos, análises bromatológicas foram feitas. Durante a lactação, a composição média do leite de coelhas em termos de sólidos totais, cinzas, gordura, proteína e energia foi de 28,80%, 1,92%, 14,44%, 10,77% e 1.694,22 kcal L^{-1}, respectivamente. A energia apresentou efeito linear decrescente ao longo da curva de lactação. O nível de cinzas apresentou efeito quadrático. Todos os macrominerais apresentaram efeito linear. Comparado a outros leites, o leite de coelha se mostrou três a quatro vezes mais rico em gordura e proteína, duas a quatro vezes mais rico em energia e duas vezes mais baixo em lactose. A metodologia de ordenha desenvolvida é eficaz. O leite de coelhas Nova Zelândia é rico em proteína, gordura e energia em comparação com o leite de cabra e vaca.

Termos de indexação: ordenha, nutrientes, produção de leite.

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Introduction

Rabbit farming is a sound livestock production alternative, especially for small and medium producers. It is a low-investment business, easier to manage than business dealing with larger animals, and it has a short-term financial return (Souza & Silva, 2022). Besides rabbit meat, entrepreneurs can benefit from its co-products (Klinger & Toledo, 2018) and even sell the animals as pets (Clauss & Hatt, 2017; Birolo, 2023).

Although not popular in Brazil, rabbit farming is in constant development (Piza et al., 2021). According to FAOSTAT, Brazil produced 1,143 tons of rabbit meat in 2022 (FAO, 2024). A remarkable characteristic of the Brazilian production is that more than 50% of rabbit farmers have other livestock as their main source of income (Falcone et al., 2022).

This small rabbit production in Brazil is a consequence of few information about rabbit farming, compared to poultry and swine's productions. It causes losses related to high mortality rates. In rabbits, mortality is highest between birth and weaning (El-Ashram et al., 2020; Machado et al., 2021). The main reasons are lack of a fully developed immune system and difficult to maintain their temperature (Miranda & Castilha, 2020).

Milk plays a major role in mortality rates. Rabbit milk is an essential food for the kits until they are 18 to 19 days old, when they start leaving the nests and consuming solid foods (El Nagar et al., 2014; Szendrö et al., 2019). This milk is important in shaping the kits' microbiota (Beaumont et al., 2020). Rabbit farmers face problems of lack of milk due to the mother's death, insufficient milk production, or even abandonment of the offspring (Leite et al., 2022).

In order to improve the survival of the kits, the development of efficient strategies such as the formulation of specific milk replacer is essential (Paul & Friend, 2017; Chankuang et al., 2020; Ozawa & Gleeson, 2024). Therefore, it is important to understand the composition of rabbit milk, which present different compositions and volumes throughout the lactation period (Maertens et al., 2006).

Regarding methods of rabbit milk collection, methodologies as the collection from the oesophagus present problems in its results. Studies have introduced some alternatives, as the manual collection using hormone induction (Maertens et al., 2006; Ludwiczak et al., 2023) and milking machines. However, such alternatives imply additional investments.

Considering the importance of rabbit milk for the production system and the scarcity of information about its nutritional composition, the aim of this study was to develop a milking methodology and to determine the nutrients of New Zealand White does' milk.

Materials and Methods

All experimentais procedures were approved by the Ethics Committee on the Use of Animals (CEUA/UEM) of the Universidade Estadual de Maringá (Process number 5542190123). Prior to the experiments, New Zealand White rabbits were crossed following the standards of the farm. Crossings and births occurred every 15 days. The nests (20x40x25 cm) were placed for the pregnant does five days before the expected delivery date. This allowed does to prepare them for the arrival of the kits. The nests were placed over paper sheets to avoid moisture and to maintain an ideal temperature. Dry pine shavings were inserted inside the nests (approximately 200 g) as nesting material. Pellets of 4.5 mm were provided *ad libitum* for the females as feed, formulated to meet the requirements of growing rabbits, according to De Blas & Mateos (2020).

Animals were placed in suspended metal cages (80x60x45 cm) used for rabbit production. They were in a closed masonry shed with canvas curtains on the sides, floor made of concrete, and fiber-cement tile roof. The cages were equipped with semi-automatic galvanised aluminium feeders and automatic nipple drinkers. Wooden nests were made of marine plywood and screened with aluminium mesh with a gap of 3 mm.

Two experiments were carried out in Universidade Estadual de Maringá, in the municipality of Maringá, state of Paraná, Brazil. In the first experiment (Experiment 1), the milk collected was used to determine the contents of total solids (TS), free fat, fat, crude protein, gross energy, mineral matter, macrominerals – Ca, P, Na, Mg, K –, and microminerals – Zn, Fe, Cu, Mn. All percent contents were TS based. Ten lactating New Zealand White does with a mean body weight (BW) of 3.5 kg and age ranging between one to two years old – parturition order between 3 and 6 – were subjected to natural breeding 11 days after last parturition. Males comprising a mean BW of 3.3 kg and ranging between one to two years old were used.

After parturition, five does (n=5) with eight kits each were identified and redistributed to new cages to be monitored. Two cages were used per doe: one to place the nest; and another one to separate the doe the day before milk collection. Milk collection was done in the morning in order to prevent kits from spending too much time without feeding since the females have the habit of feeding them during the night. Does were subjected to five milk collections on the first, 7th, 14th, 21st, and 28th day of lactation.

In the second experiment (Experiment 2), 23 lactating New Zealand White does between 18th and 21st day of lactation with a mean BW of 3.55 kg and aged from one to two years old were used. This experiment determined and compared the contents of TS, mineral matter, fat, crude protein, lactose, freezing point (FP), and gross energy of milk from does, Saanen goats, and cows. After parturition, does were identified and redistributed to new cages to be monitored. Milk collection was carried out once in each doe.

The collection method used followed the same one described for Experiment 1. Given the amount of milk needed for some analysis, the samples' sizes were different. The amount of milk produced for each animal ranged from 25 to 50 mL. For content determination of TS, ash, fat, protein, and FP, it was used n=23 because the amount of milk needed for each determination was 10 mL and it was possible to obtain that from all does. For the gross energy, only 11 does (n=11) produced enough milk (at least 20 mL) so that to allow analysis. The production of the remaining 12 does was not used for energy determination. For lactose, 200 mL of milk were needed for the analysis. The milk of five does were combined in order to obtain a unique sample (n=1). The five most productive does were selected. The sum of milk produced by the remaining animals did not reach 200 mL, therefore, their data was not used in the milking information.

The proposed methodology to collect milk from does (Figure 1) used hormonal stimulation, exposure to rabbit kits, manual massages of the teats, and mechanical suction. On the day before the milk collection, the females were separated from their kits for a period of approximately 24 h to avoid milk consumption and to accumulate it in the mammary glands. On the day of collection, 0.05 mL (10 IU mL^{-1}) of the synthetic hormone oxytocin was applied in the dorsal region of the females, with the aid of an insulin

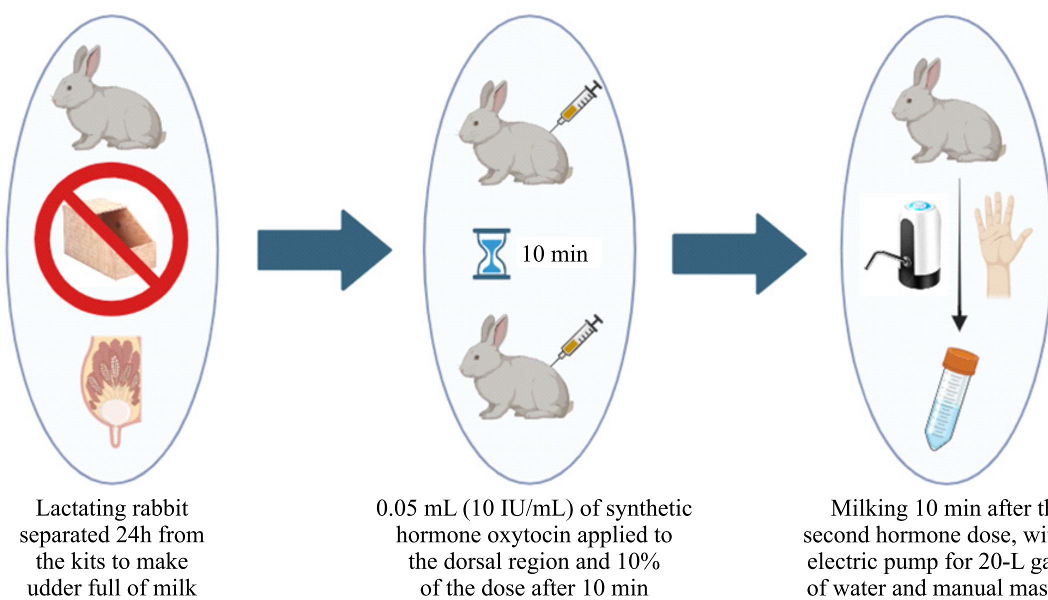

Figure 1. Illustrative representation of the milk collection protocol.

needle. The hormone dose used was of 0.01 mL kg^{-1} of BW. Ten minutes after the first dose, a booster dose of 0.005 mL kg^{-1} of BW of the same hormone was given. After 10 minutes, time sufficient for the oxytocin to take effect, the milking began.

The UT 40G electric pump (UTIMAIS+, Colombo, PR, Brazil) for a 20-L water bottle carboy was used as an adapted milking pump. The water outlet of the electric pump was attached to a water bottle for domestic cats. The water inlet of the pump was attached to a silicone hose. The other end of the hose was attached to the does' teats for suction. In order to induce milk releasing, one kit of each doe was allowed to feed itself for approximately 1 minute. During the entire milking, manual massages were performed to assist suction. Milk was collected from all pairs of teats. Two 15-mL Falcon tubes were collected from each doe. After collection, milk samples were kept in a refrigerator at 4°C.

Refrigerated samples were subjected to fat analysis and determination of freezing point using the Master Complete milk analysis equipment (Mylabor, Tatuapé, SP, Brazil). Then, the samples were subjected to chemical analysis to determine TS using Method 930.15 (Horwitz, 2006), mineral matter (MM) using Method 942.05 (Horwitz, 2006), and crude protein (CP) using Method 984.13 (Horwitz, 2006). The gross energy (GE) were determined using an adiabatic calorimeter (Parr AC 6200, Moline, IL). The analysis of macro – Ca, P Na, K, Mg – and microminerals – Zn, Fe, Cu and Mn – were carried out using Method 2011.14 (Latimer Jr., 2016).

As to Experiment 2, cow's milk and goat's milk were used as reference comparison for rabbit's milk. Three different types of cow's milk were used: whole milk, fat free milk, and lactose free milk. All types were sampled from available carton boxes of UHT milk in the local market. Fresh goat's milk were sampled from morning milking of the University's goat herd. The goats were of the Saanen breed, aged between two and four years old, receiving corn silage and concentrated made of ground corn, soybean, and vitamin-mineral supplement. The goat's production was a semi-confinement system with access to paddocks with star grass (*Cynodon aethiopicus*) and signal grass (*Brachiaria decumbens*) forage. The mean milk production was 2.5 kg day^{-1}.

Experimental unit was one doe in both experiments. Experiment 1 used a split-plot experimental design with repeated measures of different days (main units) over animals (subunits). Experiment 2 had no experimental design since it was carried out one collection of milk of each doe and the rabbits were not submitted to treatments.

Data of Experiment 1 were checked for outliers, normality of residues, homoscedasticity, independence of residues, and linearity. Outliers were verified using Student standardized residuals analysis. Standard deviations greater than three were considered outliers. Assumption of normality was checked using Shapiro-Wilk's test. Homoscedasticity was checked using Levene's test. Independence of errors was checked using Durbin-Watson's statistics. Linearity was verified through correlation coefficients. All assumptions were met. After outliers' removal, a one-way analysis of covariance (ANCOVA) and one-way analysis of variance (ANOVA) were carried out. Additionally, the variable "days of lactation" was included in the main plot and the variable "animals" was included in the subplot. A repeated-measures analysis of variance (ANOVA) was performed for modeling time as a regression variable. It was used procedure MIXED of the statistical package SAS (SAS Inst. Inc., Cary, NC, USA).

No post-hoc test was carried out on the mean results of the milk components. Instead of that, a polynomial regression model was obtained in order to describe the behavior of eight components of the rabbit milk – energy, ash, Ca, P, Na, Mg, K, and Zn – along the days of lactation. Variance inflation factor (VIF) was used to check multicollinearity among independent variables. The ones presenting VIF > 10 were removed from the regression model. The degrees of freedom referring to the day of milk collection were broken down into orthogonal polynomials to obtain the regression equations, according to the best fit. The model fit was assessed using the coefficient of determination (R^2). The criteria used to interpret R^2 were: $R^2 \geq 0.7$, "good fit"; $0.3 \geq R^2 > 0.7$, "acceptable fit"; and $R^2 < 0.3$, "poor fit". The accuracy of the estimates was assessed using standard deviations. For all analyses, $\alpha = 0.05$ was considered.

Data of Experiment 2 were submitted to descriptive analysis. Mean values and standard deviations were calculated and presented. They were not submitted to any inferential analysis.

Results and Discussion

The milking methodology proposed for milking New Zealand White does was tested and proven effective. The milk obtained was sufficient for all laboratory analyses.

Results of the bromatological analyses and energy for different does and along the lactation curve are shown in Table 1. There was no difference ($p > 0.05$) between animals, confirming that the mammary gland can modulate a constant nutrient content in milk. There was difference ($p < 0.05$) along the days of evaluation for milk composition.

Energy values decreased linearly along the lactation curve, from the first day of collection, when colostrum was collected, to the last day of collection (28th day). A slight deviation at the peak of lactation, between 14th and 21st day, and decreasing again afterwards (Figure 2).

The lactation period lasts for five weeks, sometimes extending to six weeks, depending on the type of housing and handling (Maertens et al., 2006; El Negar et al., 2014). In the present study, at the 5th week, females had already ceased or drastically reduced their milk production.

Rabbit kits consume colostrum until the 4th day of life. According to Maertens et al. (2006), colostrum is the milk with the highest dry matter (DM) during lactation period due to its high fat and protein contents. They found means of DM, fat content, and protein content of 32.6%, 16.3%, and 14.7%, respectively. Ludwiczak et al. (2020) found the same results.

In the present study, the highest total solids (TS) content was observed on the last day of collection (28th day). The protein level was highest on the 7th day. The highest fat content was found in the colostrum, but slightly below the values reported by Maertens et al. (2006). In colostrum, El-Sayiad et al. (1994) found contents of 15.9%, 13.7%, and 2%, protein, fat, and ash, respectively. In the present study, the fat content was higher when protein and ash were lower. Maertens et al. (2006) found higher contents of ash and energy in colostrum, 1.8% and 2,222.27 kcal L^{-1}, respectively.

Several factors may affect milk composition. Breed, feeding, number of kits, and duration of lactation period are some of them (Ludwiczak et al., 2020). In terms of breed, El-Sayiad et al. (1994) studied the New Zealand White and found contents of DM, fat, protein, and ash of 31.6%, 14%, 13.6% and 2.1%, respectively. Such results were close to those expressed in Table 1,

Table 1. Results of Experiment 1, mean and standard deviation (of each lactation day of collection, general mean, standard error of the mean (SEM), and p-value of the milk components of New Zealand White does during lactation period.

Components	Lactation days					General mean	SEM	p-value
	1st	7th	14th	21st	28th			
Total solids (TS, %)	28.32±4.50	26.10±3.59	28.78±2.22	28.33±6.86	32.61±5.74	28.80	0.98	0.428
Free-fat (% of TS)	28.32±4.50	26.10±3.59	28.78±2.22	28.33±6.86	32.61±5.74	28.80	0.98	0.292
Fat (% of TS)	15.15±1.82	14.61±2.29	15.14±0.70	13.81±1.87	13.77±0.72	14.44	0.35	0.136
Crude protein (% of TS)	10.91±1.77	11.44±1.89	9.47±0.18	10.66±1.71	11.32±2.16	10.77	0.34	0.385
Ash (% of TS)	1.25±0.09	2.00±0.29	2.17±0.22	2.13±0.37	2.05±0.23	1.92	0.09	<.001**
Gross energy (kcal L^{-1})	1,944±20	1,679±154	1,770±122	1,705±66	1,373±173	1,694	46.97	<.001*
Macrominerals (% of TS)								
Calcium	0.22±0.10	0.30±0.02	0.42±0.05	0.42±0.04	0.43±0.06	0.36	0.02	<.001*
Phosphorus	0.09±0.02	0.09±0.01	0.09±0.03	0.12±0.02	0.14±0.01	0.11	0.01	<.001*
Sodium	0.08±0.00	0.07±0.00	0.07±0.00	0.06±0.00	0.06±0.00	0.07	0.00	<.001*
Magnesium	0.03±0.01	0.03±0.00	0.04±0.00	0.05±0.01	0.06±0.01	0.04	0.00	<.001*
Potassium	0.02±0.00	0.02±0.00	0.02±0.00	0.01±0.00	0.01±0.00	0.02	0.00	<.001*
Microminerals (ppm)								
Zinc	25.86±9.01	23.49 ±3.81	21.12±4.17	13.02±1.73	6.24±0.84	17.95	1.79	<.001*
Iron	15.14±3.58	15.93±2.54	16.72±3.28	17.47±2.41	18.09±1.06	16.67	0.57	0.194
Copper	3.21±1.66	3.03±1.18	2.86±0.84	5.05±3.71	7.23±6.46	4.28	0.70	0.094
Manganese	2.90±0.30	2.90±0.17	2.89±0.26	2.78±0.18	2.75±0.28	2.84	0.05	0.093

*Linear regression. **Quadratic regression.

but DM and protein, which were higher. Parity order and number of kits affect milk composition and production (Zerrouki et al., 2005). Multiparous females can reach a production 30% higher than primiparous females (Zerrouki et al., 2005). This is related to the development of the mammary glands along lactation. Ludwiczak et al. (2020) evaluated milk production of females nursing eight or ten kits and observed an increase in milk production for does with ten kits when compared to those with eight. They stated that the day of lactation also affected milk composition.

Collection day affects milk composition of rabbit during lactation. Ludwiczak et al. (2023) observed such effect for fat and protein contents. Their results were slightly lower than the ones found in the present study, 8.8% and 10.7%, respectively.

Although most of the components contents show variations, fat content varies only slightly in the final third of the lactation (Table 1). Rabbit milk is high in fat, an important energy source for the kits (Maertens et al., 2006). Its composition encompasses triglycerides, diglycerides, monoglycerides, liposoluble vitamins, free fatty acids, cholesterol, and phospholipids.

The peak of milk production coincides with the moment when the kits begin to leave the nests freely and to consume solid food, which occurs between 16 to 19 days postpartum (Davies & Davies, 2003; El Nagar et al., 2014; Gidenne et al., 2020). Ludwiczak et al. (2020) found that the peak of lactation occurred on the 17 days postpartum. However, milk production consistently increases until the third week of lactation and decreases thereafter (Zerrouki et al., 2005; Xiccato & Trocino, 2020).

The lactation peak reported in the literature presents the lower contents of fat and protein. In contrast, at the end of the lactation period, while the amount of milk produced is smaller, nutrient contents are higher (Maertens et al., 2006). Between the 4th and 5th week postpartum, Maertens et al. (2006) reported a value of 37.7% of DM, and El-Sayiad et al. (1994), 32.3%. El-Sayiad et al. (1994) and Maertens et al. (2006) found out that energy was higher at the beginning of the lactation and in its end. Regarding ash content, a low content was found in the beginning of the lactation and increasing toward the end of the period.

Mineral content as function of lactation day is in Figure 3. The results showed a good linear function for

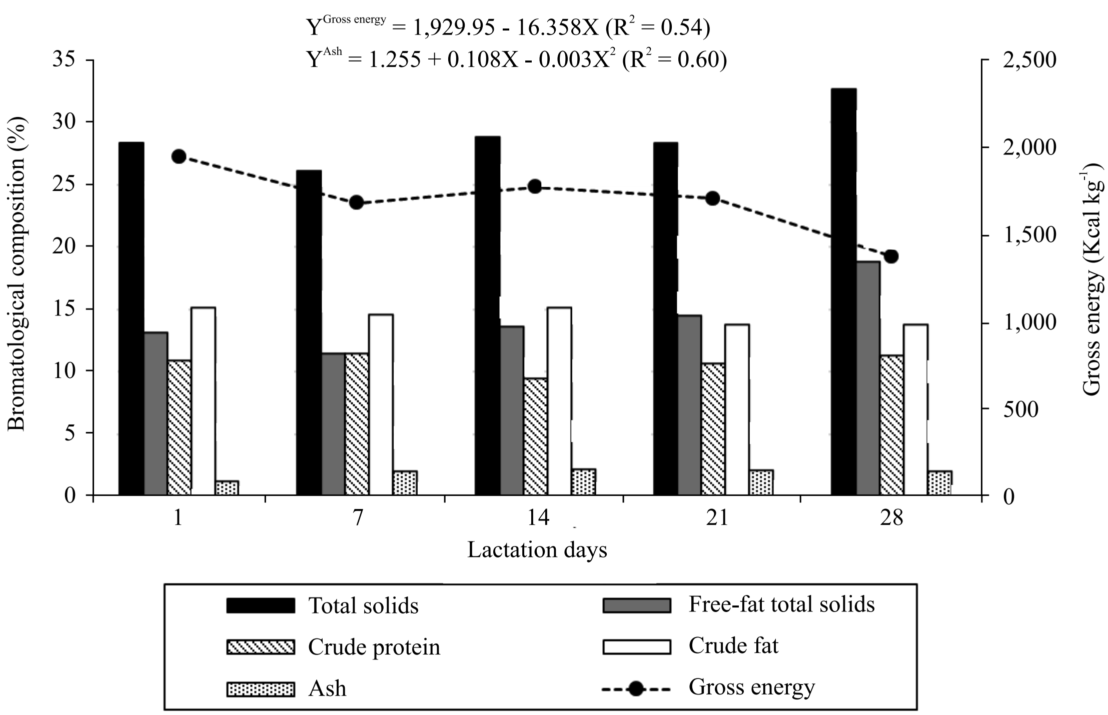

Figure 2. Bromatological and energy composition of New Zealand White does' milk over 28 days of lactation.

K, Mg, and Zn ($R^2 \geq 0.7$). The linear functions for Ca, P, and Na showed an acceptable fit ($0.3 \leq R^2 < 0.7$). Bromatological and energy composition of the milk as function of lactation day is in Figure 2. Ash content showed an acceptable fit as a quadratic function of lactation day, while energy content showed an acceptable negative linear function.

Similar to the milk of other mammalian species, rabbit milk is rich in calcium, the most common mineral, with 0.488% for New Zealand Black rabbits (El-Sayiad et al., 1994). Concerning Ca, some authors found a Ca and P ratio of 2:1 in the milk composition (El-Sayiad et al., 1994; Maertens et al., 2006; Mateos et al., 2020). However, Lebas et al. (1971) observed that this ratio is lower at beginning of lactation and increased over time reaching between 1.7:1.0 and 2.0:1.0, at 25th day. In the present study, this ratio was not observed. The results showed a ratio of approximately 3:1.

El-Sayiad et al. (1994) observed an increase in sodium content over time, while magnesium content decreased. In the present study, the results are in disagreement (Figure 3). Lebas et al. (1971) reported similar findings, such as an increasing in the magnesium content over time.

Kustos et al. (1999) observed that levels of Zn, Fe, Cu, and Mn in rabbit milk gradually decreased along lactation. However, this was not found in the present study, exception to Zn. The contents of Fe, Cu, and Mn increased or remained similar along the experiment.

Results of the bromatological analyses of fresh rabbit milk, UHT cow milk – whole, fat-free and lactose-free –, and fresh goat milk – whole – are in Table 2. The rabbit milk had three to four times more fat and protein than the other milks. It had also two to four times more energy than whole cow milk. The lactose content was two times lower than the other milks, but lactose-free milk. Freezing point of rabbit milk was lower than the other milks. Rabbit milk had the highest ash content, suggesting that it is richer in nutrients than goat and cow's milk. Rabbit milk is the lowest in lactose content. These results agree with those found by other authors (Maertens et al., 2006;

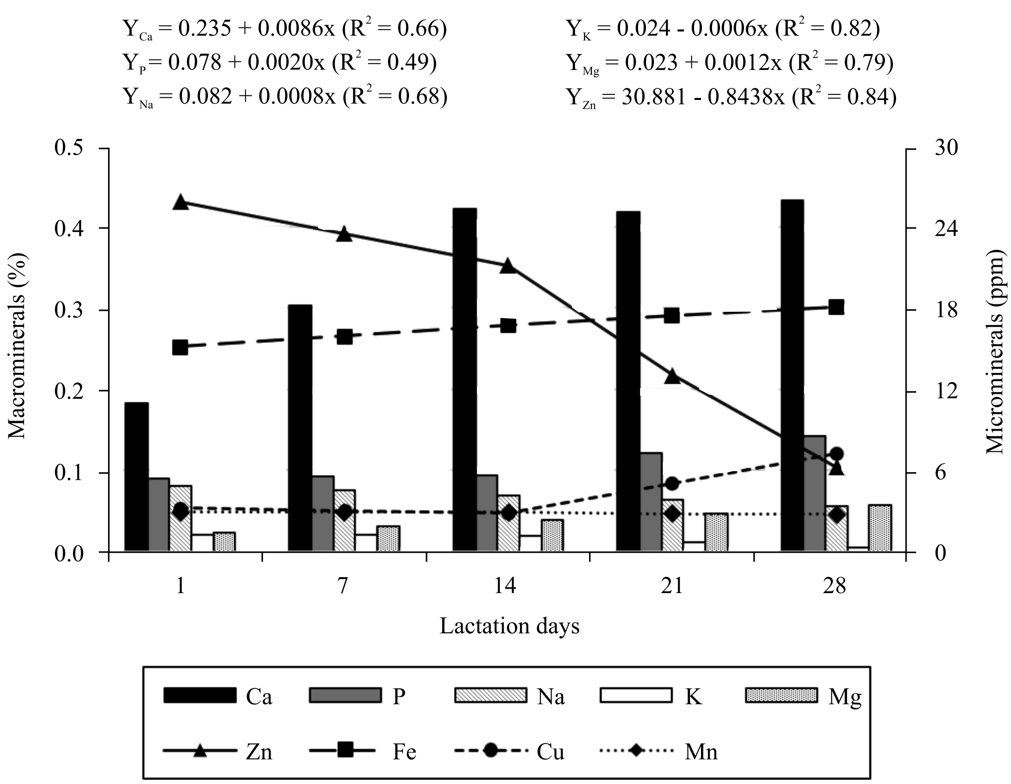

Figure 3. Macro (Ca, P, Na, K, and Mg) and micromineral (Zn, Fe, Cu, and Mn) contents in New Zealand White does'milk, over 28 days of lactation.

Table 2. Results of Experiment 2, average composition of New Zealand White does' fresh milk between 18 and 21 days of lactation (milking peak), compared to Saanen goats fresh milk, and UHT cow milk (lactose-free, skimmed, and whole milk).

Variables	Rabbit	Goat	Cow(1)		
	Fresh milk	Fresh milk	Lactose free	Skimmed	Whole
Total solids (TS, %)(2)	26.19±3.83	12.27	8.97	8.48	11.29
Mineral matter (% of TS)(2)	2.32±0.31	0.73	0.49	0.64	0.59
Fat (% of TS)(2)	14.27±1.78	3.65	0.92	0.32	3.00
Crude protein (% of TS)(2)	9.89±0.88	3.72	3.22	3.30	3.20
Lactose (% of TS)(3)	2.09	3.62	0.00	4.62	4.20
Freezing point (FP, ºC)	-1.060±0.07	-0.602	-0.593	-0.539	-0.530
Gross energy (kcal L^{-1})(4)	1,549.72±253.79	697.72	450.81	384.17	607.64

(1)Free lactose bovine milk, skimmed, and whole. (2)Sample size n=23. (3)A unique sample made of a pool of the five most productive does. (4)Analysis performed using milk from 11 does.

Chankuang et al., 2020; Ludwiczak et al., 2020, 2021, 2022; Ozawa & Gleeson, 2024).

Does produce a milk two to three times more concentrated in fat and protein than cows and sows, however comprising 1/3 of the amount of lactose (Maertens et al., 2006). Maertens et al. (2006) reported a lactose content of 1.70%, whereas El Nagar et al. (2014), 2.67%, and Ludwiczak et al. (2020), 2.69%. These results are close to the ones found in the present study.

Not only has the fat content present in rabbit milk differed from that of milk from other animals, but also the fat composition. According to Maertens et al. (2006), rabbit milk fat is characterised by a large amount of medium-chain fatty acids, mainly caprylic acid (C8:0) and capric acid (C10:0), accounting for 26.3% and 20.1%, respectively. However, the fat is composed of 70% saturated FAs (SFAs), 13% monounsaturated FAs (MUFAs) and 16% polyunsaturated FAs (PUFAs), whereas the amounts of oleic acid (11.3%) and linoleic acid (12.8%) are similar.

It is important to highlight that both lactose and sodium are part of the main constituents involved in maintaining constant milk osmolarity. Although rabbit milk has a low lactose content, this is compensated by the high concentration of sodium, even greater than that of cow and goat's milk (Maertens et al., 2006).

Conclusions

1. The milking methodology proposed was effective.

2. Milk from New Zealand White does is rich in protein, fat, and energy and poor in lactose compared to goat and cow's milks.

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