



Effect of the Use of *Brachiaria ruziziensis* on the Chemical Composition of Milk and Serum from Dairy Cattle Breeds in Vina, Cameroon

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Abstract

The demand for animal protein is expected to double by 2040 due to population growth, urbanization, and changing consumption habits [1]. Beyond the need to produce food for a growing population, health concerns call for production methods that preserve human health. This study aimed to evaluate the effect of feeding dairy cows *Brachiaria* on the mineral and cholesterol content of their milk. The objective was to evaluate the use of *Brachiaria* on the composition of milk and serum from dairy cows. Twenty-five cows aged 4 - 8 years and of different breeds (Gudali, Montbéliard, Holstein) were selected from 5 farms with natural pasture and a *Brachiaria* field and the same farming system; Milk and blood samples taken from these cows, raised either on natural pasture or improved pasture (*Brachiaria*), were subjected to assays for Na, N, P, K, Ca, and lipid profile (HDL cholesterol). It appears that regardless of the forage type, the milk calcium concentration is higher in Gudali (local breed) fed on natural pasture. The Student's T-test performed shows that between the animal groups, there is a significant difference ($P > 0.05$) between the Ca concentrations in animals on *Brachiaria* and those on natural pasture. Indeed, the mean Ca in animals on *Brachiaria* (1580 mg/L) is higher than that of animals on natural pasture (1577 mg/L). There is no significant difference in glucose levels between animals fed the different types of pasture. The HDL cholesterol concentration is identical for all animal groups regardless of the type of pasture consumed. In conclusion, there is no major difference in the composition of milk or serum from animals that consumed *Brachiaria* or natural pasture.

Subject Areas

Animal Behavior

Keywords

Brachiaria ruziziensis, Chemical Composition, Milk, Serum, Vina

1. Introduction

Milk is one of the most produced and most valuable agricultural raw materials in the world. In 2013, with a total production of 770 billion liters valued at 328 billion US dollars, milk ranked third globally in terms of production volume of agricultural raw materials and first in terms of value. Milk contributes 27% to the overall value added of livestock farming and 10% to that of agriculture. The dairy sector relies on natural resources such as land, water, nutrients, and energy. Feeding dairy cows, sheep, goats, and buffaloes requires approximately 1 billion hectares of land, which is 7% of the Earth's surface. This land primarily consists of grasslands (meadows and pastures). These grasslands provide 77% of forage grass and straw, meaning dairy herds worldwide convert inedible materials for humans into high-quality essential proteins and micronutrients. Therefore, it is necessary to provide them with high-nutritive-value forage. Among the forages meeting these conditions, researchers have selected a grass: *Brachiaria* sp., which is one of the most important known tropical plants globally. The genus *Brachiaria* includes approximately 100 recorded species, of which 7 species of African origin have been used for pasture production in South America, Asia, South Pacific, and Australia. It has a high biomass production potential and produces nutritious forage, which increases livestock productivity [2]. Beyond the need to produce food for an ever-growing population, health concerns call for production methods that preserve human health. The lipid content of milk has raised questions about the potential effect of milk fat quality on the frequency of cardiovascular diseases in humans who consume it. Indeed, the composition of milk can be largely influenced by the diet of dairy cows [3]. In ruminants, the plasma concentration of total lipids is approximately between 2.9 and 5.6 g/l [4]. Cholesterol, the fat responsible for cardiovascular diseases, is also found in milk. But its esterified plasma portion remains predominantly associated with HDL (High-Density Lipoproteins) in ruminants, while plasma LDL (Low-Density Lipoprotein) cholesterol has a low concentration [4]. The presence of certain minerals such as sodium (Na) could impact milk production levels [4]. Milk minerals, however, represent a small fraction (8 - 9 g/l) compared to other fractions; this fraction contains calcium, magnesium, sodium, and potassium as the main cations and chloride, inorganic phosphate, and citrate as the main anions. Some forages have a potassium (K) content exceeding the nutritional requirements of ruminants, but the latter have the capacity to tolerate variations in potassium absorption between 3.8 and 20 mmol per kg/day, whereas a value of 40 mmol per kg/day is considered toxic [5]. This study aimed to evaluate the effect of dairy cows consuming *Brachiaria* on the mineral and cholesterol content of milk. The objective was to evaluate the use of *Brachiaria*

on the composition of milk and serum from dairy cows.

2. Materials and Methods

2.1. Laboratory Equipment

It consisted of

- Dry test tubes;
- Laboratory gloves;
- Syringes;
- Cool box;
- Centrifuge;
- -20°C freezer.

2.2. Animals or Genetic Material

Twenty-five cows aged 4 - 8 years and of different breeds (Gudali, Montbéliard crossbreeds, Holstein crossbreeds) were selected from 5 different farms with natural pasture and a field of *Brachiaria*; each farm selected had either Montbéliard crossbreed, Holstein crossbreed or Gudali breed; and from each farm 5 healthy lactating animals were selected. The 5 groups were distributed as follows:

- Montbéliard crossbreeds fed on natural pasture, typically consisting of Andropogon;
- Montbéliard crossbreeds fed on *Brachiaria*;
- Holstein crossbreeds fed on natural pasture;
- Holstein crossbreeds fed on *Brachiaria*; and finally;
- Gudali fed on natural pasture (control group).

Brachiaria forage was rationed twice a day (At 9 o'clock and 4 o'clock in the afternoon) as hay since the experiment took place during the dry season. Animals on natural forages were exposed to natural pasture from 10 O'clock in the day till 4 O'clock in the afternoon. The supplement to forage was almost the same amount (500 g/anl/day) except for Gudali (200 g/anl/day). The complement was composed of cotton seed cake, cotton seed bran and maize bran and salt.

All the animals had water ad-libitum on the farm.

Blood was drawn early in the morning from the jugular vein (before the cows were milked) into 5 ml tubes using a syringe and stored in a cooler. Milk was collected in small 10 ml bottles and then placed in a cooler. Upon arrival at the laboratory, the blood samples were centrifuged at 3400 rpm for 5 minutes; then the serum was transferred to another tube, which was kept at -20°C . The milk bottles were kept at -20°C . The milk and blood samples were subjected to assays for Na, N, P, K, Ca, and lipid profile (HDL cholesterol).

2.3. Mineral and Cholesterol Analysis Protocols

The various milk and serum samples were analyzed for total cholesterol, HDL, nitrogen, glucose levels, phosphorus, sodium, potassium, and total proteins. The assay protocols for each element were as follows:

HDL Cholesterol

The specimen (1 ml) was measured into a centrifuge tube, then a precipitating reagent (100 µl) was added. The mixture was thoroughly mixed, then left to stand for 10 minutes at room temperature. The mixture was then centrifuged for 15 minutes at 3500 - 4000 RPM to obtain a supernatant. Next, the standard was calibrated using a pre-treated serum calibrator. 1 ml of reagent and 25 µl of demineralized water (blank), 1 ml of reagent and 25 µl of standard at 1 g/l (standard), and 1 ml of reagent and 25 µl of supernatant (assay) were mixed. The mixture was then left to stand at 37°C for 5 minutes. The color remained stable for one hour. The result was determined using the following formula:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

Glucose

The reagents and specimen were brought to room temperature, and the temperature was maintained constant throughout the measurement series. The steps for the glucose assay were as follows:

The reagents and specimen were thoroughly mixed, then left to incubate for 10 minutes at 37°C or 20 minutes at room temperature. The absorbances were read at 500 nm (460 - 560 nm) against the reagent blank. The color was stable for 15 to 20 minutes at 37°C and then gradually decreased. The result is determined using the following formula:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

Calcium (Ca)

A serum blank was performed by adding one drop (25 µl) of a 10mM EDTA solution to the test and reagent blank tubes, mixing, and reading again. This value was subtracted from the one previously read for the specimen. The result is calculated as follows:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

Phosphorus

The protocol was as follows:

The specimen was measured in clearly labeled test tubes; 1 ml of reagent, consisting of a 9 g/l NaCl solution, was mixed with 20 µl of demineralized water. The standard was 20 µl. The mixture was thoroughly mixed and incubated for 2 minutes at room temperature. The absorbance of the standard and the test sample was read using a 1 cm pathlength cuvette at 340 nm (334 - 366 nm) against the reagent blank. The specimen blank was read against the 9 g/l NaCl solution.

The result is determined using the following formula:

$$\text{Result} = \frac{\text{Abs (Sample)} - \text{Abs (Sample Blank specimen)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

Potassium

Samples were stored protected from light in the original, tightly sealed bottle at 2°C to 8°C, and were used and stored under recommended conditions. The reagents are stable for at least 30 days. The analyzer provided the results.

Sodium

Samples were stored protected from light in the original, tightly sealed bottle at 2°C to 8°C, and were used and stored under recommended conditions. The reagents are stable for at least 30 days. The following steps were followed: The sample was centrifuged and tested rapidly. The calculations were performed automatically by the analyzer.

Nitrogen

Colorimetric method described by Gornall *et al.* (1949) [6]. The peptide bonds of proteins react with Cu^{2+} in an alkaline solution to form a colored complex. The absorbance of this complex, which is proportional to the protein concentration in the specimen, is measured at 550 nm. The Biuret reagent contains sodium potassium tartrate, which complexes copper ions and maintains their solubility in alkaline solution. When stored protected from light in the original, tightly sealed bottle at 18°C - 25°C, the reagents are stable. Any reagent that is turbid or has an absorbance > 0.150 at 550 nm is discarded. Specimen collection and preparation (Serum or plasma). Analysis on

Fresh specimen or specimen stored at 2°C - 8°C for less than 72 hours. Proteins are stable in serum: 6 months at -20°C, indefinitely at -70°C.

2.4. Statistical Analyses

For the assays in milk and serum, descriptive analyses were performed to determine the quality and quantity of the measured substances. The data were then subjected to ANOVA using SPSS Software, and any existing differences among the means of the studied substances on different pastures were compared using T-test. The data on material costs were subjected to descriptive analysis using R-software.

3. Results

3.1. Concentration of Calcium, Sodium, Potassium, Phosphorus, Proteins, Glucose, and Cholesterol for the 5 Animal Groups in the Milk

Table 1 above shows the concentrations of proteins, glucose, cholesterol, and the main minerals in the milk.

Calcium concentration in the milk of Holstein crossbreeds fed on natural pasture (6.87 mmol/L or 1236 mg/L) and that of Montbéliard crossbreeds fed on the same natural pasture (9.27 mmol/L or 1668.6 mg/L) is lower than that of Gudali fed on natural pasture (10.15 mmol/L or 1827 mg/L). The calcium concentration in the milk of Holstein crossbreeds fed *Brachiaria* is (7.53 mmol/L or 1355.4 mg/L) and that of Montbéliard fed *Brachiaria* is (10.03 mmol/L or 1805.4 mg/L). It therefore appears that, regardless of the forage type, the milk calcium concentration is

Table 1. Concentration of Calcium, potassium, phosphorus, proteins, glucose and cholesterol in the milk for the 5 animal groups.

Animal group	Ca (mg/l $\pm$ SD)	Na (mg/l $\pm$ SD)	K (mg/l $\pm$ SD)	P (mg/l $\pm$ SD)	Protéines (mg/l $\pm$ SD)	Glucose (mg/l $\pm$ SD)	Cholesterol HDL (mg/l $\pm$ SD)
Gudali Natural Pasture	1827 $\pm$ 0.22 ^a	2400 $\pm$ 1.02 ^a	30.9 $\pm$ 0.02 ^{bc}	720 $\pm$ 0.52 ^a	4297 $\pm$ 0.77 ^b	200 $\pm$ 0.00 ^a	9 $\pm$ 0.01 ^a
F1_Montbeliard <i>Brachiaria</i>	1805 $\pm$ 1.38 ^a	2316 $\pm$ 0.59 ^a	27.59 $\pm$ 0.01 ^c	786.6 $\pm$ 0.05 ^a	7548 $\pm$ 2.37 ^a	300 $\pm$ 0.01 ^a	7.2 $\pm$ 0.01 ^a
F1_Montbeliard Natural Pasture	1668 $\pm$ 2.16 ^b	2631 $\pm$ 1.74 ^a	27 $\pm$ 0.01 ^c	774 $\pm$ 0.71 ^a	7582 $\pm$ 5.73 ^a	200 $\pm$ 0.01 ^a	9 $\pm$ 0.01 ^a
F1_Holstein <i>Brachiaria</i>	1355 $\pm$ 4.26 ^c	2329 $\pm$ 0.20 ^a	33.6 $\pm$ 0.02 ^b	806.4 $\pm$ 0.37 ^a	3588 $\pm$ 1.67 ^c	200 $\pm$ 0.01 ^a	9 $\pm$ 0.02 ^a
F1_Holstein Natural Pasture	1236 $\pm$ 0 ^c	2332 $\pm$ 12.96 ^a	898.2 $\pm$ 9.74	631.8 $\pm$ 0.0 ^a	5591 $\pm$ 0.00 ^b	200 $\pm$ 0.02 ^a	10.8 $\pm$ 0.06 ^a

higher in Gudali fed on natural pasture. Calcium concentrations appear lower in Holsteins crossbred than in Montbéliard crossbred and Gudali on natural pasture. Student's T-test shows that between the animal groups; there is a significant difference ($P > 0.05$) between the Ca concentrations in animals on *Brachiaria* and those on natural pasture. Indeed, the mean Ca level in animals on *Brachiaria* (1580 mg/L) is higher than that of animals on natural pasture (1577 mg/L).

In Montbéliard crossbred cattle on natural pasture, a slight difference in sodium levels is observed compared to the other groups. However, Student's T-test revealed no significant difference ($P < 0.005$) between sodium levels in the different animal groups. This level is 3 times higher in milk than in serum from Holsteins crossbred on natural pasture.

The concentration of K, which ranges from 0.08 to 0.11 mmol/L (14.4 mg/L to 19.8 mg/L) in milk from the different animal groups, shows an extremely high value in milk from Holsteins on natural pasture (9.74 mmol/L or 898.2 mg/L). This extreme value may be explained by a handling error. Nevertheless, statistical tests showed a significant difference between potassium concentrations in milk from Holsteins crossbred on natural pasture where it is higher than in all other groups (9.74 mmol/L or 898.2 mg/L) and those from Holsteins crossbred on *Brachiaria* and Gudali on natural pasture (0.18 mmol/L or 32.4 mg/L), and Montbéliard crossbred on natural pasture and on *Brachiaria* (0.15 mmol/L or 27 mg/L). However, no significant difference ($P < 0.05$) was observed between the mean K concentrations in animals on natural pasture and those on *Brachiaria*.

The main sugar contained in milk is lactose, a disaccharide composed of glucose (70 mg/L) and galactose (20 mg/L) with traces of other carbohydrates. In milk, the glucose concentration is 200 mg/L for all animals fed on natural pasture; this value ranges from 200 mg/L to 300 mg/L in Holsteins crossbred and Montbéliard's crossbred fed on *Brachiaria*. There is no significant difference between the glucose levels of animals fed on the different types of pasture.

The HDL cholesterol concentration is identical for all animal groups, regardless

of the type of pasture consumed. The value ranges from 7.2 mg/L to 9 mg/L for milk on one hand, and from 66.6 mg/L to 73.8 mg/L for serum on the other. However, Student's T-test shows a significant difference ($P > 0.05$) between HDL concentrations in animals on natural pasture and those on *Brachiaria*. Indeed, the mean concentration for animals on natural pasture is 9.6 mg/L compared to 8.1 mg/L for animals on *Brachiaria*.

Regarding phosphorus (P) levels for the three breeds, they are practically identical for all animal groups on natural pasture. Specifically, it is 3.51 mmol/L for Holstein crossbreeds on natural pasture, 4.30 mmol/L for Montbéliard crossbreeds on natural pasture, and 4 mmol/L for Gudali on natural pasture. The same trend is observed for animals on *Brachiaria*, with 4.48 mmol/L and 4.37 mmol/L for Holstein and Montbéliard crossbreeds, respectively. However, the concentration is higher in Holstein crossbreeds on natural pasture, decreases slightly on *Brachiaria*, and the opposite is observed for Montbéliard crossbreeds. Nevertheless, Student's T-test revealed a significant difference ($P > 0.05$) between the mean P concentrations of animals on natural pasture and those on *Brachiaria*. Indeed, the mean P concentration for animals on natural pasture is 708.6 mg/L, compared to 796.5 mg/L for animals on *Brachiaria*. Regarding phosphorus levels for the three breeds, they are practically identical for all animal groups on natural pasture. Specifically, it is 3.51 mmol/L for Holstein crossbreeds on natural pasture, 4.30 mmol/L for Montbéliard crossbreeds on natural pasture, and 4 mmol/L for Gudali on natural pasture. The same trend is observed for animals on *Brachiaria*, with 4.48 mmol/L and 4.37 mmol/L for Holstein and Montbéliard crossbreeds, respectively. The concentration in serum does not differ. However, it is higher in Holstein crossbreeds on natural pasture, decreases slightly on *Brachiaria*, and the opposite is observed for Montbéliard crossbreeds. Nevertheless, Student's T-test revealed a significant difference ($P > 0.05$) between the mean P concentrations of animals on natural pasture and those on *Brachiaria*. Indeed, the mean P concentration for animals on natural pasture is 708.6 mg/L, compared to 796.5 mg/L for animals on *Brachiaria* (See **Table 1**).

Milk from Gudali cattle on natural pasture contains 4297 mg/L of protein, or 4.297 g/L. This concentration is close to, though numerically higher than, that of Holstein crossbreeds on *Brachiaria*. In contrast, Holstein crossbreeds on natural pasture have a milk protein concentration of 5.591 g/L, approaching the value obtained for Gudali on natural pasture. Montbéliard crossbred has very similar protein concentrations, at 7.548 and 7.582 g/L on *Brachiaria* and natural pasture, respectively.

3.2. Concentration of Calcium, Sodium, Potassium, Phosphorus, Protein, Glucose, and HDL Cholesterol and Protein for the 5 Animal Groups in the Serum

Table 2 below shows the concentrations of protein, glucose, cholesterol, and the main minerals in serum.

Table 2. Concentration of Calcium, sodium, potassium, phosphorus, protein, glucose, and HDL cholesterol in the serum (mg/L) for the 5 animal groups.

Animal groups	Ca (mg/l $\pm$ SD)	Na (mg/l $\pm$ SD)	K (mg/l $\pm$ SD)	P (mg/l $\pm$ SD)	Protéines (mg/l $\pm$ SD)	Glucose (mg/l $\pm$ SD)	Cholesterol HDL (mg/l $\pm$ SD)
Gudali Natural Pasture	766 $\pm$ 0.07 ^a	6840 $\pm$ 1.74 ^c	43.2 $\pm$ 0.01 ^b	849.6 $\pm$ 0.24 ^a	1327 ^b $\pm$ 0.09	500 $\pm$ 0.01 ^a	70.2 $\pm$ 0.06
F1_Montbeliard <i>Brachiaria</i>	909 $\pm$ 0.61 ^b	6697.8 $\pm$ 0.41 ^c	37.7 $\pm$ 0.03 ^c	946.8 $\pm$ 0.73 ^a	1350 ^b $\pm$ 0.12	400 $\pm$ 0.02 ^a	73.8 $\pm$ 0.07
F1_Montbeliard Natural Pasture	759 $\pm$ 0.04 ^a	7117 $\pm$ 0.12 ^b	37.8 $\pm$ 0.03 ^c	856 $\pm$ 0.15 ^a	1057 ^c $\pm$ 0.18	500 $\pm$ 0.01 ^a	72 $\pm$ 0.02
F1_Holstein <i>Brachiaria</i>	779.4 $\pm$ 0.17 ^a	7039 $\pm$ 1.50 ^b	52.2 $\pm$ 0.01 ^a	847.8 $\pm$ 0.06 ^a	1091 ^c $\pm$ 0.08	500 $\pm$ 0.01 ^a	59.4 $\pm$ 0.05
F1_Holstein Natural Pasture	808 $\pm$ 0.0 ^a	808 $\pm$ 0.0 ^a	43.2 $\pm$ 0.24 ^b	912 $\pm$ 0.0 ^a	5051 ^a $\pm$ 0.00	400 $\pm$ 0.04 ^a	66.6 $\pm$ 0.37

In serum, the calcium concentration for Holstein crossbreeds, Montbéliard crossbreeds, and Gudali fed on natural pasture was 4.49, 4.22, and 4.26 mmol/L, equating to 808.2 mg/L, 759.6 mg/L, and 766.8 mg/L, respectively. Although the difference was not significant ($P < 0.005$) for all breeds on natural pasture, the concentration was highest in Holstein crossbreeds, followed by Montbéliard crossbreeds, and then Gudali. For the concentrations of exotic crossbreeds fed on *Brachiaria*, the highest value was found in Montbéliard crossbreeds (5.05 mmol/L or 909 mg/L), while Holstein crossbreeds had a value of (4.33 mmol/L or 779.4 mg/L). This latter concentration is lower than that of Holstein crossbreeds on natural pasture. The calcium concentration in Montbéliard crossbreeds was higher on *Brachiaria* than on natural pasture. Student's t-test showed that there is no significant difference ($P < 0.05$) between serum calcium concentrations, regardless of the type of pasture to which the animals are subjected. The results obtained indicate that calcium concentration in milk and serum may not be linked to the type of forage consumed but to other factors.

Sodium (Na) concentration in the sera of the different groups was nearly identical, regardless of the type of forage they were subjected to (ranging from 6.6 g/L to 7.2 g/L), except in Holstein crossbreeds on natural pasture, where it was lower. Nevertheless, it should be noted that sodium concentrations in the various sera were three times higher than those in milk from the different breeds, regardless of pasture type, except in Holstein crossbreeds. Student's t-test showed a significant difference ($P > 0.05$) between Na concentrations in animals fed natural pasture and those fed *Brachiaria*. Indeed, the mean Na concentration was higher in animals on *Brachiaria* (6868 mg/L) than in animals on natural pasture (4921 mg/L).

Potassium (K) concentration in serum of the different groups was almost identical; it ranged from 0.20 to 0.29 mmol/L, or 36 mg/L to 52.2 mg/L, across all groups, irrespective of forage type. Student's t-test showed a significant difference ($P > 0.05$) between the mean potassium concentrations in animals on natural pas-

ture and those on *Brachiaria*. Indeed, the mean K concentration on natural pasture was 41.4 mg/L, while it was 44.95 mg/L for animals on *Brachiaria*. Holstein and Gudali have nearly similar concentrations on natural pasture. Meanwhile, Montbéliard cattle have similar potassium concentrations regardless of pasture type.

Serum phosphorus (P) concentrations in animals on natural pasture ranged from 849 mg/L to 912 mg/L. On *Brachiaria*, they ranged from 847.8 mg/L to 946.8 mg/L. The student's t-test showed a significant difference ($P > 0.05$) between animals on natural pasture and those on *Brachiaria*. Specifically, the mean P concentration for animals on natural pasture was 872.5 mg/L, while for those on *Brachiaria* it was 897.3 mg/L, with the mean concentrations on *Brachiaria* being numerically higher. In the milk of animals on natural pasture, concentrations ranged from 66.6-72 mg/L, with the lowest value found in Holstein crossbreeds and the highest in Montbéliard crossbreeds. For those on *Brachiaria*, the range was 59-73.8 mg/L for Holstein crossbreeds and Montbéliard crossbreeds, respectively.

HDL level in the serum of Gudali cattle is nearly identical to that of Montbéliard crossbreeds, regardless of pasture type. Holstein crossbreeds exhibited the lowest levels, irrespective of the type of pasture consumed.

In the various sera, protein concentrations were lower than those in the milk of the different groups. Specifically, the serum of Holstein crossbreeds on natural pasture had a higher concentration (5.051 g/L) than any other group and was approximately 5 times higher than that of Montbéliard crossbreeds (1.057 g/L) on natural pasture. Conversely, the Montbéliard crossbred cattle on *Brachiaria* had a higher protein concentration (1.350 g/L) than Holstein cattle (1.091 g/L) on *Brachiaria*. But the protein concentration in the serum of Gudali cattle is higher than that of Montbéliard cattle on natural pasture. The test of St-test showed that there is a significant difference between the protein concentration in milk and in serum.

4. Discussion

Calcium concentration in milk cows in this trial ranged from 4.49 mmol/L to 4.33 mmol/L, and in serum from 3.7 mmol/L to 4.10 mmol/L. Calcium level in milk of these animals (Holstein crossbreeds on *Brachiaria* and on natural pasture) approaches the normal level (approximately 120 mg/L) [7]. The others, that is to say the crossbred Montbéliard and Gudali have rates ranging from 160 mg/L to 182,7 mg/L, a rate higher than the normal rate. ANOVA showed a significant difference between treatments (animal groups on different pastures) ($P > 0.05$); Regarding the media (milk and serum), no significant difference appeared between the media ($P < 0.05$). The combination between animal groups and media showed that the means presented a significant difference between animal groups and the media from which the element was sampled ($P > 0.05$). It appears that the calcium level in blood or milk is a trait linked to heritability, that is to say it is a hereditary characteristic that is highlighted by the type of pasture to which the animals are subjected to. In a preliminary study on the genetic parameters of calcium levels,

[8] showed that the calcium level in milk presents moderate to high heritability; and much higher than those found by [9]. The difference would also be due to the type of feed received by the cows. Indeed, the ration consisted mainly of grasses (*Brachiaria* or natural pasture with high floristic diversity) while in the morning during milking or in the evening, cows received a certain quantity of supplement composed either of cottonseed cake, or a mixture of cake, husk, and corn bran.

The phosphorus concentration in milk ranged from 3.31 mmol/L to 4.48 mmol/L, or 102 mg/L to 138.8 mg/L for all animal groups. In serum, it ranged from 4.71 to 5.26 mmol/L, or 146 mg/L to 163 mg/L. This level is higher in serum than in milk, although the analyses showed no significant difference. According to [10], the production requirements for dairy cows standardized at 4% fat are 0.90 g for all breeds. The values obtained in this study are similar to those obtained by [8] on Friesian, Normandy and Red Pied (or Holstein) cows. However, the values obtained in this study are lower than those proposed by the NRC. If we consider the NRC values, dairy cows in Vina would require additional phosphorus supplementation. However, according to [9] these values are completely normal. Regarding nitrogen (protein) concentration, the concentrations in milk for Holstein cross-bred cows were 3888 mg/L and 5591 mg/L for animals fed on *Brachiaria* and natural pasture, respectively. Montbéliard crossbred cows, regardless of feed type, had protein concentrations of 7548 mg/L and 7582 mg/L on *Brachiaria* and natural pasture, respectively. Gudali cows had a concentration of 4297 mg/L. It should be noted that the protein content in milk is higher in animals on natural pasture than those on *Brachiaria*. If the normal concentration of protein in milk is 32 to 35 g/L, the protein concentration of milk in Vina Division is very low. This means that milking cows should be supplemented with more protein.

In serum of animals from the 5 groups, the concentration of protein was higher in Holstein cows on natural pasture than in any other group. For the remaining 4 groups, the concentrations were almost identical, ranging from 1057 to 1327 mg/L. These levels are higher than those obtained by [10] on exotic and local cows (Boran, Nguni, Tuli, Afrikaner...). However, these levels are lower than those observed in animals in tropical environments like in this study by [11]. Indeed, the latter found concentrations of 64,500 to 89,200 mg/L in the serum of tropical animals (cows). However, he notes that variations in serum protein levels are due either to season or breed...But during the dry season, the levels decrease before starting to rise again at the beginning of the rainy season. We collected our samples during the dry season (specifically in January). The values obtained for both milk and serum could also be explained by the scarcity of forage in the pastures. The protein levels, represented here by nitrogen levels, also vary depending on the breed and type of pasture. However, it should be noted that for exotic breeds, protein levels are generally 30,000 - 35,000 mg/L. Protein concentrations in cows of Vina, regardless of breed and forage type, are therefore very low. This may be due to the fact that the amount of energy or protein provided to the animals through their feed is low and thus does not allow the microbial flora to increase signifi-

cantly. Moreover, during the period when the samples were taken, the animals were primarily fed straw, which has low nutritional value. Hence the supplementation that is often provided to animals in general and to dairy cows in particular. But most often, for economic reasons, this supplementation is insufficient.

In the serum, the lowest concentrations were those of Montbéliard crossbred fed on natural pasture (1057 mg/L) and Holstein fed on *Brachiaria* (1091 mg/L). The protein content of *Brachiaria* in the Vina department is around 8.5%. Nevertheless, the Student's T-test revealed that there is no significant difference between the nitrogen (protein) levels in animals from the different groups. The fact that animals on *Brachiaria* have lower protein levels than those on natural pasture can be explained by the floristic and Ecophysiological diversity of forages in natural pastures. Indeed, in natural pastures, one finds both legumes and grasses, and the animal feeding on all these species also benefits from their richness in nutrients. This indicates the need for farms seeking improvement through the selection of high-yield forages for animals to enrich rations with protein ingredients such as oilseed cakes or tropical alfalfa (*Stylosanthes hamata*). However, it should be noted that the Protein Content of milk is largely determined by the cow's genetics [12]. Indeed, the supply of crude protein in the ration, across a range of different feeding systems, appears to have an inconsistent effect on the protein content (PC) of milk [12]. However, the PC of milk can be reduced when rations are supplemented with UIP (Undegraded intake Protein) that is not well balanced in amino acids (AA) relative to the cow's requirements [12]. The availability of energy in the rumen also influences the milk PC. Indeed, experiments (11) have shown that the inclusion of corn silage significantly increases milk total protein TP. Also, during experiments [12] during which diets with high starch content were fed to cows, milk production and milk TP increased. The authors of these studies believe that a greater microbial production (Pmi) would be the cause of these results. Also, there are factors such as fat supplementation that can reduce milk TP [12]. Low fat concentrations in cow's milk are often reported in the case of range-grazing cows; this is attributable to a high intake of linoleic acid which leads to the formation of trans isomers which are associated with the decrease in milk fat [11]. This phenomenon could be amplified by the fact that less mature forages contain a lower NDF rate than mature forages, leading to less pronounced chewing [12].

The sodium content in milk and serum is very high compared to the levels obtained by [13], who reported values of 400 mg/L. In the latter's study, milk used was either a small batch milk or a large batch milk. The authors were unable to draw a conclusion about the limits within which normal sodium concentration should be judged. Indeed, the sodium content in milk varies from 2.3 to 2.6 g/kg while that in serum varies from 6.8 to 7 g/kg, regardless of the type of pasture on which the animals were raised. These results are higher than those obtained by [9] for the Normande, Friesian, and Red Pie breeds regarding milk. On the other hand, [8] gives milk sodium content equal to 500 mg/L. According to a study by [14], the lower the flow rate of milk, the richer the milk is in ion. This means

Gudali the local breed has lower milk ion content than Holstein and Montbeliard crossbreds. The lower the flow rate, the richer the milk is in ions. This means that if the cow produces less milk, its milk is richer in ions. In our study, Gudali milk, which is supposed to produce less milk than exotic breeds (Holstein and Montbeliard), contains less sodium than these breeds, both in serum and in milk. The Student's T- test showed that there is a significant difference ($P < 0.005$) between sodium concentrations in milk and serum. These differences could be due the fact that exotic cows receive slightly more salt than Gudali cows. This means, salt provided to cows in the Vina department should be reduced.

Potassium (K) concentrations in milk from animals of all groups and pasture types combined range from 0.08mmol/L to 0.11mmol/L, or 14 to 19.8 mg/L, except for Holstein crossbred on natural pasture where it is 9.74 mmol/L or 1700 mg/L. However, according to [15], the average potassium concentration in cows would be 1 g/l while [8] found a value in milk of 1400mg/L. The value is high in Holsteins crossbred on natural pasture and very low for all animal groups on all other types of pasture. The value for Holstein crossbred on natural pasture is close to that of Red Pie (1.73 g/l or 1730 mg/L) and French Friesian (1.62 g/l or 1620 mg/L) found by [9]. [13] found concentrations ranging from 1433 mg/L to 1760 mg/L, which is the same result as in Holstein crossbred on natural pasture. The hypokalemia observed in the 4 other groups may be due to several causes. Indeed, according to literature, the forage that constitutes the basis of these animals' diet should provide them with sufficient potassium. But its low level could be explained either by the intake of medications that act as laxatives, or by a low magnesium level in the blood of these animals. Potassium levels as low as these should cause muscle cramps leading to complete cardiac arrest. However, we did not observe anything like this in any of these animals. Another cause of hypokalemia could be stress. Regardless of breed or pasture type concentrations vary from 0.20 to 0.24 g/l, or 200 mg/L to 240 mg/L. The normal potassium level in blood is 1.26 g/l. However, the results obtained in our trial show very low concentrations compared to this normal level. These low concentrations may be due to high concentrations of Ca [9]. It is known that potassium plays an important role in muscle contraction, particularly those of the heart. A deficiency or excess of this element in the blood could therefore cause cardiac disorders in cows, while a deficiency in milk could cause a deficiency in humans if they are not supplemented with potassium. Hence, the supplementation of dairy cows in Vina should take potassium supplementation into account. In serum, it ranges from 0.059 g/l to 0.07 g/l; with the lowest concentration found in crossbred Holstein on *Brachiaria*. However, the normal value of HDL cholesterol (or good cholesterol) in dairy cow blood ranges between 1.063 and 1.21 g/l [15] and in milk would be 14.5mg/L. Low HDL concentrations contribute to the development of atherosclerosis (with the occurrence of complications such as hypertension, myocardial infarction, and stroke) whose causes can be genetic or secondary (dietary) [16]. Yet it has been demonstrated that the fat content of *Bos indicus* (Gudali) milk is higher than that of *Bos Taurus* (Montbeliard and Holstein) [17]. In this trial, there is no significant difference

between HDL cholesterol concentrations in all these animals, regardless of pasture type. The results obtained here are much lower than those reported by [4]. Indeed, in the article by [4], the concentration of HDL in the plasma of lactating dairy cows is 3270 mg/L. Indeed, plasma is much more concentrated than serum, which is the liquid portion of the former. The majority of plasma lipids in ruminants is transported in the HDL fraction ($d = 1.063 - 1.21$ g/ml). Although ruminants are not an exception in the animal world, they differ from humans in whom the majority of lipids are transported in light lipoproteins [17]. The low concentrations observed here could indicate that these animals' diet is low in fat. Hence the need to review the fat content in the supplement given to dairy cows. The difference could also be due to the medium from which HDL is measured.

The concentration of glucose in milk ranges from 200 mg/L for all breeds to 300 mg/L for Montbéliard crossbred cows on natural pasture. In serum, it ranges from 400 mg/L to 500 mg/L regardless of breed or forage type. According to a study by [18], the normal concentration in cow's milk 4 weeks after calving would oscillate around 3 mmol/L and less than 3.5 mmol/L, or approximately 540 mg/L. This shows that glucose level is very low in the analyzed milk and serum samples. Part of the explanation would come from the fact that the amount of forage provided or gleaned by the animals is insufficient while production is high. Therefore, forage quantities should be increased or the amount of supplements should be increased, especially since the effect of prolonged hypoglycemia could have consequences such as the cessation of milk production [18].

5. Conclusion

In conclusion, we can say that there is no major difference in the composition of milk or serum from animals that consumed *Brachiaria* or natural pasture. Therefore, *Brachiaria* can safely substitute natural pasture with the advantage that it produces more biomass. But in both cases, the diet should be supplemented with certain minerals like K. Na should be reduced. The low HDL concentration may indicate that the animals are accumulating more LDL. And the glucose level in the sera and milk clearly shows that the amount of energy ingested and produced by these animals is lower than their needs. Hence the need to increase energy sources in the diet of dairy cattle in Vina Division Cameroon.

Conflicts of Interest

The authors declare no conflicts of interest.

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