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Composition and Yield of Colostrum and Milk from Murrah and "Murrah x Carabao" Crosses in the Philippines

O. L. Bondoc[a,*], T. Almendral-Saludes[b], A. G. Tandang[b], A. R. Bustos[c], A. R. Ramos[a], & A. O. Ebron[a]
[a]Institute of Animal Science, College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna 4031, Philippines
[b]Philippine Carabao Center at UP Los Baños, Department of Agriculture, College, Laguna 4031, Philippines
[c]Institute of Human Nutrition and Food, College of Human Ecology, University of the Philippines Los Baños, College, Laguna 4031, Philippines
[*]Corresponding author: olbondoc@up.edu.ph
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ABSTRACT

This study compared the composition, yield, and freezing point of buffalo colostrum and milk collected on the 30th, 60th, and 90th day of lactation from purebred Murrah and its crosses with the Philippine Carabao. A total of 133 milk samples were collected from 36 buffaloes (20 purebred Murrah and 16 "Murrah x Carabao" crosses) and evaluated for fat, protein, and lactose content, solids non-fat (SNF), total solids, and freezing point. Colostrum contained significantly (p<0.05) more protein, SNF, total solids, and higher freezing point, but less moisture and lactose, and were produced in lower amounts than milk obtained on different days of lactation. Fat percentage was not significantly different (p>0.05) between colostrum and milk. Colostrum from Murrah buffaloes had more protein but less fat and colostrum yield than "Murrah x Carabao" crosses. Among crossbred buffaloes, the F2 "75% Murrah – 25% Carabao" crosses produced more colostrum than F1 "50% Murrah – 50% Carabao" (p<0.05). Older buffaloes also produced more colostrum. Milk parameters were similar for Murrah and "Murrah x Carabao" crosses, except for test-day milk yield, which was significantly higher in "Murrah x Carabao" crosses. The F1 crossbred buffaloes had milk containing more lactose and SNF, but lower freezing point than milk from F2 crossbred buffaloes. Buffaloes, already with more lactations, had higher test-day milk yield but with lower fat and total solids. High monthly temperature reduced test-day milk yield. In conclusion, breed differences, age at calving, number of lactations, and high monthly temperature may have caused changes in the composition and yield of buffalo colostrum and milk.

Keywords: colostrum; milk components; Murrah buffalo; Philippine carabao

INTRODUCTION

The water buffalo inventory in the Philippines, mostly of the swamp-type called the carabao, is about 2.9 million heads. In 2015, the dairy buffalo population in the Philippines was 17,802 heads. A total of 7.121 million liters (LME) of milk were produced from 4,983 buffaloes in the milking line (Philippine Statistics Authority, 2016). Total milk produced from buffaloes of various breeds was 38.49% by individual smallholder producers (who consume and sell locally what they produce), 30.17% by cooperatives (who deliver their milk to a collection point for transport to a processing plant), 24.80% by institutional farms (which supply school and rural community feeding programs), and 7.11% by commercial farms (which supply processors).

Milk production from carabaos is very low at about 2.8 kg/day/lactation, while the small population of the river-type Murrah breed have an average milk production of 7.2 kg/day/lactation (Philippine Statistics Authority, 2016). In this regard, the Philippine Carabao Center (PCC) leads the national carabao upgrading program, which aims to accelerate the herd build-up of dairy buffaloes. While continuous upgrading of the carabao is known to increase milk productivity, its effects on the composition and yield of colostrum and milk at different days of lactation have not been studied.

Similar studies were conducted to compare milk from purebred Murrah and crossbred buffaloes in China (Sun *et al*., 2014; Ren *et al*., 2015; Han *et al*., 2017; Zhou *et al*., 2018). The composition and nutrient data on colostrum in comparison to milk, however, are not commonly reported. In addition to the analysis of milk components, information on the freezing point of buffalo milk can also be studied as it is now widely used not only to indicate adulteration but also as a quality criterion for calculating the price of raw milk purchased and processed into dairy products (Pesce *et al*., 2016). Such important information can be used to promote buffalo colostrum and milk as a rich source of nutrients for adult men and women. They may also contribute to the development of local reference materials used by health and nutrition professionals engaged in nutrition education and medical nutrition therapy.

This study aimed to compare the composition and yield of colostrum and milk collected on the 30th, 60th, and 90th day of lactation from purebred Murrah and its crosses with the carabao in the institutional herd of the PCC located inside the University of the Philippines Los Baños (UPLB) campus, Laguna, Philippines. The effects of calving and previous lactation records and monthly weather parameters–temperature, relative humidity, and rainfall on various colostrum and milk parameters were likewise determined.

MATERIALS AND METHODS

Animal Care

This study was conducted in compliance with the requirements of the Institutional Animal Care and Use Committee of the University of the Philippines Los Baños, Laguna, Philippines with Assigned Protocol No. 2019-0034.

Experimental Animals and Data

A total of 133 milk samples were collected in the period between April 4, 2019 and July 27, 2020 from 36 buffaloes (i.e., 20 purebred Murrah and 16 "Murrah x Carabao" crosses) at the Philippine Carabao Center dairy buffalo farm in UPLB, Laguna, Philippines (Table 1). The crossbred buffaloes consisted of 3 F1 "50% Murrah – 50% Carabao", 2 F2 "75% Murrah – 25% Carabao", 9 F3 "87.5% Murrah – 12.5% Carabao", and 2 F4 "93.75% Murrah – 6.25% Carabao". Buffaloes were equally managed to fulfill all welfare requirements and were kept in individual parturition pens about 2 weeks before calving. Buffaloes were fed with forage, and commercial lactating feed concentrates. The nutritional content of the lactating feeds was analyzed at the Animal Nutrition Division, Institute of Animal Science, College of Agriculture and Food Science, UPLB, and found to contain 15.52% crude protein, 5.87% crude fat, 11.99% crude fiber, 7.90% moisture, 8.74% ash, 1.02% calcium, and 0.58% phosphorus using the Semi-micro Kjeldahl distillation, Soxhlet extraction, Weende method, oven drying, ashing at 600^{o}C, Titrimetric, and Colorimetric-UV-Vis method, respectively.

Colostrum and milk samples (approximately 500 mL) were collected by hand or milking machine on the 30th, 60th, and 90th day of lactation, placed in plastic bottles, and immediately frozen at –20°C until further analysis. The MilkoScan Mars (FOSS Analytical A/S, Hillerod, Denmark) using the Fourier-transform infrared spectroscopy (FTIR) technology was used to determine % fat, % protein, % lactose, % solids non-fat (SNF), % total solids, and freezing point (°C). Other data were also collected, including colostrum yield, test-day milk yield, calving and previous lactation records (i.e., age at calving, parity, average calving interval, total milk yield, lactation length, and daily milk yield) and weather parameters (i.e., monthly average temperature and relative humidity, and total monthly rainfall) corresponding with each colostrum or milk sample.

Statistical Analysis

Pearson product-moment correlation coefficients among the proportion and yield of milk components (fat, protein, and lactose) and freezing point, and their relationships with calving and previous lactation records, and with weather parameters were initially determined separately for colostrum and milk samples using the CORR procedure (SAS, 2009). Calving and lactation records and weather parameters found to be consistently and significantly correlated with proportion and yield of milk components were included as covariates in the statistical models.

The general least squares procedures for unbalanced data (SAS, 2009) were used to examine the principal sources of variation affecting each colostrum/milk component and freezing point. Statistical significance was set at $p<0.05$. The final statistical models were determined separately to compare colostrum and milk, analyze colostrum, and analyze milk.

Statistical model (1) used to compare colostrum and milk was: $\mathbf{y1_{ijkl} = \mu + BType_i + MType_j + (BType \times MType)_{ij} + TDMY_k + e_{ijkl}}$ where $y1_{ijkl}$ is the proportion and yield of components and freezing point of all colostrum and milk samples, μ is the overall mean, $BTypei_j$ is the fixed effect of the i^{th} type of breed – purebred Murrah and "Murrah x Carabao" crosses, $MType_j$ is the fixed effect for the j^{th} type of milk – colostrum, milk collected on the 30th, 60th and 90th day of lactation, $(BType \times MType)_{ij}$ is the interaction effect between i^{th} type of breed and j^{th} type of milk, and $TDMY_k$ is the k^{th} covariate effect of colostrum or test-day milk yield (kg), and e_{ijkl} is the error term assumed to be normally distributed with the variance of errors as constant across observations.

Table 1. Number and distribution of buffaloes and milk samples (by breed and milk type)

Breed type	Milk type				No. of samples	No. of buffaloes
	Colostrum	Milk 30-d	Milk 60-d	Milk 90-d		
Purebred Murrah	18	19	18	15	70	20
"Murrah x Carabao" Crosses	16	16	16	15	63	16
F1 50% Murrah – 50% Carabao	3	3	3	3	12	3
F2 75% Murrah – 25% Carabao	2	2	2	2	8	2
F3 87.5% Murrah –12.5% Carabao	9	9	9	8	35	9
F4 93.75% Murrah – 6.25% Carabao	2	2	2	2	8	2
Total	34	35	34	30	133	36

Statistical model (2) used to analyze colostrum components, and the freezing point was: $\mathbf{y2}_{ijkl} = \mu + \mathbf{BType}_i + \mathbf{CY}_j + \mathbf{AC}_k + \mathbf{e}_{ijkl}$ where $y2_{ijkl}$ is the proportion and yield of components and freezing point of colostrum samples, μ is the overall mean, $BTypei_j$ is the fixed effect of the i^{th} type of breed – purebred Murrah and "Murrah x Carabao" crosses, CY_j is the j^{th} covariate effect of colostrum yield on the day of calving (kg), AC_k is the k^{th} covariate effect of age at calving (yr), and e_{ijkl} is the error term assumed to be normally distributed with the variance of errors as constant across observations.

Statistical model (3) used to analyze milk components. Freezing point was: $\mathbf{y3}_{ijklmno} = \mu + \mathbf{BType}_i + \mathbf{MTDate}_j + \mathbf{TDMY}_k + \mathbf{P}_l + \mathbf{Temp}_m + \mathbf{RH}_n + \mathbf{e}_{ijklmno}$ where $y3_{ijklmno}$ is the proportion and yield of components and freezing point of milk samples, μ is the overall mean, $BTypei_j$ is the fixed effect of the i^{th} type of breed – purebred Murrah and "Murrah x Carabao" crosses), MTD_j is the fixed effect of the i^{th} milk test date – 30^{th}, 60^{th} and 90^{th} day of lactation, $TDMY_k$ is the k^{th} covariate effect of test-day milk yield (kg), P_l is the l^{th} covariate effect of parity number, $Temp_m$ is the m^{th} covariate effect of the average monthly temperature (°C), RH_n is the n^{th} covariate effect of the average monthly relative humidity (%), and $e_{ijklmno}$ is the error term assumed to be normally distributed with the variance of errors as constant across observations.

Comparisons among "Murrah x Carabao" crosses were based on the statistical models (2) and (3) above, but replacing $BType_i$ with $Breed_i$ (i.e., i^{th} breed, namely 100% Murrah, F1 "50% Murrah – 50% Carabao", F2 "75% Murrah – 25% Carabao", F3 "87.5% Murrah – 12.5% Carabao", and F4 "93.75%Murrah – 6.25% Carabao).

RESULTS

Correlations Among Colostrum Parameters

Percent fat in buffalo colostrum was positively correlated with fat yield (r= 0.66). Percent protein was positively correlated with protein yield (r= 0.65). However, percent lactose was not correlated with lactose yield (Table 2). Percent fat was negatively correlated with % lactose (r= –0.54) but not correlated with % protein and % lactose. Percent total solids was positively related to % protein (r= 0.88), but negatively correlated to % lactose (r= –0.43). Percent total solids were not related to % fat.

Freezing point of colostrum was positively correlated with % moisture (r= 0.47) and % fat (r= 0.37), but negatively correlated with % protein (r= –0.60), % SNF (r= –0.69) and % total solids (r= –0.47). Freezing point was not correlated with % lactose and lactose yield.

Correlations among Milk Parameters

Percent fat in buffalo milk was positively correlated with fat yield (r= 0.80). Percent protein was positively correlated with protein yield (r= 0.83). Percent lactose was correlated with lactose yield (r= 0.53), see Table 3. Percent fat was not correlated with % protein and % lactose. Percent protein was positively correlated with % lactose (r= 0.24). Percent total solids was positively

Table 2. Pearson correlation coefficients among buffalo colostrum composition, yield, and freezing point and their relationships with calving and previous lactation records and with monthly weather parameters

	Buffalo colostrum parameters									
	% Moisture	% Fat	% Protein	% Lactose	% solids non-fat	% Total solids	Fat yield	Protein yield	Lactose yield	Freezing point
% Moisture	–	ns	–0.88**	0.43*	–0.88**	–1.00**	ns	–0.58**	ns	0.47**
% Fat		–	ns	ns	ns	ns	0.66**	ns	ns	0.37*
% Protein			–	–0.54**	0.99**	0.88**	ns	0.65**	ns	–0.60**
% Lactose				–	–0.41*	–0.43*	ns	ns	ns	ns
% Solids non-fat					–	0.88**	ns	0.65**	ns	–0.69**
% Total solids						–	ns	0.58**	ns	–0.47**
Fat yield							–	0.50**	0.64**	–0.48**
Protein yield								–	0.61**	ns
Lactose yield									–	ns
Colostrum yield	ns	ns	ns	ns	ns	ns	0.70**	0.85**	0.88**	ns
Age at calving	ns	ns	ns	ns	0.38*	ns	ns	0.40*	0.38*	–0.55**
Parity	ns	ns	0.34*	ns	0.40*	ns	ns	0.41*	0.34*	–0.56**
Age at first calving	ns	0.37*	ns	ns	ns	ns	ns	ns	ns	0.36*
Ave. calving interval	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Ave. total milk yield	ns	ns	ns	ns	ns	ns	ns	0.37*	0.44*	ns
Ave. lactation length	ns	ns	ns	ns	ns	ns	ns	ns	0.38*	ns
Average daily milk yield	ns	ns	ns	ns	ns	ns	ns	0.39*	0.40*	ns
Ave. monthly temperature	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Ave. monthly rel. humidity	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Total monthly rainfall	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: ns= not significant (p>0.05); *= means r is significantly different from zero (p<0.05); **= means r is significantly different from zero (p<0.01).

related to % fat (r= 0.84), % protein (r= 0.52), and lactose (r= 0.51).

Freezing point of buffalo milk was positively correlated with % moisture (r= 0.27), but negatively correlated with % protein (r= –0.40), % lactose (r= –0.83), % SNF (r= –0.77), and % total solids (r= –0.27). Freezing point was not correlated with % fat.

Correlations of Colostrum and Milk Parameters with Calving and Previous Lactation Records and with Weather Parameters

Colostrum yield in buffaloes was positively correlated with fat yield (r= 0.70), protein yield (r= 0.85), and lactose yield (r= 0.88). Percent lactose and % total solids were not correlated with colostrum yield, calving, and the previous lactation records monthly temperature, monthly relative humidity, and monthly total rainfall.

Test-day milk yield was also positively correlated with fat yield (r= 0.52), protein yield (r= 0.70), and lactose yield (r= 0.82). Percent lactose, SNF, and % total solids in buffalo milk were not correlated with test-day milk yield, calving, and previous lactation records. Fat yield, protein yield, and lactose yield were not correlated with monthly temperature, monthly relative humidity, and monthly total rainfall.

Factors Affecting Colostrum and Milk Parameters

In this study, percent fat is the most variable of the components of colostrum/milk with a coefficient variation (CV) of 47.00%, followed by percent protein (CV= 35.52%), percent lactose (CV= 18.72%), and percent moisture (CV= 4.08%). Among the major component yields, protein has the highest coefficient of variation, followed by fat and lactose (Table 4).

The type of breed had significant effects (p<0.01) on most colostrum parameters except fat yield and lactose yield (Table 5). Colostrum yield had significant effects (p<0.05) on its fat, protein, and lactose yield. A higher colostrum yield is thus likely to produce more protein, fat, and lactose. The age of the buffalo at calving also had a significant effect (p<0.05) on freezing point and colostrum yield. This implies that older buffaloes are likely to produce more colostrum on the day of calving (i.e., additional 0.29 kg colostrum produced on the day of calving for each year increase in age at calving), but with a lower freezing point.

The type of breed also had significant effects (p<0.01) on test-day milk yield (Table 6). The proportion and yield of milk components were also affected by the test-day milk yield, age at calving, parity, average monthly temperature, and average monthly relative humidity. Higher test-day milk yield means more fat, protein, and lactose yields. Older buffaloes at calving produced milk with a higher percentage of fat and total solids. Buffaloes, already with more lactations, produced more milk (i.e., additional 0.57 kg milk yield per day for each unit increase in parity number), but with lower percentage fat and total solids. Higher average monthly temperature reduced the amount of milk produced (i.e., 0.69 kg decline in milk yield per day for every 1°C increase in monthly temperature), but with higher percentage lactose and SNF. Higher average monthly relative humidity reduced percentage protein, SNF, and total solids in milk (i.e., 0.05% protein, 0.08%

Table 3. Pearson correlation coefficients among buffalo milk composition, yield, and freezing point and their relationships with calving and previous lactation records and with monthly weather parameters

	Buffalo milk parameters									
	% Moisture	% Fat	% Protein	% Lactose	% solids non-fat	% Total solids	Fat yield	Protein yield	Lactose yield	Freezing point
% Moisture	-	–0.84**	–0.52**	–0.51**	–0.62**	–1.00**	–0.73**	–0.33**	–0.32*	0.27**
% Fat		-	ns	ns	ns	0.84**	0.80**	ns	ns	ns
% Protein			-	0.24*	0.81**	0.52**	ns	0.83**	0.26*	–0.40**
% Lactose				-	0.76**	0.51**	ns	ns	0.53**	–0.83**
% Solids non-fat					-	0.62**	ns	0.58**	0.50**	–0.77**
% Total solids						-	0.73**	0.33**	0.32*	–0.27**
Fat yield							-	0.33**	0.54**	ns
Protein yield								-	0.56**	ns
Lactose yield									-	–0.41**
Test-day milk yield	ns	ns	0.23*	ns	ns	ns	0.52**	0.70**	0.82**	ns
Age at calving	ns	0.24*	ns	ns	ns	ns	ns	ns	ns	0.24*
Parity	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.23*
Age at first calving	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Ave. calving interval	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Ave. total milk yield	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Ave. lactation length	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Average daily milk yield	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Ave. monthly temperature	ns	ns	ns	0.24*	ns	ns	ns	ns	ns	ns
Ave. monthly rel. humidity	0.22*	ns	–0.29**	ns	–0.31**	–0.22*	ns	ns	ns	0.22*
Total monthly rainfall	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: ns= not significant (p>0.05); *= means r is significantly different from zero (p<0.05); **= means r is significantly different from zero (p<0.01).

Table 4. Mean square F tests results for the effects of breed type, milk type, "Breed type x milk type" interaction, and covariate (test-day milk yield) on different buffalo colostrum/ milk parameters

	Breed type	Milk type	"Breed type x milk type" interaction	Test-day milk yield	CV (%)
% Moisture	ns	**	ns	ns	4.08
% Fat	ns	ns	ns	ns	47.00
% Protein	ns	**	ns	*	35.52
% Lactose	ns	**	ns	ns	18.72
% Solids non-fat	ns	**	ns	*	20.98
% Total solids	ns	**	ns	ns	20.68
Freezing point	ns	*	ns	ns	<0.00
Fat yield	ns	ns	ns	**	46.85
Protein yield	ns	**	ns	**	50.81
Lactose yield	ns	**	ns	**	22.50
Test-day milk yield	**	**	ns	-	31.93

Note: CV= coefficient of variation; ns= not significant (p>0.05); *= means significant effect of independent variable (p<0.05); **= means highly significant effect of independent variable (p<0.01).

Table 5. Mean square F test results for the effects of breed type and regression coefficients (zero-intercept model) for significant co-variate effects of colostrum yield and age at calving on different buffalo colostrum parameters

Colostrum parameters	Breed type	Covariates		CV (%)
		Colostrum yield	Age at calving	
% Moisture	**	ns	ns	5.84
% Fat	**	ns	ns	50.11
% Protein	**	ns	ns	31.24
% Lactose	**	ns	ns	26.44
% Solids non-fat	**	ns	ns	23.38
% Total solids	**	ns	ns	21.05
Freezing point	**	ns	*b= –0.012	<0.00
Fat yield	ns	**b= 0.038	ns	53.16
Protein yield	**	**b=0.162	ns	36.76
Lactose yield	ns	**b=0.018	ns	24.93
Colostrum yield	**	-	*b= 0.290	43.27

Note: CV= coefficient of variation; ns= not significant (p>0.05);); b= regression coefficient; *= means significant effect of independent variable (p<0.05). **= means highly significant effect of independent variable (p<0.01).

Table 6. Mean square F test results for the effects of breed type and milk test date and regression coefficients (zero-intercept model) for significant covariate effects of test-day milk yield, age at calving, parity, monthly temperature, and monthly relative humidity on milk collected on different buffalo milk parameters

Milk parameters	Breed type	Milk test date	Covariates					CV (%)
			Test-day milk yield	Age at calving	Parity	Monthly temperature	Monthly rel. humidity	
% Moisture	ns	ns	ns	**b= –0.905	*b= 0.998	ns	*b= 0.143	3.20
% Fat	ns	ns	ns	**b= 0.776	*b= –0.770	ns	ns	43.99
% Protein	ns	ns	**b= 0.145	ns	ns	ns	*b= –0.054	23.33
% Lactose	ns	ns	ns	ns	ns	*b= 0.259	ns	16.82
% Solids non-fat	ns	ns	*b= 0.176	ns	ns	*b= 0.445	*b= –0.077	14.99
% Total solids	ns	ns	ns	**b= 0.905	*b= –0.998	ns	*b= –0.143	18.56
Freezing point	ns	ns	ns	ns	ns	ns	ns	<0.00
Fat yield	ns	ns	**b= 0.048	**b= 0.045	*b= –0.047	ns	ns	43.74
Protein yield	ns	ns	**b= 0.055	ns	ns	ns	*b= –0.005	37.29
Lactose yield	ns	ns	*b= 0.046	ns	ns	*b= 0.021	ns	18.99
Test-day milk yield	**	ns	-	ns	*b= 0.571	**b= –0.690	ns	27.02

Note: CV= coefficient of variation; ns= not significant (p>0.05);); b= regression coefficient; *= means significant effect of independent variable (p<0.05). **= means highly significant effect of independent variable (p<0.01).

SNF, and 0.14% total solids decrease for every 1% increase in monthly relative humidity).

DISCUSSION

Comparisons between Colostrum and Milk

Colostrum had a significantly higher percentage of protein, SNF, and total solids, protein yield, and freezing point than milk obtained on the 30th, 60th, and 90th day of lactation. On the other hand, colostrum had lower % moisture, % lactose, lactose yield, and amount of colostrum produced than milk. Percent fat and fat yield were not significantly different between colostrum and milk (Table 7). Differences in the proportion and yield of milk components of milk obtained on the 30th, 60th, and 90th day of lactation were not significant.

Percent protein. Buffalo colostrum had a significantly higher percentage of protein than milk. Colostrum is rich in antibodies (immunoglobulins) and therefore has a high percent protein. Immunoglobulins are proteins that provide passive immunity to calves. In this study, protein content in colostrum is about 3.4 times higher than in milk. A similar percent protein was reported by Arain *et al.* (2008) for buffalo colostrum which can be as high as 18.75% (0-4 h after calving) and decreases to 12.01% (4-12 h after calving), and further reduced to 8.75% (12-24 h after calving). On the other hand, the protein content of milk in this study (3.61% to 4.20%) is similar to the average protein percentage (4.0%) and range (2.7% to 4.5%) reported by Medhammar *et al*. (2011) in their comprehensive review of the nutrient composition of milk from more than 16 buffalo breeds.

Percent lactose. Lactose (milk sugar) was higher in milk (4.58% to 4.84%) than in colostrum (2.12% to 2.40%). The lactose content in buffalo colostrum reported by Arain *et al.* (2008) ranges from 2.70% (0–4 h after calving) to 3.42% (12-24 h after calving), while the average lactose content in buffalo milk reported by Medhammar *et al*. (2011) was 4.4%, ranging from 3.2% to 4.9%.

Freezing point. The freezing point of buffalo colostrum was higher than that in milk. This may have been caused by the combined effect of breed and the differences in the content of lactose, and mineral composition (chloride salts, calcium, potassium, and magnesium), which have been shown to contribute to freezing point depression in milk (Jennes & Patton, 1959 as cited by Henno *et al*., 2008). Large differences in protein content may also be related to the higher freezing point since variation in the mineral composition of milk can be influenced by the protein content (Henno *et al*., 2008). However, this study found no significant differences ($p>0.05$) in the freezing point of milk obtained on the 30th, 60th, and 90th day of lactation. In dairy cattle, Henno *et al*. (2008) reported that the freezing point of cow's milk was affected by the stage of lactation. The cow's milk freezing point was at its highest level during the second and third months of lactation when milk protein was at its lowest level.

The freezing point of buffalo milk in this study, ranging from –0.517°C to –0.480°C was higher than that reported by Khedkar *et al.* (2016) in India (i.e., –0.590°C to –0.518°C) and Pesce *et al.* (2016) for the Mediterranean breed in Italy (i.e., –0.527 to –0.545°C). Incidentally, Khedkar *et al.* (2016) showed that the freezing point of buffalo milk is related to its soluble constituents and is affected by season (–0.528°C and –0.531°C in warm and cold weathers, respectively), farm size (–0.532°C and –0.519°C in small and large farms, respectively), and between organic and conventional farming methods (–0.526°C and –0.537°C, respectively). Pesce *et al.* (2016) added that the freezing point is usually lower with the presence of milk solids and the difference in solute concentrations, especially % lactose.

Comparisons Between Murrah and Its Crosses with the Carabao

Colostrum. Colostrum from purebred Murrah buffaloes had significantly ($p<0.05$) higher protein but lower fat, fat yield, and colostrum yield than "Murrah x Carabao" crosses (Table 8). Percent moisture, % lactose, % SNF, % total solids, protein yield, lactose yield, and freezing

Table 7. Composition, yield, and freezing point in buffalo colostrum and milk collected on different days of lactation

Variables	Colostrum	Buffalo milk collected on the		
		30th day of lactation	60th day of lactation	90th day of lactation
% Moisture	78.04 ± 0.62^b	85.97 ± 0.59^a	85.68 ± 0.59^a	84.38 ± 0.63^a
% Fat	4.34 ± 0.41	4.69 ± 0.39	5.11 ± 0.40	5.49 ± 0.42
% Protein	12.96 ± 0.40^a	3.61 ± 0.38^b	3.65 ± 0.38^b	4.20 ± 0.40^b
% Lactose	2.26 ± 0.14^b	4.62 ± 0.13^a	4.58 ± 0.13^a	4.84 ± 0.14^a
% Solids non-fat	16.15 ± 0.42^a	9.24 ± 0.40^b	9.13 ± 0.40^b	10.01 ± 0.43^b
% Total solids	21.96 ± 0.62^a	14.03 ± 0.59^b	14.32 ± 0.59^b	15.62 ± 0.63^b
Fat yield, kg	0.27 ± 0.02	0.29 ± 0.02	0.32 ± 0.02	0.34 ± 0.03
Protein yield, kg	0.76 ± 0.03^a	0.20 ± 0.03^b	0.21 ± 0.03^b	0.26 ± 0.03^b
Lactose yield, kg	0.16 ± 0.01^b	0.29 ± 0.01^a	0.29 ± 0.01^a	0.30 ± 0.01^a
Colostrum yield, kg	4.95 ± 0.34^b	6.82 ± 0.34^a	6.64 ± 0.34^a	6.69 ± 0.36^a
Freezing point, °C	–0.440 ± 0.017^a	–0.487 ± 0.016^b	–0.480 ± 0.016^b	–0.517 ± 0.017^b

Note: Means in the same row with different superscripts differ significantly ($p<0.05$).

point were not significantly different (p>0.05) between purebred Murrah and "Murrah x Carabao" crosses.

The colostrum parameters were generally not significantly different between the F1 "50% Murrah – 50% Carabao" and F2 "75% Murrah – 25% Carabao" crosses (p>0.05), except for colostrum yield (Table 9). The F2 crossbred buffaloes had significantly higher (p<0.05) colostrum yield (8.53 kg vs. 3.72 kg) than F1 crossbred buffaloes.

Milk collected on different days of lactation. The composition and freezing point of milk collected on the 30th, 60th, and 90th day of lactation were generally similar for Murrah and "Murrah x Carabao" crosses (Table 8). This suggests that the proportion and yield of milk components and freezing point are determined by additive genes and may be improved through selection. The "Murrah x Carabao" crosses had significantly (p<0.05) higher test-day milk yield (7.27 kg vs. 6.15 kg) than purebred Murrah. Assuming low milk productivity of carabaos (not studied in this investigation), this implies that test-day milk yield is probably determined by non-additive genes and can be improved by crossbreeding. This suggests that the carabao upgrading (crossbreeding) program can be a good strategy to accelerate the herd build-up of dairy buffaloes in the country. This may not only increase milk productivity but also improve colostrum/milk composition parameters similar with those of the Murrah breed.

The fat content, protein content, and lactose content of milk from the Murrah breed in this study were generally lower than published reports for Murrah in India (Yadav *et al*., 2013; Sarkar *et al*., 2016) and Murrah in China (Ren *et al*., 2015; Zhou *et al*., 2018). Higher fat, protein, and lactose content of milk were also reported for other buffalo breeds such as the Nili-Ravi breed in China (Sun *et al*., 2014; Ren *et al*., 2015; Zhou *et al*., 2018), the Mediterranean breed in Italy (Pesce *et al*., 2016), and the Anatolian breed in Turkey (Cinar *et al*., 2019).

The milk from F1 crossbred buffaloes had significantly (p<0.05) higher % lactose and % SNF, but a lower freezing point than that in F2 crossbred buffaloes (Table 10). Percent moisture, % fat, % protein, % total solids, fat yield, protein yield, lactose yield, and test-day milk yield were not significantly different (p>0.05) between the F1 and F2 crossbred buffaloes.

Table 8. Composition, yield, and freezing point of buffalo colostrum and milk from purebred Murrah and "Murrah x Carabao" crosses

Variables	Buffalo colostrum		Buffalo milk	
	Purebred Murrah	"Murrah x Carabao" crosses	Purebred Murrah	"Murrah x Carabao" crosses
% Moisture	77.97 ± 1.16	78.61 ± 1.24	85.40 ± 0.39	85.13 ± 0.41
% Fat	3.74 ± 0.56[b]	5.20 ± 0.60[a]	4.97 ± 0.32[a]	5.13 ± 0.33[a]
% Protein	13.54 ± 1.01	11.70 ± 1.08	3.92 ± 0.13	3.96 ± 0.14
% Lactose	2.21 ± 0.15	2.38 ± 0.16	4.68 ± 0.12	4.65 ± 0.12
% Solids non-fat	16.68 ± 0.94	14.97 ± 1.01	9.52 ± 0.20	9.60 ± 0.22
% Total solids	22.03 ± 1.16	21.39 ± 1.24	14.60 ± 0.39	14.87 ± 0.41
Fat yield, kg	0.18 ± 0.03[b]	0.26 ± 0.03[a]	0.33 ± 0.02[a]	0.34 ± 0.02[a]
Protein yield, kg	0.71 ± 0.06[a]	0.56 ± 0.06[b]	0.27 ± 0.01[a]	0.27 ± 0.01[a]
Lactose yield, kg	0.11 ± 0.01	0.11 ± 0.01	0.31 ± 0.01	0.31 ± 0.01
Test-day milk yield, kg	3.79 ± 0.50[b]	6.13 ± 0.53[a]	6.15 ± 0.25[b]	7.27 ± 0.26[a]
Freezing point, °C	–0.454 ± 0.018	–0.422 ± 0.019	–0.499 ± 0.013	–0.490 ± 0.014

Note: Means in the same row (for the same milk type) with different superscripts differ significantly (p<0.05).

Table 9. Composition, yield, and freezing point of buffalo colostrum from different "Murrah x Carabao" crosses

Colostrum parameters	F1 50% Murrah – 50% Carabao	F2 75% Murrah – 25% Carabao	F3 87.5% Murrah – 12.5% Carabao	F4 93.75% Murrah – 6.25% Carabao
% Moisture	75.68 ± 2.88[c]	75.40 ± 3.50[bc]	79.38 ± 1.67[ab]	83.82 ± 3.29[a]
% Fat	4.70 ± 1.49	5.97 ± 1.81	5.53 ± 0.86	4.18 ± 1.70
% Protein	15.13 ± 2.48[a]	14.04 ± 3.02[ab]	10.59 ± 1.44[b]	7.64 ± 2.83[b]
% Lactose	1.77 ± 0.37	2.00 ± 0.45	2.56 ± 0.21	3.14 ± 0.42
% Solids non-fat	17.70 ± 2.38[a]	16.89 ± 2.89[ab]	14.12 ± 1.38[ab]	11.55 ± 2.72[b]
% Total solids	24.32 ± 2.88[a]	24.60 ± 3.50[ab]	20.62 ± 1.67[bc]	16.17 ± 3.29[c]
Fat yield, kg	0.25 ± 0.08	0.32 ± 0.09	0.27 ± 0.04	0.19 ± 0.09
Protein yield, kg	0.76 ± 0.15[a]	0.73 ± 0.18[ab]	0.49 ± 0.08[b]	0.33 ± 0.17[b]
Lactose yield, kg	0.09 ± 0.02[b]	0.08 ± 0.02[bc]	0.12 ± 0.01[b]	0.16 ± 0.02[a]
Colostrum yield, kg	3.72 ± 1.28[c]	8.54 ± 1.42[a]	6.35 ± 0.70[b]	6.68 ± 1.44[ab]
Freezing point, °C	–0.421 ± 0.048	–0.432 ± 0.057	–0.422 ± 0.030	–0.423 ± 0.053

Note: Means in the same row with different superscripts differ significantly (p<0.05).

Table 10. Composition, yield, and freezing point of buffalo milk from different "Murrah x Carabao" crosses

Milk parameters	F1 50% Murrah – 50% Carabao	F2 75% Murrah – 25% Carabao	F3 87.5% Murrah – 12.5% Carabao	F4 93.75% Murrah – 6.25% Carabao
% Moisture	84.94 ± 1.04	85.25 ± 1.12	85.68 ± 0.58	83.17 ± 1.13
% Fat	4.59 ± 0.84^{b}	6.15 ± 0.91^{ab}	4.72 ± 0.47^{b}	6.48 ± 0.91^{a}
% Protein	4.30 ± 0.35	3.56 ± 0.38	3.86 ± 0.20	4.22 ± 0.38
% Lactose	4.87 ± 0.30^{a}	4.16 ± 0.32^{b}	4.59 ± 0.17^{ab}	5.01 ± 0.32^{a}
% Solids non-fat	10.19 ± 0.54^{a}	8.59 ± 0.58^{b}	9.46 ± 0.30^{a}	10.28 ± 0.58^{a}
% Total solids	15.06 ± 1.04^{ab}	14.75 ± 1.12^{ab}	14.32 ± 0.58^{b}	16.83 ± 1.13^{a}
Fat yield, kg	033 ± 0.06	0.39 ± 0.06	0.31 ± 0.03	0.41 ± 0.06
Protein yield, kg	0.29 ± 0.04	0.23 ± 0.04	0.27 ± 0.02	0.28 ± 0.04
Lactose yield, kg	0.32 ± 0.02^{ab}	0.27 ± 0.02^{b}	0.31 ± 0.01^{ab}	0.34 ± 0.02^{a}
Test-day milk yield, kg	7.14 ± 0.69	6.53 ± 0.75	7.65 ± 0.38	6.61 ± 0.75
Freezing point, °C	–0.556 ± 0.036^{c}	–0.412 ± 0.037^{a}	–0.489 ± 0.019^{b}	–0.488 ± 0.037^{b}

Note: Means in the same row with different superscripts differ significantly (p<0.05).

Milk from F1 "50% Murrah – 50% Carabao" and F2 "75% Murrah – 25% Carabao" crossbreeds had lower fat, protein, and lactose contents than milk from F1 and F2 "Murrah x local swamp buffalo (Guangxi/Yunnan)" crosses in China (Sun *et al*., 2014; Ren *et al*., 2015; Han *et al*., 2017). Incidentally, the milk from the Chinese F1 and F2 crossbreeds had better nutritional value in terms of fat, protein, and lactose contents than those of the Murrah breed. The fat, protein, and lactose content of milk were, however, not significantly different between crossbred F1 and F2 buffaloes.

The discrepancy between our results and those of other studies in other countries may be due to the different maintenance and feeding conditions, as well as in experimental methods. Nevertheless, the results of this study add to widen the biodiversity knowledge base, which is important to the conservation and sustainable use of milk from purebred Murrah and "Murrah x Carabao" crosses in the Philippines.

Implications

This study provided evidence that the Murrah x Carabao crossbreeding program can lead to improvements in milk composition parameters similar to those of the Murrah breed. Since test-day milk yield in buffaloes was positively correlated to yield of fat, protein, and lactose, the local herds having the Murrah breed, "Murrah x Carabao" crosses, or a mix of both, that already have above average milk components may focus on increasing milk yield. However, test-day milk yield was positively correlated to % protein but not related to % fat and % lactose. Herds that are below the breed average for protein may thus seem to benefit from including protein yields in the sire selection criteria for the Murrah herd. While changes in the herd's milk protein concentration may be achieved through genetic selection, the basic production and husbandry practices to increase milk yield should be improved further. Meanwhile, the detrimental effects of a high average monthly temperature on test-day milk yield and high average monthly relative humidity on protein yield can both be mitigated to achieve higher than average levels of proteins in buffalo milk.

Furthermore, by integrating the characteristics of milk from Murrah and "Murrah x Carabao" crosses with existing "Food Composition Tables" and "Food Exchange Lists", consumers will benefit from a better understanding of buffalo milk and determining the adequacy of nutrients when drinking buffalo milk. Alternatively, buffalo colostrum, especially rich in protein compounds, may offer extra benefits as a functional food for adult humans. In this case, higher protein content in colostrum may be obtained from purebred Murrah buffaloes, albeit lower colostrum yield compared to "Murrah x Carabao" crosses.

CONCLUSION

This study showed significant breed differences (Murrah *vs.* "Murrah x Carabao" crosses) for colostrum yield (including its protein yield and fat yield) and for test-day milk yield. The composition and freezing point of colostrum and milk were, however, similar for Murrah and "Murrah x Carabao" crosses. While new information on breed (genetic) similarities and differences are important in managing the local dairy buffalo farms, this study also showed that non-genetic factors such as age at calving, number of lactations (or parity), and high average monthly temperature may also cause changes in the composition and yield of buffalo colostrum and milk.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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