



ABSTRACT

To determine the relationship between udder traits and peak milk yield, udder parameters of 203 Dehong crossbred dairy buffaloes were measured. The average peak milk yield (kg/day) of buffaloes was 9.60 ± 2.73 , with average udder dimensions of 16.51 ± 10.05 , 8.07 ± 3.26 , 8.68 ± 3.92 , 50.74 ± 8.82 , 11.38 ± 3.08 , 7.18 ± 2.18 and 7.73 ± 2.15 cm for udder depth, rear udder width, rear udder height, udder length, distance of fore teats, distance of rear teats, and distance of fore-rear teats, respectively. The peak milk yield was negatively correlated with udder depth (r=-0.28, P<0.01) and positively correlated with other mammary parameters (rear udder r=0.24, P<0.01; rear udder height r=0.32, P<0.01; udder length r=0.34, P<0.01; distance of rear teat length r=0.29, P<0.01; right fore teat length r=0.22, P<0.01 and right rear teat length r=0.25, P<0.01). However, no significant correlation was found between peak milk yield and mammary vein and teat conformation. The results of the current study indicated that udder length has the greatest association with peak milk yield. Due to udder parameters fitting a normal distribution, it is valid to build evaluation procedures for the early selection of high yielding dairy buffaloes based on the experiences of Holstein cows scoring rules.

KEY WORDS dairy buffaloes, peak milk yield, udder and teat dimensions.

INTRODUCTION

Buffalo milk is rich in nutrients with a percentage of milk fat, protein, lactose and solid 6.05-7.73%, 4.15-4.49%, 5.57-5.63% and 16.72-18.07%, respectively (Tanpureet *et al.* 2012; Nasr, 2016; Nasr *et al.* 2016). These components are of higher percentages when compared with those found in the milk of Holstein cows, generating popularization of buffalo milk in the consumer market (Kanwal *et al.* 2004; Ahmad *et al.* 2008). Swamp Dehong buffalo is a famous

native breed in China, with a large body size, high fiber digestion ability and strong disease resistance (Mei *et al.* 2008). Based on the popularity of agricultural mechanization, Dehong buffaloes have become an important source of milk rather than the traditional draught power. They have been crossbred with imported Murrah and Nili-Ravi buffaloes (frozen-thawed sperm) to improve their milk production (a project sponsored by the European Union; Wang *et al.* 2007). The population of milked crossbred dairy buffaloes is about 30000 China. However, variations in milk

yield and udder dimensions of Dehong crossbred dairy buffaloes may cause evaluation deviation that is based on the scoring criterion used for Holstein cows. Body shapes of dairy cows have a close relationship with milk yield, reproductive and growth performance. Compared to the body shape traits, udder as a milk synthesis and storage organ is an important factor influencing milk yield. Sieber et al. (1987) reported that characteristics of the mammary system make great impacts on milk yield in American Holstein cows, and those cows with well-developed rear udders and strongly supported udders produced a high milk yield. Tilki et al. (2005) also reported udder system features, including distance of teats, teat length, udder height and other udder traits, had positive effects on milk yield in Brown Swiss cows. Peak milk yield is the greatest milk produced in the lactation period with a range of 12.99-15.31 kg/day (Nasr, 2016; Nasr, 2017).

Evaluation criteria have been developed for Holstein cows (Li et al. 2016). Some of them were unsuitable for the selection of crossbred dairy buffaloes. Italy has built systematic procedures for their native buffalo selection (Bosselli et al. 2010), but some parameters are unsuitable for the evaluation and early selection of Dehong dairy buffaloes in China for breed differences. Dairy buffaloes are mainly found in peasant households, family farms and farming communities, and they are manually milked, causing difficulties in collecting milk yield data throughout the lactation. Fortunately, peak milk yields (kg/d) for each buffalo were accurately recorded by farmers. Singh and Kumar (2007) reported it is appropriate to predict 305-day milk yield using peak milk yield estimations. Information of the udder parameters should be incorporated into the evaluation criteria for dairy buffaloes. To our knowledge, there are no reports in the literature describing the relationship between peak milk yield and udder traits. Therefore, udder and teat measurements were performed to assess their effect on peak milk yield for Dehong crossbred dairy buffaloes.

MATERIALS AND METHODS

Animals, housing and management

The study was conducted in the Dehong State (24.43° North latitude, 98.57° East longitude and 870 m altitude). The Animal Ethics Committee under the Yunnan Province Animal Welfare Act 2007, China, approved animal handling procedures. 203 healthy crossbred buffaloes (134 Murrah×Nili-Ravi×Dehong swamp buffaloes, 58 Murrah×Dehong swamp buffaloes and 11 buffaloes with other crossbred types) were reared in farming communities. Groups of 5 buffaloes were housed open-sided barns (8×2.4 m) with an anti-slippery concrete floor, and a loafing area (8×10 m) with a concrete surface that was connected to the open-sided barns. Buffaloes were given silage *ad libitum* silage grass and fresh drinking water.

Udder parameters measurements

All the udder parameters (rear udder, rear udder, udder depth, udder length, teat length, and teat conformation) were measured for each animal. Each animal had all parameters measured. Rear udder height was the distance between the top edge of the mammary gland and the bottom of the basilar part of cunnus. Rear udder width was the distance of the top of the crease between the leg and the udder. Udder depth was the distance between the hocks and the bottom point of the udder. Udder length was the distance from the fore to rear attachment of the mammary gland. Teat length and teat distance between any pair of teats were measured with a ruler. Teat conformation was evaluated through teat length, teat perimeter and distance between different teats. Udder vein was evaluated visually with the thickness of the mammary vein (thick and obvious, thin and obvious, thick and unobvious, thin and unobvious and invisible). Buffaloes stood on a horizontal concrete floor, and all measurements were performed by the same person.

Statistical analysis

The data were analyzed using SPSS 22.0 (SPSS, 2013). Regression of peak milk yield on udder parameters (continuous variables), including udder depth, rear udder height, udder length, distance of fore-rear teats and teat length, was performed using multiple or simple linear regressions. Stepwise regression through backward elimination was performed to obtain regression equations. When some udder parameters had no significant effects on the regression models (P>0.05), they were eliminated. Linear regression models were calculated to verify the relationships between peak milk yield (dependent variable) and udder depth, rear udder height, udder length, distance of fore-rear teats and teat length (independent variable) of different ranges. Due to peak milk yield fitting a normal distribution, one-way analysis of variance was performed to analyze the peak milk yield of different ranges of udder parameters, and the significance was determined using Duncan's test. All data represented were mean values and error bars (\pm) presented the standard deviation.

RESULTS AND DISCUSSION

Udder measurements of dairy buffaloes with different ranges of peak milk yield (kg/day) were shown in Table 1. All udder parameters indicated significant differences between different ranges of peak milk yield (P<0.05), indicated that udder traits have a close relationship with peak milk yield.

Table 1 Udder size of dairy buffaloes in different ranges of peak milk yield (kg/day)

Peak milk yield (kg)	4-5.9	6-7.9	8-9.9	10-11.9	12-13.9	14-15.9
Month	5.33	7.46	7.83	7.56	8.52	8.50
Parity	1.31	2.59	3.09	3.33	3.52	3.75
Udder depth, cm	18.54 ^a	20.88 ^a	18.31 ^a	13.00 ^b	12.34 ^b	13.62 ^b
Rear udder width, cm	6.48 ^a	7.49 ^{ac}	7.30 ^a	9.24 ^b	8.53	9.39 ^{bc}
Rear udder height, cm	6.77 ^a	6.86 ^a	8.42 ^{ac}	9.69 ^{bc}	9.69 ^{bc}	11.61 ^b
Udder length, cm	42.46 ^a	49.64 ^{bc}	50.16 ^b	53.35°	52.61 ^{bc}	54.40 ^{bc}
Distance of fore teats, cm	9.46 ^a	10.48 ^{ab}	11.22 ^{bc}	13.06 ^d	11.88 ^{bc}	11.35 ^{bc}
Distance of rear teats, cm	5.85ª	6.55 ^{ac}	7.17 ^{bc}	7.98 ^b	7.58 ^{bc}	7.29 ^{bc}
Distance of fore-rear teats, cm	5.91ª	7.48 ^{bc}	7.28 ^c	8.23 ^{bd}	8.80^{d}	9.26 ^d
Left-fore teat length, cm	6.46	5.76 ^a	5.89 ^a	6.29 ^{ac}	6.93 ^{bc}	7.69 ^b
Left-rear teat length, cm	6.79 ^{ac}	6.76 ^a	7.04^{a}	7.31 ^a	8.28 ^{bc}	8.73 ^b
Right-fore teat length, cm	6.16	6.42 ^{ac}	6.51 ^a	6.92	7.52 ^b	7.75 ^{bc}
Right-rear teat length, cm	6.68	6.80 ^a	6.92 ^a	7.56	7.78	8.15 ^b
Average teat length, cm	6.53 ^{ac}	6.44 ^a	6.59 ^a	7.02 ^{ad}	7.63 ^{bcd}	8.08 ^b

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Regression relationship between peak milk yield and udder parameters

Highly significant positive correlation existed between peak milk yield and other measured mammary parameters. Their correlation coefficients were shown in Table 2. Dahiya (2006) reported cows with greater udder depth produce more milk than those with a shallow udder. This study found a significant negative correlation between udder depth and peak milk yield for dairy buffaloes (r=-0.28, P<0.01).

We speculated that there is a difference in using udder depth to predict milk yields between cows (*Bos taurus*) and buffaloes (*Bubalus bubalis*) due to different species. Another study suggested that greater udder depth is associated with higher risk of mastitis (Singh *et al.* 2014).

A multiple linear regression equation was constructed to determine the relationship between peak milk yields and the udder parameters (Table 2). The multiple regression equation was determined to be:

Peak milk yield= 0.86-0.05 udder depth + 0.15 rear udder height + 0.08 udder length + 0.31 distance of fore-rear teats + 0.28 right fore teat length (R²=0.35, P<0.05)

Peak milk yield and udder sizes

Buffaloes with udder depth ranging between 20 and 25 cm showed higher peak milk yields than buffaloes with udder depth above 25 cm (Figure 1a). Peak milk yields slowly increased with the increase of rear udder width (Figure 1b). Buffaloes with rear udder width less than 6 cm produced low peak milk yields, while no difference was found of peak milk yield if the rear udder width was less than 6.0 cm. Peak milk yield significantly increased for buffaloes with rear udder width above 9.0 cm (Table 3). Study performed by Liu *et al.* (2001), udder width had a small direct effect on milk yield for Holstein cows.

Peak milk yield increased with the increase of rear udder height, but no significant difference in peak milk yield was found for buffaloes with rear udder height less than 9.0 cm. However, peak milk yield rapidly increased when the rear udder height was greater than 9.0 cm (Figure 1c and Table 3). Negative values for udder depth represented the udder hanging down under the buffalo's hock, and the number of buffaloes with this trait accounted for a low percentage. The peak milk yield (9.14 \pm 2.23 kg) of buffaloes with negative values of udder depth was around the median in our field investigation. Peak milk yield gradually decreased with the increase of absolute value of udder length (Figure 1d). Buffaloes with 0 to 10 cm udder depth showed the highest peak milk yield.

Peak milk yield increased with the increase of udder length. The udder length of Dehong dairy buffaloes are mainly within this range from 30 to 70 cm. Buffaloes with udder length above 50 cm showed higher peak milk yields than buffaloes with udder length less than 50 cm (Figure 1d, Table 3). A linear relationship was found between the distance of teats and milk yield (Lin *et al.* 1987). This agreed with Tilki *et al.* (2005) who reported there was a significant positive relationship between 305-day milk yields and the distance of teats. Teat length and diameter also affected the 305-day milk yield of Brown Swiss cows (Tilki *et al.* 2005). Rear udder height, udder width and udder length also influenced 305-day milk yields in Holstein cows (Zhang *et al.* 2005).

Peak milk yield and teat length

Peak milk yield slowly increased with the increase of left fore teat length (Figure 2a). Buffaloes with 8.1-10 cm left fore teat length had higher peak milk yields (11.30 ± 3.07) compared to buffaloes with less than 8 cm, and no significant difference was found with a left fore teat length less than 8 cm (Table 4).

 Table 2 Correlation between peak milk yield and different udder indices

	PMY	UD	RUW	RUH	UH	DFT	DRT	DFRT	LFTL	LRTL	RFTL	RRTL	MV	ТС
PMY	1	-0.28**	0.24**	0.32**	0.34**	0.19*	0.20**	0.40**	0.25**	0.29**	0.22**	0.251**	-0.09	0.09
UD	-	1	-0.07	-0.25**	-0.21**	-0.23**	-0.18**	-0.14*	-0.01	-0.07	-0.00	-0.06	0.11	-0.01
RUW	-	-	1	-0.03	0.33**	0.22**	0.14*	0.34**	0.20**	0.12	0.19**	0.12	16*	0.19**
RUH	-	-	-	1	0.08	0.14*	0.02	0.18*	0.04	0.17*	0.01	0.14*	-0.03	0.06
UH	-	-	-	-	1	0.20**	0.13	0.35**	0.04	0.17*	-0.04	0.06	-0.10	0.11
DFT	-	-	-	-	-	1	0.67**	0.41**	-0.12	-0.09	-0.04	-0.01	-0.19**	-0.09
DRT	-	-	-	-	-	-	1	0.28**	-0.03	-0.03	0.12	0.06	-0.06	-0.03
DFRT	-	-	-	-	-	-	-	1	0.19**	0.20**	0.14	0.16*	-0.19**	-0.07
LFTL	-	-	-	-	-	-	-	-	1	0.68**	0.71**	0.58**	-0.03	0.20**
LRTL	-	-	-	-	-	-	-	-	-	1	0.43**	0.69**	0.08	0.23**
RFTL	-	-	-	-	-	-	-	-	-	-	1	0.56**	-0.05	0.21**
RRTL	-	-	-	-	-	-	-	-	-	-	-	1	0	0.21**
MV	-	-	-	-	-	-	-	-	-	-	-	-	1	0.12
TC	-	-	-	-	-	-	-	-	-	-	-	-	-	1
** (P<0.01) and * (P	<0.05).												

PMY: peak milk yield; UD: udder depth; RUW: rear udder width; RUH: rear udder height; UH: udder height; DFT: distance of fore teats; DRT: distance of rear teats; DFRT: distance of fore-rear teats; LFTL: left fore teat length; LRTL: left rear teat length; RFTL: right fore teat length; RRTL: right rear teat length; MV: mammary vein and TC: teat conformation.



Figure 1 Relationship between udder sizes showing a) depth; b) width; c) height and d) length and peak milk yield for dairy buffaloes

Table 3 Peak milk yield (kg/d) with different ranges of udder depth and rear udder width (cm)

UD		ŀ	RUW	RUH		UL	
Range	PMY	Range	PMY	Range	PMY	Range	PMY
< 0	9.14±2.23ª	0.0-3.5	8.66±2.74 ^a	0.0-3.0	8.86±2.33ª	≤ 40.0	$8.26{\pm}2.80^{a}$
0-9.9	11.99±2.37 ^b	3.6-5.9	8.53±1.98 ^a	3.1-6.0	8.94±2.12 ^a	40.1-45.0	$8.08{\pm}2.75^{a}$
10-14.9	10.52±2.71°	6.0-7.4	9.67 ± 2.72^{b}	6.1-9.0	8.82±3.15 ^a	45.1-50.0	8.71±2.12 ^a
15-19.9	10.00±2.95°	7.5-9.0	9.24±2.81 ^b	9.1-12.0	10.02 ± 2.49^{b}	50.1-55.0	10.15±2.76 ^b
20-24.9	$8.63{\pm}2.02^{a}$	9.1-11.3	10.35±2.87°	12.1-15.0	10.70 ± 2.40^{b}	55.1-60.0	10.60±2.06 ^b
≥ 25	6.94±1.18 ^d	≥ 11.4	10.49±2.72°	> 15.0	13.00±1.90°	> 60.0	11.12±2.68°

PMY: peak milk yield; UD: udder depth; RUW: rear udder width; RUH: rear udder height and UL: udder length. The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Peak milk yields increased with the increase the left rear teat length (Figure 2b). Buffaloes with left rear teat length greater than 10 cm produced higher peak milk yields than their counterparts with length shorter than 10 cm (Table 4). No significant differences in peak milk yields were found with left rear teat lengths. Peak milk yield increased with the increase of right fore teat length, and the trend of peak milk yield slowly increased when the teat length exceeded 10 cm (Figure 2c). Buffaloes with right fore teat length above 7.6 cm produced high peak milk yields when compared with buffaloes of a teat length less than 7.6 cm. The 7.5 cm right rear teat length was an important inflexion point of peak milk yield, and buffaloes with more than 7.5 cm right rear teat length produced higher peak milk yields than those with a teat length of less than 7.5 cm (Table 4).

Overall, peak milk yields increased with the increase of right rear teat length (Figure 2d), which isn't in agreement with other (Coffie *et al.* 2015).

Contrary to our study, some researchers found a negative correlation between milk yield and teat length (Erdem *et al.* 2010; Tilki *et al.* 2005). One possible reason could be the species difference between buffaloes and Brown Swiss cows or Jersey cows, and a correlation between teat length and peak milk yield was determined, but not milk yield not considered.

Peak milk yield and distance of teats

Peak milk yield increased with the increase of distance between the fore teats, and the highest peak milk yield was found within the range of 10 to 12 cm.



Figure 2 Relationship between teat length showing a) left fore teat; b) left rear teat; c) right fore teat and d) right rear teat and peak milk yield for dairy buffaloes

Table 4	Peak milk	yield (kg/d)	in different ranges	of teat length (cm)
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LFTL	LRTL				RFTL]	RRTL		
Range	PMY	Range	PMY	Range	PMY	Range	PMY		
2.1-4.0	8.87±2.43ª	2.1-4.0	9.04±2.34ª	≤ 4.5	8.98±2.76 ^a	≤ 4.5	8.87±3.04 ^a		
4.1-6.0	9.14±2.37 ^a	4.1-6.0	8.57 ± 2.22^{b}	4.6-6.0	9.18±2.39 ^{ab}	4.6-6.0	8.67±2.18 ^a		
6.1-8.0	9.57±2.62ª	6.1-8.0	$9.44{\pm}2.70^{a}$	6.1-7.5	9.37±2.66 ^{ab}	6.1-7.5	$9.24{\pm}2.48^{ab}$		
8.1-10.0	11.30±3.07 ^b	8.1-10.0	9.99±2.64 ^{ac}	7.6-9.0	10.07 ± 2.61^{b}	7.6-9.0	9.97 ± 2.50^{bc}		
> 10.0	10.57±4.64 ^b	>10.0	11.15±3.10 ^c	9.1-10.5	11.19±3.54°	9.1-10.5	11.63±3.28°		
-	-	-	-	> 10.5	10.20 ± 2.88^{b}	> 10.5	9.78 ± 3.16^{bc}		

PMY: peak milk yield; LFTL: left fore teat length; LRTL: left rear teat length; RFTL: right fore teat length and RRTL: right rear teat length.

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Peak milk yield slowly increased and demonstrated a decreasing trend when the distance between the fore teats exceeded 12 cm (Figure 3a). Buffaloes with 13.3-14.8 cm distance between their fore teats produced higher peak milk yields when compared with their counterparts of a distance between fore teats of less than 13 cm. Meanwhile, no significant difference was found of peak milk yields between 13.3-14.8 cm and more than 15 cm (Table 5).

Peak milk yields increased with increasing the distance between the rear teats. The highest peak milk yield didn't increase when the distance of rear teats exceeded 10 cm (Figure 3b).

There was no significant difference of peak milk yield when the distance of rear teats exceeding 6 cm (Table 5). Peak milk yields rapidly increased with the increase of forerear teats distance, but the yield was smaller when the distance of fore-rear teats exceeded 9 cm (Figure 3c). Erdem *et al.* (2010) found a positive correlation between milk yields and teat distance, which is in agreement with our findings. Our results indicated no significant difference in peak milk yields of buffaloes with fore-rear teat distances exceeding 7 cm (Table 5).

Mammary vein and teat conformation

Mammary vein plays an important role in milk production through the draining of blood to support milk synthesis in the mammary gland and high yielding dairy cows have characteristic thicker mammary veins.

Mammary veins are an indicator to assess the milk yields in Holstein cows. Abdullah *et al.* (2013) reported mammary vein size affects milk yield for Nili-Ravi buffaloes. High yielding crossbred dairy buffaloes, however, are characteristic with discrete mammary veins, as seen in our study (Table 6).

One possible explanation is that they studied the relationship between milk yield and mammary vein size, but we evaluated the effect of mammary vein size on peak milk yield of crossbred buffaloes. No difference in the peak milk yield was observed for buffaloes with different characteristics of mammary veins. Braun and Forster (2012) found greater blood flow speed in high yielding Brown Swiss cows when compared with low yielding or dry cows. The blood flow speed of mammary vein was estimated and thought to be a suitable indicator to evaluate the milk yields of dairy buffaloes.



Figure 3 Relationship between distance of teats showing a) fore teats; b) rear teats; and c) fore and rear teats and peak milk yields for dairy buffaloes

Table 5 Peak milk yield (kg/d) in different ranges of teats distance (c	m)
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DFT			DRT		DFRT
Range	PMY	Range	PMY	Range	PMY
4.0-8.8	9.18±3.37 ^a	2.1-4.0	9.25±3.59 ^a	≤ 5.0	7.15±1.82 ^a
9.0-11.5	9.27±2.56 ^a	4.1-6.0	8.54±2.85	5.1-7.0	8.76 ± 2.10^{b}
11.6-13	9.31±2.75 ^a	6.1-8.0	9.99±2.34 ^b	7.1-9.0	10.06±2.83°
13.3-14.8	10.60±2.45 ^b	8.1-10.0	9.99±2.73 ^b	9.1-11.0	10.72±2.49°
15.0-19.0	10.15±1.97 ^b	> 10.0	10.58±2.28 ^b	> 11.0	11.54±3.39°

PMY: peak milk yield; DFT: distance of fore teats; DRT: distance of rear teats and DRFT: distance of fore-rear teat. The means within the same column with at least one common letter, do not have significant difference (P>0.05).

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mill mald (k	a/d) at dittarai	it mammary vein	and teat co	ntormation

Mammary vein		Teat conformation				
Apparent shape	PMY	Apparent shape	PMY			
Thick and obvious	9.20±2.02 ^a	Similar length and thickness	9.21 ± 2.50^{a}			
Thin and obvious	10.43 ± 2.40^{b}	Similar length, but not thickness	10.42 ± 2.88^{b}			
Thick and unobvious	9.88±2.91 ^{ab}	Dissimilar length and thickness	9.62±2.93 ^a			
Thin and unobvious	10.03±2.59 ^b	-	-			
Invisible	9.41±2.74 ^a	-	-			
PMV: neak milk vield						

PMY: peak milk yield.

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

For teat conformation, buffaloes with similar length but not thickness of teats produce higher peak milk yields when compared with buffaloes of similar length and thickness (Table 6). It has been suggested that the mammary vein may not influence milk yield throughout the lactation. Gračner *et al.* (2015) reported Simmental cows with a high internal diameter surface of mammary vein had high milk yield only during the second lactation. Liu *et al.* (2001) reported that mammary vein diameter is an effective factor of 305-day yield for Holstein cows.

CONCLUSION

In the current study, udder and teat measurements were performed to assess their effect on peak milk yield for Dehong crossbred dairy buffaloes. Our result showed udder depth had negative impacts on peak milk yield. Other mammary parameters, including rear udder width, rear udder height, udder length, distance of rear udder, distance of fore-rear teats, left fore teat length, left-rear teat length, right fore teat length and right rear teat length, had significant positive correlations with peak milk yields. Udder parameters used to determine evaluation criteria for the selection of high yielding dairy buffaloes. This offers guidance to early selection of high yielding crossbred dairy buffaloes and designing milking machines for dairy buffalo teat length.

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