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Parameters of abdominal vein and their association with milk production in dairy cows

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Abstract: *In this observational study we investigate morphological and blood flow parameters of milk vein and their associations with milk production stage in high milk-producing Holstein Friesian cows. Morphological parameters: distance of the milk vein from skin surface (parameter 1); vertical diameter from intima to intima of milk vein (parameter 2) in cm; and vein area in cross-section (parameter 3) in cm2 were measured from B-mod images. Blood flow parameters of milk vein were investigated with Spectral Doppler. Linear regression model was used to evaluate relationship between diameter of milk vein and blood flow volume (BFVol) in end of lactation, dry period and early lactation stages of milk production of dairy cows. Also, the same procedure was employed to investigate relationship between diameter, BFVol and maximum velocity (MaxV) of milk vein and milk yield in early lactation stage. Parameter 1 and Parameter 2 of milk vein had constant values in each lactation stage. Parameter 3 of milk vein in early lactation stage was significantly different compared with that in dry period. A very strong direct relationship was found between diameter of milk vein and BFVol in each lactation stage. R–Squared (R2) means that 84.1%, 78.5% and 95.4% of the variability of BFVol of milk vein in each lactation is explained by its diameter. An increase of the diameter of milk vein by 1 cm the value of BFVol increases by 3.315 l/min in the end of lactation, 2.9 l/min in dry period and 1.09 l/min in the early lactation stage of dairy cows. A very strong direct relationship was found between diameter, BFVol and MaxV of milk vein and milk yield of dairy cows in early lactation stage (R = 0.867, 0.924, and 0.947 respectively). R-Squared (R2 = 0.752, 0.853, and 0.898 respectively) means that 75.2%, 85.3%, and 89.8% of the variability of milk yield is explained by diameter, BFVol and MaxV of milk vein respectively of milk vein in early lactation of dairy cows. An increase of this parameters by 1 unit increases milk yield by 7.335 kg, 6.982 kg and 0.210 kg respectively.*
This study showed that ultrasonography is a useful tool in calculating morphological and blood flow parameters of milk vein as indicators of high milk production in Holstein Friesian cows.

• Introduction

A key driver of dairy intensification was the genetic selection for increased milk yield. The modern dairy cows produce much higher amounts of milk compared with dairy cattle of several years ago, and this may have an influence on blood flow variables in the milk vein at different stages of lactation of dairy cows.
The milk vein is a large subcutaneous vein that extends along the lower side of the abdomen of a cow and returns blood from the cow udder. The milk vein exists the mammary gland at the anterior end of the front quarters and passes along abdominal wall and enters the body cavity at the xiphoid process via milk well, and empties into interval thoracic vein.
According to Huth (1995) [1], approximately 90% of blood is carried out of mammary gland by milk vein and the rest (10%) drains via the perineal vein.
Cardiac output caused by the increase in the heart rate and heart volume represents the basic mechanism to meet the increased demand for blood supply during lactation. The amount of milk supply is closely related to the level of milk production [2].
Years ago, the easy accessibility and measurements of milk vein’s diameter and shape were practical tools for predicting the milk yield in lactating cows [3, 4].
Today, modern technologies could be applied, and one of this is ultrasonography. Ultrasonography is a noninvasive method to visualize the udder parenchyma and blood flow on farm [5, 6, 7, 8, 9, 10].
Udder echotexture refers to the appearance, structure and arrangement of parts of mammary gland within an ultrasonographic image.
Blood flow of the mammary gland has been investigated with invasive techniques [11] and with non-invasive color Doppler ultrasonography [12, 13, 10].
The morphology and blood flow of the milk vein have been investigated with collar Doppler ultrasonography in lactating Brown Swiss cow [14, 15], Simmental cows [6] and Holstein cows [10].
The term „milk vein“ originates from its direct association with the cows ability to produce milk. This vein’ prominent visual characteristics and essential function in milk production have earned it this name. The term combines „milk“ referring to lactation and „vein“ indicating a blood vessel.
The milk vein is particularly malleable to ultrasonographic examination because of its superficial lactation, and cows tolerate examination. Also, this vein has clinical importance in inflammatory changes, such as thrombophlebitis, puri-phlebitis or cellulitis after injections or infusions into the vein or after injuries.
Congestion of the milk vein may occur in association with right-slide cardiac insufficiency [14].
The milk vein size indicates milking capacity of dairy cows. A high-yielding cow produces a massive amount of milk has a large supply of blood, and having it, must have a large channel to return that blood back to the heart. It is estimated that for production of 20 kg of milk/day approximately 9000 L of blood needs to be circulated through the mammary gland of a dairy cow.
The vascular system is well suited for evaluation by ultrasonography. The fluid filled vessels are anechoic and easily visualized.
The lactation of a cow appears to affect the entire circulatory system including blood flow volume in the subcutaneous abdominal vein (milk vein) [15]. In the last decades a few studies have used ultrasonography for the examination of morphology and blood flow of milk vein in lactating or dry dairy cows of various health and milk production profiles [16, 15, 17, 14, 6, 10].
To our knowledge there is scarce data on the fluctuation of blood flow parameters in the milk vein and their association with milk production stages.
The present observational study utilized spectral ultrasonography to examine morphology and blood flew parameters of milk vein and their associations with milk production stages (End of lactation, Dry period and Early lactation).

• Material and method

2.1. Animals

This observational study was performed on the research dairy farm of Agricultural Research and Development Station (ARDS) Șimnic-Craiova, Romania. The dairy farm is located in the South-West region (Oltenia, 182 m above sea level, 44°19’ N, 23°8’ E), and has a 140 Holstein Friesian cows. The initial dairy herd was imported from Denmark (1977-1978) as Danish Black and White (DBW) dairy cattle. Today the most genes from the original DBW cattle have been replaced by Holstein Friesian. The investigation was performed in compliance with European Union Directive 86/609/EC, and national guidelines for animal research.
Initially a total of 16 clinically healthy pregnant multiparous cows were selected. Forage crops, a small amount of concentrates, and a trace mineral salt constituted the dry cow ration during early dry period (DP). Subsequently a close-up ration was fed which provided additional nutrients. All feed rations were calculated to provide recommended nutrients dry and lactating cows. At 3 weeks before due date, cows were moved into the close-up pen, where the farm worker and research moved into the close-up pen, where the farm worker and research personnel monitor the calving process. Peri- and postpartum clinical events are recorded and described. All lactating cows are fed a partial mixed ration based on corn silage and concentrate supplement in the morning and afternoon. The water was always available ad libitum. The lactating cows were milked two times per day at 05:00 am and 17:00 pm, in a DeLaval milking system and were housed free in barn bedded with straw.
At dry off an intramammary infusion with long-acting antibiotics was applied. At the beginning of this study 6 cows were at the end of second lactation, 6 cows at the end of third lactation and 4 cows at the end of fourth lactation. Their anterior milk production was: 8908±66.6 kg SD, 9220±160.4 kg SD, for 1st, 2nd and 3rd lactation respectively.

2.2. Study design and clinical examination

In this study 16 repeated measurements on each cow during late lactation and dry period and 14 repeat measurements on each cow during early lactation period were included in this evaluation. All measurements occurred between 11:00 and 12:00 am. Before each measurement a general physical examination was conducted [18, 19]. Also, clinical examination included inspection and palpation of the mammary gland, and examination of the milk from each quarter using California Mastitis Test (CMT). Cows diagnosed with pathological condition after calving were excluded. In this study among the 16 cows initially selected 2 cows were excluded in the early lactation stage, because all 2 cows were excluded in the early lactation stage, because all 2 cows were diagnosed with clinical mastitis.

2.3. Ultrasonography of the milk vein

Using a cattle crush all cows were examined at standing position and handled gently. Morphology of subcutaneous abdominal veins (milk veins) was investigated with B-display mode of a portable Veterinary Ultrasound Scanner Ecoson 800v. Blood flow parameters were investigated with spectral Doppler ultrasonography.
The procedure for B-mode and spectral Doppler ultrasonography has been previously described [16, 15]. The area around mid-point of milk vein (was washed, shaved) decreased with alcohol and ultrasound gel was put over skin, and on transducer. The transducer was positioned in cross-section of the vein without touching the skin. Distance of the milk from skin surface (parameter 1, cm), vertical diameter from intima to intima (parameter 2, cm), of the vein and vein area in cross section (parameter 3, cm3) were determined from B-mode images. Blood flow parameters (time-averaged) mean velocity (TAMV, cm/s), maximum velocity (Vmax, cm/s) and milk vein blood flow volume (BFVol, L/min) were measured from optimal spectral Doppler images. Left and right milk vein diameters in each animal were summarized and divided by 2 in order to obtain the mean. If a milk vein cross-section has an oval shape, the mathematical formula II a.b was used (a = the smaller milk vein radius, b = the larger milk vein radius, II = constant-3.14) for calculating vein area in cross section [6].

Statistical analysis

All data were entered into Microsoft Excel computer program 2007. Stata version 14 was used into summarize data, and descriptive statistics was used to express the results. One-way ANOVA for repeated measurements was used to test for differences between production stages. Also, a linear regression model was used to investigate the linear relationship between morphological and Blood Flow parameters, and relationship of diameter, Blood Flow Volume and Maximum Velocity in milk vein and milk production in early lactation stage. Linear regression was reported in APA format. All p-values were two-sided and significance level was set at $p \leq 0.05$.

• Results and discussions

Morphological and blood flow parameters of the milk vein (subcutaneous abdominal vein) during study period are presented in table 1.
Distance of the milk vein from skin surface had constant values in each production stage (0.51, 0.53, 0.58 cm for end lactation, dry period and early lactation, respectively) (table). Also, the diameter of the milk vein had constant values (table 1). Cross-sectional area of milk vein in Early Lactation (EL) stage was significantly different compared with that in Dry period (DP) (p=0.009), table 1. In B-mode, the milk veins appeared as a blood vessel in the circulatory system of dairy cows that carry blood towards the heart.
Left and right milk vein morphological parameters in each cow were summarized and divided by 2 to get the average and median of the measurements.
Blood flow parameter (Blood flow volume) was significantly different between production stage ($p<0.001$) (table 1). Also, TAMS’ and Vmax parameters were significantly different (table 1). Cows in early lactation (EL) had the highest blood flow volume (BFvol) 7.64 L/min compared with cows in end of lactation (5.21 L/min), or with cows in dry period (2.60 L/min) (table 1). A large BFvol variability was found in dry period (37%) compared with end of lactation (18%) and with early lactation (4%).
The time-averaged mean velocity (TAMV) of blood flow was also different in each production stage of the dairy cows. The highest value was found in EL stage 19.54 cm/s compared with the values in End of lactation with the values in End of lactation (14.27 cm/s) and in DP (7.25 cm/s) (table 1).
The maximum velocity of blood flow was found in cows in EL (64.90 cm/s) compared with values in end of lactation (44.13 m/s) and DP stage (23.76 m/s) (table 1).
Diameter of milk vein and its association with blood flow volume in each production stage of dairy cows.
The results of the linear regression model investigating the linear relationship between a scalar response (Blood Flow Volume) and independent variable (diameter of milk vein), in each production stage are presented in table 2. A very strong direct relationship was found between Diameter of milk vein and Blood flow volume in each production stage (R=0.917, 0.886, and 0.976 in End of lactation, Dry period and Late lactation respectively).
R-Squared (R2; 0.841, 0.785 and 0.954) means that 84.1%; 78.5% and 95.4% respectively of the variability of blood flow volume of milk vein in each production stage is explained by its diameter. Also, a increase of the diameter of milk by 1 cm the value of blood flow volume increases by 3.315 l/min in the end of lactation stage, 2.9 l/min in dry period and 1.09 l/min in the early lactation stage of the dairy cows.

Table 1. Morphology and blood flow parameters during end of lactation (End L), dry period (DP) and early lactation (EL)

Parameters		End of lactation N=64	Dry period N=128	Early lactation N=224	p = value	
					End x DP	EL x DP
Morphology:						
Parameter 1[1] (cm)	Average (SD)	0.51 (0.13)	0.53 (0.13)	0.58 (0.12)	0.67	0.28
	Median (IQR)	0.51 (0.22)	0.52 (0.23)	0.58 (0.21)		
Parameter 2 (cm)	Average (SD)	2.49 (0.27)	2.41 (0.30)	2.52 (0.26)	0.43	0.29
	Median (IQR)	2.55 (0.32)	2.50 (0.43)	2.60 (0.22)		
Parameter 3 (cm^2)	Average (SD)	5.30 (0.76)	4.88 (0.61)	5.41 (0.39)	0.09	0.009
	Median (IQR)	5.10 (1.05)	4.80 (0.90)	5.30 (0.70)		
Blood Flow (L/min)						
BFVol (L/min)	Average (SD)	5.21 (0.98)	2.60 (0.97)	7.64 (0.30)	<0.0001	<0.0001
	Median (IQR)	4.90 (1.72)	2.50 (1.73)	7.65 (0.27)		
TAMV (cm/s)	Average (SD)	14.27 (2.50)	7.25 (2.51)	19.54 (2.37)	<0.0001	<0.0001
	Median (IQR)	13.90 (3.17)	6.80 (3.50)	19.20 (3.35)		
Vmax (cm/s)	Average (SD)	44.13 (9.31)	23.76 (9.37)	64.90 (10.23)	<0.0001	<0.0001
	Median (IQR)	39.40 (17.9)	19.20 (15.65)	62.20 (19.25)		

Parameter 1 = Distance between milk vein and skin surface.
Parameter 2 = Diameter of the milk vein.
Parameter 3 = Cross-sectional area of the milk vein.
TAMV = Time-averaged mean velocity of the milk vein.
BF Vol = Mean blood flow volume of the milk vein.
V max = Mean maximum velocity of the milk vein.
N = Number of measurements in each production stage.
SD = Standard deviation.
IQR = Interquartile range.
p – value: comparison of means.

Tabel 2. Linear relationship between diameter of milk vein and blood flow volume of milk vein in each production stage of dairy cows

Parameters of milk vein	Production stage of dairy cows	Linear regression APA format:								
		R	R^2	F			β		α	
				Value	df	p=value	Value	p=value	Value	p=value
Diameter vs. Blood Flow Volume	End of lactation	0.917	0.841	74.02	1.14	<0.001	3.32	<0.001	-3.05	=0.007
Diameter vs. Blood Flow Volume	Dry period	0.886	0.785	51.08	1.14	<0.001	2.9	<0.001	-4.39	<0.001
Diameter vs. Blood Flow Volume	Early lactation	0.976	0.954	246.3	1.12	<0.001	1.9	<0.001	-4.89	<0.001

Table 3. Linear relationship between diameter of milk vein, blood flow volume and maximum velocity and milk yield in early lactation stage of dairy cows

	Production stage of dairy cows	Linear regression APA format:								
		R	R^2	F			β		α	
				Value	df	p=value	Value	p=value	Value	p=value
Diameter vs. Milk yield	Early lactation	0.867	0.752	36.32	1.12	<0.001	7.33	<0.001	15.1	<0.001
Blood Flow Volume vs. Milk yield	Early lactation	0.924	0.853	69.69	1.12	<0.001	6.98	<0.001	-19.77	=0.009
Maximum Velocity vs. Milk yield	Early lactation	0.947	0.898	105.37	1.12	<0.001	0.21	<0.001	19.93	<0.001

Also, we investigated the linear relationship between a scalar response (milk yield), and independent variables (diameter pf ilk vein, blood flow volume, and maximum velocity of blood flow) in the early lactation of dairy cows. The results are presented in table 3.
A very strong direct relationship was found between diameter, blood flow and maximum velocity and milk yield of dairy cows (R = 0.867, 0.924 and 0.947 respectively). R-squared (R2 = 0.752) means that 75.2% of variability of milk yield is explained by diameter (cm) of milk vein. Also, R2 = 0.853 means that 85.3% of variability of milk yield is explained by blood flow volume (L/min) of milk vein, and R2 = 0.898 means that 89.8% of variability of milk yield is explained by maximum velocity of blood flow in milk vein. An increase of diameter of milk vein 1 cm increases milk yield by 7.335 kg of milk. Also, a increase of blood flow volume by 1L/min increases milk yield by 6.982 kg, and a increase of maximum velocity of blood flow by 1 cm/s increases milk yield by 0.210 kg.

Discussion

We used ultrasonography to investigate morphological and blood flow parameters from end of lactation to the dry period and to the early lactation of dairy cows. With increasing milk production, the cows’ udder health is challenged during all stages of milk production. Also, this period is very critical for the udder as it transitions from a condition of milk synthesis to involution and back to high milk production.
In this study we recorded morphological and blood flow parameters in end of lactation, dry period and early lactation stages of dairy cows. Diameter of milk vein remained constant in all lactation stages cross-sectional area of milk vein was different (p-value 0.009) in early lactation stage compared with dry period stage, probably as a result of morphologic in the milk vein wall. Blood vessel have a complex layered structure. The media layer contributes more significantly than the intima or adventitia to the vessel properties.
Veins have tremendous tendency to remodel in responses to a change in flow leading [20].
Blood flow parameters (Blood flow volume, time-averaged mean velocity, and maximum velocity) of milk vein differed between lactation stages (end of lactation, dry period and Early lactation). Similar results were reported by Themistokleous et al., 2023 [10] for Holstein cows by Braun et al., 2013 for Swiss Braunvieh cows [14].
Gračner et al., 2015 [6] reported a value of 4.21 ± 0.139 cm2 for milk vein internal diameter surface in Simmental cows.
Using linear regression model a strong direct relationship was found between diameter of milk vein and blood flow volume in each lactation stage.
It is known that milk yield of dairy cows is directly corelated with the blood flow through the mammary gland and milk veins drain 90% of the total blood supplying this organ.
The differences of layered structure of blood vessels are directly related to different functions of arteries and veins: arteries must accommodate the influx of blood from the heart and accompanying pressure pulse, while veins return blood to the heart using a system of valves.
In this report lactating cows had a 2.9 – fold higher blood flow volume than dry cows and this highlights the importance of this vessel for venous drainage of the mammary gland.
The average of 7.64 l/m of blood flow volume of milk vein in the early lactation stage means approximately 11000 l of venous blood drainage by one milk vein in 1 day. And this represents 90% of the total venous blood of udder. Total venous blood was 22244 l, and to produce 33.5 l of milk 664 l of venous blood was needed per 1 l of milk yield. Estimates of the amount of blood required for the production of one liter of milk vary from 300 to 600 [21, 22, 15]. Braun and Forster (2012) [15] reported 445 l for Swiss Braunvieh cows to produce 1 l of milk. Our results confirm that large milk veins of dairy cows indicate high milk production.
In this study, large diameter of milk vein, high blood flow volumes and high maximum velocity of blood flow were strong and direct correlated with milk yield of dairy cows in early lactation stage. This morphologic and blood flow parameter can be used as new traits in selection of dairy cows for high milk production. Also, milk veins integrity must be preserved, and iatrogenic complications must be avoided in order to maintain in high milk production.

• Conclusions

This study showed that large morphologic and blood flow parameter of milk vein are needed to produce high milk yield of dairy cows.
Ultrasonography is a useful tool in calculating morphological and blood flux parameters of milk vein as indicators of high milk production in Holstein Friesian cows.
Blood flow parameters in milk vein changed significantly through end of lactation stages while distance of milk vein from skin surface and diameter of milk vein remained constant and cross-sectional area of milk vein changed in early lactation stage corresponding to milk production needs.

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