In vivo measures of mammary development in gestating gilts¹

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ABSTRACT: Potential links between measures of udder morphology obtained in live pregnant gilts and mammary gland development and composition measured in mammary tissue collected at slaughter were studied. Thirty-three gilts were used. In vivo measures of gland morphology using a tape or ultrasound imaging (parenchymal area measured by ultrasound [AREA]) were obtained on d 108 ± 1 of gestation. Gilts were then slaughtered on d 110 ± 1 of gestation to collect mammary glands for dissection and compositional analyses. The various tape measures were the distance between each teat on one side of the udder (DIST-TEAT), the distance between each teat pair (DIST-PAIR), the length of the udder (sum of all DIST-TEAT), the distance between the base of the teat and the ventral midline section of the udder (MID), and the distance between the base of the teat and

the exterior junction of the udder with the abdomen (EXT). The variables MID, DIST-TEAT, DIST-PAIR, and length had very poor correlations with parenchymal weight, extraparenchymal weight, or any of the measured compositional variables. On the other hand, both AREA and EXT were correlated ($P < 0.01$) with the weight of parenchymal tissue, total parenchymal protein, total DNA, and total RNA. The ultrasound measure AREA and the tape measure EXT were also correlated with each other ($P < 0.05$). These measures could, therefore, be helpful to estimate mammary development in studies where animals cannot be slaughtered. The tape measure EXT seemed to better reflect the volume of the gland than MID, and it provided as reliable an estimate of parenchymal weight as the measure of parenchymal area using ultrasound while being much easier and cheaper to obtain.

Key words: gestation, gilt, mammary development, pig, udder morphology, ultrasound imaging

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INTRODUCTION

With the current use of hyperprolific lines, sows must produce very large quantities of milk to sustain the growth of all their piglets. Taking into account the positive relation between the number of milk secretory cells at the onset of lactation and sow milk yield (Head et al., 1991), mammary development has become an essential component of the management of gilts and sows. Nutrition (Head and Williams, 1991; Farmer et al., 2014), body condition (Farmer et al., 2016a,b, 2017), breed (Farmer et al., 2000a), and hormonal

status (Farmer et al., 2000b; Farmer and Petitclerc, 2003) were shown to affect mammary development in late-pregnant gilts, yet such studies necessitate the slaughter of animals to collect mammary tissue and determine its composition. Recently, the potential use of measures obtained on live animals to estimate mammary development or milk yield was evaluated in dairy heifers (Nishimura et al., 2011; Albino et al., 2015; Esselburn et al., 2015; Geiger et al., 2016), ewes (Emediato et al., 2008), goats (Atay and Gokdal, 2016), and sows (Balzani et al., 2016a). Three types of live measures were taken, namely tape measures, computer-assisted tomography (CAT) scan (or X-ray absorptiometry), and ultrasound imaging. In sows, an early study showed that the CAT scan technique could accurately predict the weight and volume of mammary extraparenchymal tissue but not parenchymal tissue mass (Petitclerc and Farmer, 2003). A recent trial looked at the link between udder morphology and sow performance; however, actual measures of mammary

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Figure 1. Different mammary gland measures obtained with tape. (A) The distance between subsequent teats (using rigid tape; mean of 18.06 cm [SD 1.77]). (B) The distance between teat pairs (using rigid tape; mean of 9.16 cm [SD 0.91]). (C) The distance between the base of the teat and the ventral midline section of the udder (using soft tape; mean of 8.61 cm [SD 0.96]). (D) The distance between the base of the teat and the exterior junction of the udder with the abdomen (using soft tape; mean of 9.38 cm [SD 0.86]).

development were not investigated (Balzani et al., 2016c). We hypothesized that tape measures providing an estimate of gland volume could be used as indicators of mammary parenchymal tissue mass. The objective of the current study was to determine if measures of udder morphology obtained in late-pregnant gilts with the use of a tape or with ultrasound imaging could be used to estimate mammary gland development and composition.

MATERIALS AND METHODS

Animals were cared for according to the national guidelines for the care and use of animals (CCAC, 2009), and procedures were approved by the Institutional Animal Care Committee of the Sherbrooke Research

and Development Centre of Agriculture and Agri-Food Canada (Sherbrooke, Quebec, Canada).

Management of Gilts and Mammary Examination Procedures

Thirty-three Yorkshire $\times$ Landrace gilts were used. They were bred with semen from a pool of Duroc boars and housed in individual stalls (0.6 by 2.1 m) throughout gestation. They were fed a conventional gestation diet (3,072 kcal/kg DE, 14.28% CP, and 0.64% lysine) according to BW at mating. Gilts weighing 140, 145, 150, 155, 160, and 165 kg were fed 2.21, 2.11, 2.00, 1.90, 1.85, and 1.80 kg/d, respectively, from mating until d 90 of gestation. Thereafter, all gilts were fed 2.5 kg/d until slaughter on d 110 ± 1 of gestation. Gilts were weighed and had their backfat depth ultrasonically measured (WED-3000; Schenzhen WELLD Medical Electronics Co., Ltd., Schenzhen, China) at P2 of the last rib at mating and on d 108 ± 1 of gestation, at which time in vivo measures of udder morphology were obtained with a measuring tape (soft or rigid, depending on the measure taken) and with ultrasound imaging on one side of the udder. The various measures taken with a tape (Fig. 1) were always recorded by the same person and were the distance between each teat on one side of the udder (**DIST-TEAT**; using rigid tape; average for all teats), the distance between each teat pair (**DIST-PAIR**; using rigid tape; average for all teat pairs), the length of the udder (sum of **DIST-TEAT**), the distance between the base of the teat and the ventral midline section of the udder (**MID**; using soft tape following the contours of the gland) for 2 teats (with no abnormality) in the median area, and the distance between the base of the teat and the exterior junction of the udder with the abdomen (**EXT**; using soft tape) for the same 2 selected teats.

Ultrasound measures of mammary parenchymal tissue were obtained with the same scanner used for backfat thickness measurements but using a microconvex transducer (C1-12; Schenzhen WELLD Medical Electronics Co., Ltd.) operated at 5.0 MHz. The probe was placed at a 45° angle in relation to the teat base (Fig. 2), after applying ultrasound gel (EcoGel 200; Eco-Med Pharmaceutical Inc., Mississauga, ON). Images (3 to 6 for each of the 2 teats per animal selected for the tape measures) were saved in bitmap (BMP) format and subsequently transferred to the software ImageJ (National Institutes of Health, Bethesda, MD; <http://imagej.nih.gov/ij/index.html> [accessed February 2017]). The circumference area around parenchymal tissue for each image was drawn by 2 persons in triplicate, and the 2 images with the greatest average surface area and a CV $< 5\%$ were kept for statistical analy-

Figure 2. Location of the probe at a 45° angle in relation to the teat base.

ses. Pixel areas squared were converted to millimeters squared using the proper scale setting for each image.

Mammary Gland Collection and Compositional Analyses

Gilts were slaughtered on d 110 ± 1 of gestation to collect mammary glands. At slaughter, mammary glands from both sides of the abdominal wall were excised. Those from the side of the udder used for live measurements were placed directly in the freezer at -20°C and, once frozen, were cut into 2-cm slices and stored again at -20°C under vacuum. Each slice was later trimmed of skin, muscle tissue, lymph nodes, and visible blood vessels. Mammary parenchymal tissue was dissected from the surrounding adipose tissue (i.e., extraparenchymal tissue) at 4°C . Parenchymal and extraparenchymal tissue weights from the 2 mammary glands selected for the tape and ultrasound measurements were individually recorded. Parenchymal tissue from all dissected and sliced glands was then homogenized, and a representative sample was used for determination of composition by chemical analysis. The RNA content of parenchymal tissue was measured by UV spectrophotometry (Volkin and Cohn, 1954), and the DNA content of parenchymal tissue was evaluated in all samples using a method based on the fluorescence of a DNA stain (Labarca and Paigen,

1980). Dry matter, protein, and lipid contents were also determined (methods 950.46, 928.08, and 991.39, respectively; AOAC, 2005) in parenchyma.

Statistical Analyses

For the ultrasound measures, a concordance analysis was performed to compare results from 2 persons using the same image. All analyses and data manipulations were done with the SAS system (release 9.4; SAS Inst. Inc., Cary, NC) using PROC CORR for correlation and PROC REG for regression analyses. Concordance analyses were done according to Lin (1989). Pearson correlation coefficients between the various measures of mammary development using tape, ultrasound imaging, and dissection and compositional analyses were determined. Correlations with the ultrasound measure were first done per teat (2 per gilt; $n = 66$) using parenchymal and extraparenchymal weights for those 2 teats. Correlations were also done per gilt ($n = 33$) using the average ultrasound values from the 2 selected teats and the parenchymal weight, extraparenchymal weight, and chemical measures for the whole side of the udder. Stepwise regression analyses were done to obtain an equation estimating parenchymal weight based on either parenchymal area measured by ultrasound (**AREA**) or the EXT measure taken with a soft

tape. The contribution of the sow's characteristics BW and backfat thickness was assessed by determining if the coefficient of determination increased when these variables were included in the regression equations.

RESULTS

Mean BW and backfat thickness of gilts were 147.5 kg (SD 9.1) and 16.2 mm (SD 1.2), respectively, at mating and 216.8 kg (SD 7.5) and 17.2 mm (SD 1.7), respectively, on d 108 $\pm$ 1 of gestation. Comparison of the parenchymal area obtained from 2 persons using ultrasound imaging based on the same image (104 observations) showed a concordance of 0.942 between operators. When comparing data using all images obtained for a teat (total of 66 observations), the concordance remained high, at 0.953. Averages of data obtained from the 2 operators were, therefore, used for all remaining analyses. When considering the 2 selected teats per gilt ($n = 66$), correlation coefficients between ultrasound parenchymal area and extraparenchymal and parenchymal tissue weights obtained via dissection were -0.026 ($P > 0.10$) and 0.373 ($P < 0.01$), respectively. There was a significant positive correlation between backfat and parenchymal weight (0.267 ; $P < 0.05$), and adding this variable to the regression equation increased its prediction level ($P < 0.05$). The coefficient of determination was 0.139 without considering backfat and 0.218 with backfat included. The final regression equation was as follows:

$$\text{parenchymal weight} = -86.45 + (0.26 \times \text{ultrasound area}) + (13.54 \times \text{backfat})$$

Average, minimum, and maximum values for the various tape measures obtained are shown in Table 1. There was no correlation between the MID and EXT measures (concordance of 0.164). Values for DIST-PAIR (mean of 9.01 [SD 1.03]) were greater than those for DIST-TEAT (mean of 4.77 [SD 0.55]), indicating that the circumference of each gland is not circular. There also seemed to be no relation between the side of the teat on the udder or teat number and either EXT or MID (data not shown). Correlations between AREA (average of 2 teats per gilt), various tape measures, and the weights of parenchymal and extraparenchymal tissues (for one side of the udder) as well as various chemical composition measures (for one side of the udder) are shown in Table 2. The variables MID, DIST-TEAT, DIST-PAIR, and length showed very poor correlations with parenchymal weight, extraparenchymal weight, or any of the measured compositional variables. On the other hand, both AREA and EXT were significantly correlated with weight of parenchymal tissue, total parenchymal protein, total DNA, and total

Table 1. Average, minimum, and maximum values for the following in vivo mammary measures obtained on d 108 $\pm$ 1 of gestation: parenchymal area measured by ultrasound (AREA), the distance between the base of the teat and the ventral midline section of the udder (MID; average for 2 selected teats), the distance between the base of the teat and the exterior junction of the udder with the abdomen (EXT; average for 2 selected teats), the distance between each teat on one side of the udder (DIST-TEAT; average for udder side), the distance between each teat pair (DIST-PAIR; average for udder), and the length of the udder (sum of DIST-TEAT for udders with 7 teats)

Item	n	Mean	SD	Minimum	Maximum
AREA, mm ²	33	558.41	115.64	333.64	805.59
MID, cm	33	8.61	0.96	7.00	11.00
EXT, cm	33	9.38	0.86	8.00	11.00
DIST-TEAT, cm	26	18.06	1.77	14.50	22.75
DIST-PAIR, cm	33	9.16	0.91	7.37	10.87
Length, cm	23	108.12	8.72	93.50	130.00

RNA (Table 2). In the case of EXT, no significant correlation was present with any of the percent compositional measures in parenchyma. This was not the case for AREA, which was negatively related ($P < 0.01$) with the percentages of DM and fat in parenchyma and positively related ($P < 0.05$) with protein percent and RNA concentration. The correlation between the ultrasound measure AREA and the tape measure EXT, performed on an individual teat basis, was 0.31 ($P < 0.05$). Correlations between AREA and MID, DIST-TEAT, and DIST-PAIR were 0.106 ($P > 0.10$), -0.012 ($P > 0.10$), and 0.074 ($P > 0.10$), respectively.

The stepwise regression analyses estimating parenchymal weight based on the EXT tape measure was improved by the addition of backfat thickness ($P < 0.01$) and BW ($P = 0.07$) of the gilts. The coefficient of determination was 0.247 without considering these 2 variables and 0.355 with them being included. The final regression equation was as follows:

$$\text{parenchymal weight} = -773.14 + (38.87 \times \text{EXT}) + (2.07 \times \text{BW}) + (14.52 \times \text{backfat})$$

Such an equation could not be developed for estimating extraparenchymal tissue weight due to the lack of linear correlation between that variable and the EXT measure.

DISCUSSION

The current study is the first to investigate the potential relations between in vivo measures and actual

Table 2. Correlation coefficients ($n = 33$) between in vivo measures obtained on d 108 ± 1 of gestation (i.e., parenchymal area measured by ultrasound [AREA] for 2 selected teats, the distance between the base of the teat and the ventral midline section of the udder [MID] for 2 selected teats, the distance between the base of the teat and the exterior junction of the udder with the abdomen [EXT] for 2 selected teats, the average distance between each teat on one side of the udder [DIST-TEAT], the average distance between each teat pair [DIST-PAIR], and the length of the udder [sum of DIST-TEAT]) and mammary measures obtained after slaughter (i.e., parenchymal and extraparenchymal weights for one side of the udder and chemical composition)

Item	AREA	MID	EXT	DIST-TEAT	DIST-PAIR	Length
Extraparenchymal tissue, g	0.330†	-0.085	0.337†	0.053	0.294†	-0.208
Parenchymal tissue, g	0.531**	0.367*	0.598**	0.242	-0.061	0.376†
DM, %	-0.527**	-0.157	-0.193	0.141	-0.045	-0.084
Fat, ¹ %	-0.453**	-0.026	-0.069	0.201	0.118	-0.011
Fat, g	0.165	0.297†	0.515**	0.360†	0.041	0.383†
Protein, ¹ %	0.499*	0.008	0.017	-0.206	-0.110	-0.007
Protein, g	0.511**	0.310†	0.473**	0.144	-0.149	0.296
DNA, ¹ mg/g	0.306†	-0.153	0.000	-0.271	-0.090	-0.137
DNA, g total	0.488**	0.214	0.472**	0.106	-0.112	0.220
RNA, ¹ mg/g	0.401*	-0.106	0.013	-0.287	-0.077	-0.138
RNA, g total	0.582**	0.237	0.487**	0.085	-0.137	0.251

¹Expressed on a DM basis.

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$.

dissection and chemical measures of mammary development of gestating gilts. Over 10 yr ago, an attempt was made to relate mammary gland development in lactating sows with measures obtained via CAT scan (Petitclerc and Farmer, 2003). It was demonstrated that the CAT scan technique could accurately predict weight and volume of mammary extraparenchymal tissue but not parenchymal tissue mass. Similar results were recently obtained in dairy heifers (Geiger et al., 2016). Another interesting avenue to estimate mammary development is ultrasound imaging. This was used in dairy heifers, and it was shown that such a method allows visualization of the internal structures of the udder and could be a useful tool to evaluate mammary glands (Nishimura et al., 2011). Esselburn et al. (2015) reported that ultrasound measures are related with the amount of parenchymal tissue in heifers, suggesting it is an adequate noninvasive tool to quantitatively monitor mammary development. This method was indeed used to assess the impact of diets on mammary development of heifers, and it was suggested that it provides a good measure of tissue development within the fat pad (Albino et al., 2015). Sadeghi et al. (2016) also reported that udder morphology traits using ultrasound imaging are related to daily milk yield in sheep.

In sheep (Emediato et al., 2008) and goats (Atay and Gokdal, 2016), physical measurements of udders during lactation were used to assess potential relations with milk yield. It was indeed demonstrated that udder measures were correlated with milk yield. Interestingly, Chandrasekar et al. (2016) reported that milk production of primiparous buffaloes could be assessed on the basis

of prepartum udder and teat measurements, and Huntley et al. (2012) saw a relation between physical attributes of the udder and lamb growth. In swine, Balzani et al. (2016a) recently developed a methodology to describe udder conformation in prepartal sows with the goal of relating these variables to piglet survival and performance. They reported that measurements did not differ between sides of the udder, which was also observed in the present study, and they also showed that measures using anatomical landmarks as reference points were more reliable than those using the floor or the pen as landmarks. Accordingly, all measures obtained in the current experiment used anatomical reference points. The significant positive correlations observed between 2 in vivo measures (AREA and EXT) and parenchymal tissue weight is of great interest. Even though these correlations were between 0.53 and 0.60, thereby explaining approximately 28 to 36% of the variance in parenchymal tissue weight, such measures could be helpful to estimate mammary development in future studies where animals cannot be slaughtered. It is also of importance that the EXT measure with a soft tape provided as reliable an estimate of parenchymal weight as the measure of parenchymal area using ultrasound, the former measure being much easier and cheaper to obtain. Also of interest is the fact that the measure from the teat base to the exterior junction of the udder with the abdomen was more useful than that from the teat base to the midline of the udder. This indicates that the EXT measure may better reflect the volume of the gland than the MID measure. This is also suggested by the greater values for EXT compared with those for MID and the lack of

correlation between MID and EXT values that indicate a laterally asymmetric conformation of the glands.

The fact that only middle glands were used in the current study likely reduced the variability in the measures, because Balzani et al. (2016c) saw that the distance from the teat base to the abdominal midline (equivalent to the MID value) was shorter in anterior and posterior teats compared with middle teats. Interestingly, they reported that first- and second-parity sows had smaller teats than multiparous sows. A parity effect on teat pair distance and teat size in lactating sows was also reported by Ocepek et al. (2016). Teat pair distance, teat length, and teat diameter all increased with sow parity. Adegoke et al. (2017) recently reported similar findings in lactating ewes, but they also noted that parity had no effect on udder volume derived using an equation. However, to the best of our knowledge, such a measure was never determined in sows. Effects of lactation number and lactation month on various udder morphological traits (including front teat diameter) were observed in Holstein cows (Ceyhan et al., 2015). Furthermore, Sharma et al. (2017) noted that udder morphological traits (including teat shape and size) could have an impact on milk leukocyte counts, and, hence, udder health, in crossbred cows. Nevertheless, no relation was reported between teat characteristics and mammary development per se. Measures of teat length and diameter were not recorded in the present study because the goal was to relate variables to mammary development and not to piglet performance. In mammary tissue from pregnant swine, all parenchymal tissue is found underneath, and not within, the teat so that no correlation between teat size and mammary development was expected.

Both AREA and EXT were significantly correlated with weight of parenchymal tissue, total parenchymal protein, total DNA, and total RNA. In the case of EXT, the significant correlations with total amounts of the various components of parenchyma were due to the significant correlation with total parenchyma because there was no relation with any of the percent compositions. For AREA, however, there were also significant correlations with some measures of parenchymal content, namely, percent DM, percent fat, percent protein, and RNA concentration. Nevertheless, this may only be an artifact, because it is unlikely that an ultrasound measure of parenchymal area could predict its composition.

Balzani et al. (2016b) stated that udder quality traits can be used in selection and breeding practices. Ocepek et al. (2016) specifically suggested that the distance between teat pairs is important for teat use by the piglets and should be included in breeding programs to ensure colostrum intake and maintain teat functionality. Indeed, the proportion of unsuckled functional teats increased with greater teat pair distance.

In conclusion, even though chemical measures are undoubtedly the best method to determine mammary composition, current results indicate that some easily obtainable in vivo measures can give an indication of mammary development in late-pregnant sows. More specifically, AREA obtained via UV imaging and EXT obtained with a tape measure were significantly correlated with parenchymal tissue weight. Such measures could, therefore, be helpful to estimate mammary development in future studies where animals cannot be slaughtered. Taking into consideration that the EXT measure provided as reliable an estimate of parenchymal weight as the measure of parenchymal area using ultrasound and the fact that it is also much easier and cheaper to obtain, this measure should be considered as a potential selection criteria to improve sow milk yield.

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