Role of the Brix refractometer in predicting piglet survivability in relation to colostrum intake

DVM project report

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Abstract

Newborn piglets must acquire maternal antibodies from colostrum in the first 24 hours after birth to achieve passive immunity. However, in a busy farrowing house, it can be difficult to determine who has received sufficient colostrum. Since piglet serum gamma-globulins are strongly correlated with Brix percentage, the Brix refractometer has the potential to be a reliable pen side tool for identifying compromised piglets. The present study evaluated the utility of the Brix refractometer in predicating piglet pre-weaning survivability and other production measures.

A total of 227 piglets from 19 sows (parities 1-5) over 3 batches were weighed, and tail-docked 24 hours after birth. Blood was collected from the tail docking wound and Brix percentage recorded. Piglet mortalities were recorded and the remaining piglets were weighed at weaning.

Sow progeny weighed more at weaning (P = 0.001) and grew faster than gilt progeny (P = 0.001). There was a tendency for males to grow faster than females (P = 0.096). There was a weak positive relationship between Brix percentage and average daily gain (R = 0.16, P = 0.051) and Brix percentage and weight at 24 hours (R = 0.07, P = 0.722). There was no relationship between Brix percentage and piglet sex (P = 0.897) nor Brix percentage and parity group (P = 0.467). The pre-weaning mortality rate was 9.79%. Ninety-four percent of the samples had a Brix percentage ≥ 7.9% indicating successful transfer of immunity. Brix percentage had no effect on piglet pre-weaning survivability (P > 0.05).

In conclusion, the Brix refractometer was a poor predictor of piglet survivability. However, there were weak positive relationships with growth and weight at 24 hours suggesting Brix percentage may help staff determine which piglets are more likely to thrive.

List of Abbreviations

ADG	Average daily gain
BW	Body weight
FPT	Failure of passive transfer
Ig	Immunoglobulin
IgG	Immunoglobulin G
PWM	Pre-weaning mortality
RID	Radial immunodiffusion
Sn	Sensitivity
Sp	Specificity
TIA	Turbimetric immunosassay
TS	Total solids

Introduction

Piglet pre-weaning mortality (PWM) is a substantial issue in the swine industry due to its economic impact and the welfare of the pigs (1). PWM rates vary globally, but in the major pig producing countries (China, EU, USA, and Brazil), PWM is between 10-20% (1, 2) and 80% of these deaths occur between parturition and 4 days post-parturient (3). Piglet PWM is highly multifactorial (sow, piglet, environment) (1), but the largest contributor is colostrum quality and quantity (1, 4, 5).

Colostrum provides the piglets with energy due to its high fat content (25 mg/mL, 40-60% of total colostrum energy), which the piglets are reliant on as they are born with negligible amounts or inaccessible energy stores (4). Colostrum also provides immunoglobulin G (IgG) which piglets are dependent on for passive immunity as they are born agammaglobulinaemic due to the epitheliochorial structure of the porcine placenta, which prevents transfer of immune cells in utero (5). To achieve this passive immunity, it has been established that piglets need to consume a minimum of 200 g of colostrum (2, 5). This industry recommendation advises on the quantity of colostrum to prevent failure of passive transfer of immunity (FPT) but does not state the quality (IgG concentration) required. Hasen et al. (2016) categorised sow colostrum quality based on IgG content (poor <14.5 +/- 1.8 mg/mL, borderline 43.8+/- 2.3 mg/mL, adequate 50.7 +/- 2.1 mg/mL, very good 78.6 +/- 8.4 mg/mL) (6). There are also multiple studies (4, 7, 8) that looked into the relationship between serum IgG 24 hours post-parturient and mortality during lactation, but there is still no set threshold for FPT in piglets. This is in contrast to calves and foals which do have a specific 24 hour post-parturient serum/plasma IgG content that indicates FPT (calves: <10g/L (9), foals: <8

g/L (10)). Another important consideration is timing of colostrum ingestion as the gut lining of the small intestine of the piglet will close to immunoglobulins after 24 hours (4). Therefore, to ensure passive immunity, the appropriate quality and quantity of colostrum must be consumed within 24 hours.

Currently, colostrum intake can be roughly estimated on farm by the difference in piglet weight at birth and 24 hours later (4), however, there are more accurate techniques that also provide additional information such as transfer of IgG. ELISA is the gold standard for IgG analysis (6) but there is also RID (3, 11) and TIA (12). The main concern with these methods is expense as well as practicality as they cannot be performed pen-side, meaning results cannot be obtained instantaneously. The Ig immunocrit method is cheaper and can be performed pen-side unlike the IgG analysis methods, but the methodology is complex and results are still not instantaneous (13, 14). Lastly is the Brix refractometer (digital or optical) which is a handheld device that measures the percent sucrose in liquids or measures total solids (TS) as a percent in non-sucrose containing liquids (6). When used on colostrum, TS can approximate Ig content as they make up a mean of 63.3% of the total protein in colostrum; 80% of this Ig is IgG (3, 7, 15). When Brix was used as a tool to determine piglet plasma protein 24 hours post-parturient as an indicator of IgG intake, there was a 98.8% correlation between the Brix value and that of the measured total protein percentages in piglet plasma (3). The efficacy of the Brix refractometer's ability to accurately measure serum IgG was also demonstrated in foals. The sensitivity of the Brix was adequate to label it as an accurate screening test for FPT when using serum in foals (10). It should be noted that there are conflicting results for the accuracy of the Brix estimating IgG

concentration in calf serum making it an unsuitable method for assessing colostrum quality or FPT in calves (9, 16). Overall, the Brix refractometer is a good alternative to measure IgG transfer in piglets due to its practicality being a pen-side test, low cost to purchase and maintain, and the production of rapid results (3, 6, 17).

It is known that the Brix refractometer is an accurate method of estimating gamma-globulin concentration in piglet plasma, but there was no threshold for these concentrations until recently. A study by Schoos et al. (2021) suggested three gamma-globulin concentration cut-offs using the best sensitivity (Sn) and sensitivity (Sp) (Table 1) (18). Even though the Brix has been used to measure IgG in various species, after extensive review of the literature, no studies have correlated results from the Brix refractometer with production measures (i.e. mortality rates, growth, etc). Therefore, it is hypothesized that the Brix refractometer will be an accurate pen side test to confidently predict piglet survivability and ability to thrive using blood samples taken from tail docking wounds 24 hours after birth.

Table 1.
Piglet plasma gamma-globulin (g/L) thresholds developed based on studies by Devillers et al., (2021), Canbrera et al., (2012) and Hendrix et al., (1978) using the best combination of Sn and Sp determined by ROC curves. Brix % >7.9 (>30 g/L gamma-globulins) suggests adequate transfer of passive immunity.

% Brix	y-globulin (g/L)	Sn (%)	Sp (%)
5.4	10	100	98.5
7	20	100	89.3
7.9	30	90.1	80.6

Materials and methods

Study Population

This experiment was approved by the Animal Ethics Committee, Murdoch University (R3225/20). The study took place at a commercial farrow-finish pig farm in Yarloop, Western Australia. Data was collected over three batches from October-November 2020. Nineteen sows (Pig Improvement Company) of mixed parity (range: 1-5) were included (5, 7 and 7 within each batch respectively). The sows were selected if they farrowed 24 hours prior to the allocated data collection days within each of the batches. Data was collected from 227 piglets and the criteria for their selection was as follows: ≥ 1kg and no obvious sign of morbidity (respiratory signs, diarrhea, hernia, lameness, wounds). The study aimed to include approximately 300 piglets which was determined based off of a similar study by Vallet et al (2013) which measured piglet immunocrit ratios and pre-weaning survival data (14). The 300 was not achieved as regional restrictions due to COVID-19 limited travel to the farm which delayed the start of data collection. Data collection also ended prematurely as the farm implemented a new iron product in mid-December which had the potential to influence the results.

Sample Collection

The order the litters were processed was determined based on which sow was the closest to 24 hours post-farrowing. The time from birth to data collection across the three batches was 27.6 hours ± 3.24 (mean ± SD). All piglets who met the inclusion criteria were weighed in a zeroed bucket on a digital scale then ear tagged with an individually identifiable number (1-227) on the right ear.

The piglets were tail docked one at a time and blood was collected from the pig side of the tail docking wound via a capillary tube and plugged with plasticine in one end. Given tail docking is a routine husbandry procedure for the farm it presented a good opportunity to collect a blood sample from the piglets. This method also prevented undue stress that would be associated with blood collection by venepuncture. The capillary tubes were taped onto a board labelled with the piglet's identifiable number. After the samples were collected from two-three litters, the blood was spun at the farm's laboratory in a LW Scientific V24T centrifuge at 12,000 rpm for three minutes to obtain the serum. No more than three litters were collected at a time to try and limit the amount of time the samples were spent sitting in a hot environment before analysis. Fifty microlitres of serum was placed directly onto the digital handheld Brix refractometer (Misco, PA-202X-003-105) which provided a Brix percentage.

Monitoring

The sows and the piglets were housed in conventional farrowing crates (shed 1: 2400mm x 600mm x 950mm, shed 2: 2150mm x 650mm x 1000mm) for the lactation period. Farm staff monitored the piglets over the lactation period and recorded any mortalities; piglets not fit for weaning at the end of the lactation cycle were also recorded as a mortality. Weaning took place at 20 ± 0.4 days of age (mean ± SD). The study piglets were re-weighed on the day of weaning.

Statistical Analysis

Continuous variables were analysed using the t-test and MIXED procedures of SPSS (version 24, Armonk NY, USA). The individual piglet was the experimental unit for all analyses, with the exception of total born alive, where sow was the experimental unit. A paired t-test was used to analyse piglet weight at 24 hours, piglet weaning weight, piglet ADG, and piglet 24-hour Brix percentage with parity (sow vs gilt) as a fixed effect. A paired t-test was also used to analyse total born alive with parity (sow vs gilt) as a fixed effect. MIXED procedures were used to examine ADG and Brix percentage with parity group (sow vs gilt), sex, 24-hour weight and wean weight (Brix percentage analysis only) as fixed effects and batch and sow ID as random effects.

The remaining analyses were conducted in R (R Core Team, 2020). The utility of Brix percentage as a predictor of pre-weaning survivability was explored using a logistic regression with sex, parity group (gilt vs sow), weight at 24 hours after birth and Brix percentage (continuous or using ≥7.9 as a cut-off point for adequate transfer of immunity in 24-hour old piglets (18)) as fixed effects. Sow ID and replicate were also included in the model as random effects.

Results

Samples with visibly lipaemic serum (2.2%) were excluded as lipaemia significantly ($P<0.05$) increases the refractive index which leads to a false elevation in Brix percentage (19). Samples with visibly haemolysed serum (12.3%) were also excluded as a precaution. Various concentrations of haemoglobin can decrease the accuracy in reading an optical refractometer as the junction between the light and dark lines are

hard to distinguish, but the refractive index was not affected (19). Fourteen percent of the study piglets were also excluded as not enough blood was able to be collected from them. For the purpose of this study, piglets not fit to wean were all classified as a mortality. This was done for ease of statistical analysis and detailed records were not kept during the lactation period. Piglets under the classification of mortality could have been found dead, fostered to a nurse sow to extend the lactation period, not found at weaning, or had a morbidity.

Gilt vs sow progeny performance

Born alive was lower in gilt litters (*n*=7) compared to sow litters (*n*=12), 10.7 +/- 0.78 vs 13.3 +/- 0.45 (*P* = 0.006). There was no difference between the weight at 24 hours and Brix % between gilt and sow progeny (Table 2). Sow progeny weighed more at weaning and grew faster than gilt progeny (Table 2).

Table 2.
Comparison of progeny performance between gilts and sows represented as mean +/- SE.

Item	Gilts[1]	Sows	P Value
Weight @24hr (kg)	1.4 ± 0.02	1.4 ± 0.03	0.461
Weaning weight (kg)	5.5 ± 0.12	6.1 ± 0.09	0.001
ADG[2] (g/piglet/day)	214 ± 5.5	239 ± 4.8	0.001
Brix %	9.2 ± 0.13	9.4 ± 0.09	0.192

[1] Gilts = gilt progeny (n = 75) Sows = sow progeny (n = 119). Sow parities ranged from 2 to 5.
[2] ADG = average daily gain

Performance

There was a tendency for male progeny to grow faster than female progeny (P = 0.096) (Table 3). Progeny from gilts grew slower than progeny from sows (*P* =0.049) (Table 3).

Table 3.
Relationship between sex and parity group to piglet ADG using mean +/- SE.

Item	ADG[3] (g/piglet/day)	P Value
Sex		
M[1]	0.2 ± 0.01	0.096
F	0.2 ± 0.01	
Parity group		
Gilt[2]	0.2 ± 0.01	0.049
Sow	0.2 ± 0.01	

[1] M = male progeny (n= 102), F = female progeny (n = 92)
[2] Gilts = gilt progeny (n = 75) Sows = sow progeny (n = 119). Sow parities ranged from 2 to 5.
[3] ADG = average daily gain

The relationship between Brix % and ADG is represented in Figure 1A. There is a weak, positive, linear relationship (R = 0.16, P = 0.051) between Brix % and ADG. The relationship between weight at 24 hours and ADG is represented in Figure 1B. There is a very weak, positive, linear relationship (R = 0.08, P = 0.556) between weight at 24 hours and ADG.

Figure 1A.
Relationship between Brix % and average daily gain (**ADG**) (g/piglet/day).

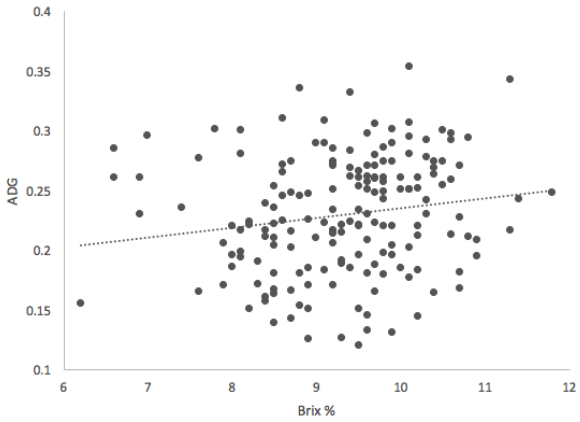
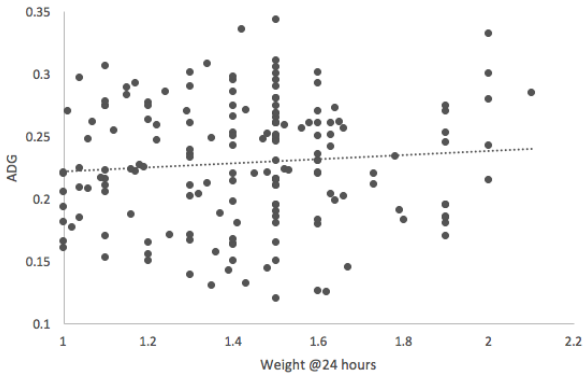


Figure 1B.
Relationship between weight at 24 hours and average daily gain (**ADG**) (g/piglet/day).



Brix %

There was no relationship between piglet sex and Brix percentage (*P* = 0.897) (Table 4). There was no relationship between parity group and Brix percentage (*P* = 0.467) (Table 4).

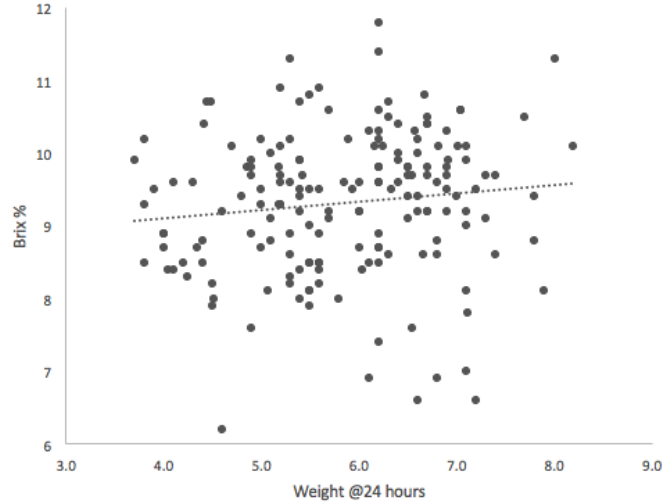
Table 4.
Relationship between sex and parity group to Brix % using mean +/- SE.

Item	Brix %	P Value
Sex		
M[1]	9.3 ± .10	0.897
F	9.3 ± .10	
Parity group		
Gilt[2]	9.2 ± .13	0.467
Sow	9.4 ± .09	

[1] M = male progeny (n= 102), F = female progeny (n = 92)
[2] Gilts = gilt progeny (n = 75) Sows = sow progeny (n = 119). Sow parities ranged from 2 to 5.

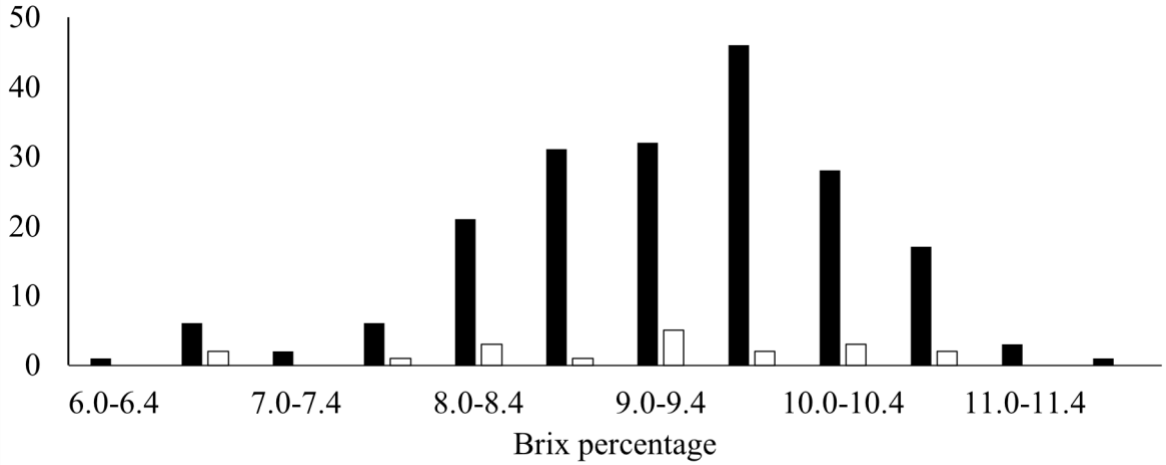
The relationship between weight at 24 hours and Brix % is represented in Figure 2. There is a very weak, positive, linear relationship (R = 0.07, P = 0.722) between weight at 24 hours and Brix % (Figure 2.).

Figure 2.
Relationship between weight at 24 hours and Brix %.



The pre-weaning mortality rate was 9.79%. Ninety-four percent of the piglets had a Brix % ≥ 7.9 which is the cut-off for adequate transfer of immunity determined by Schoos et al., (2021) and the average Brix % was 9.2 (± 1.15) (Figure 3.).

Figure 3.
Histogram for Brix percentage and piglet mortalities. Black = total piglets, white = total mortalities.



Logistic regression

There was no effect of Brix as a continuous variable or at a dichotomous transformation of 7.9% (on weaning mortality (P >0.05). There was also no effect of sex or weight at 24 hours on weaning mortality (P > 0.05).

Discussion

In this study, the Brix refractometer was not a reliable predictor of piglet survivability. However, most of the piglets had adequate transfer of passive immunity (Brix % ≥ 7.9, >30 g/L gamma-globulins) as suggested by Schoos et al. (2021). In this case the Brix % is the percentage of solids in the piglet's plasma which approximates their gamma-globulin levels (3, 6, 18). Schoos et al., (2021) developed these gamma-globulin cut-

off concentrations based on studies by Devillers et al. (2011), Cabrera et al. (2012) and Hendrix et al. (1978) (Table 1.). The ROC curves were also created in this study to determine the best combination of Sn and Sp for Brix % (Table 1.). The method of determining these cut-off concentrations from multiple studies was advantageous as it increased the sample size and showed its applicability across different farms, breeds, and countries. However, there are some disadvantages to this approach as different collection time points of the piglet serum (Ig concentration peaks at 12-16 hours post-partum then starts declining) makes it difficult to compare results between the studies. Lastly, the relationship between these thresholds and FPT and clinical outcomes was not investigated in this study. In this present study, the Brix % was not a good predictor for piglet survivability which was most likely due to adequate transfer of passive immunity as the litter size of the experimental sows at this farm was low, 12.4 +/- 2.1 (mean ± SD). In Denmark, and other countries around the world that use more prolific genetics, the average sow has 19.4 total born piglets/litter and this is predicted to increase as well (20). Brix % may still be a valid predictor for piglet survivability on farms that have problems with adequate colostrum transfer or quality and higher litter sizes.

Brix % could not be used to predict piglet survivability in this study, but Urie et al. (2018) demonstrated a dose-dependent decrease in morbidity and mortality in dairy calves with a higher Brix score (Brix %) using Kaplan-Meier survival curves (21). Piglet survivability is highly multifactorial and even though colostrum plays a large role, other piglet, sow, and environmental factors should also be considered (1). The main piglet factors affecting PWM are birth weight and vitality (1). Piglets with lower birth weights

are prone to hypothermia (smaller piglet = higher surface area-volume ratio) and often have poor energy reserves (smaller piglet = lower body-mass index). Both of these consequences will hinder the piglet's ability/time to suckle (1, 7), increase risk of crushing (22); and low body weight alone already makes them less competitive for a teat than their heavier littermates (1). Piglet vitality or vigour is also very important as it contributes to their ability to compete for a teat, colostrum intake, and odds of crushing (1). Piglets can be assigned a vitality score directly after birth which is based on heart rate, muscle tone, onset of respiration, and attempts to stand. A poor score is usually related to intra-partum hypoxia which can be caused by birth order, posterior presentation at birth, and long farrowing duration (>30 minutes) (1). Congenital malformations such as splay-leg will also result in poor neonatal vitality (1).

Other than colostrum, the other sow factors that affect PWM are litter size, nutrition, and maternal stress (1). Litter sizes have been increasing due the industry taking advantage of the naturally high rate of fertility of sows to create hyperprolific sows (7, 22, 23). The price of the increased economy and efficiency are lower birth weights and increased complications during farrowing which negatively impacts PWM in addition to an increased risk of FPT (discussed above) (1). Sow nutrition also contributes to PWM as it affects fetal development, piglet BW (body weight) at birth, colostrum yield and composition (1). To reduce PWM, gestation diets should be balanced and not restricted (especially protein and amino acid profile) but not overindulgent either as it negatively affects mammogenesis (1). A diet high in dietary fiber during gestation may also improve colostrum intake in the piglets (24). Lastly, maternal stress impacts PWM as it negatively impacts colostrum production and can cause aggression leading to an

increase in crushing (1). This stress is often caused by housing (type, size, hygiene), environment (temperature, humidity), and management practices (farrowing supervision) (1). The difficulty with providing the ideal environment for the sow is that this is not often the ideal environment for the piglets. Sows are prone to heat stress and require an atmospheric temperature between 18 C - 20 C, whereas piglets require a temperature of at least 34 C until they can thermoregulate on their own (1). As in sows, stressors should also be limited in piglets as they are associated with an increase in PWM due to a decrease in resistance to pathogens (22). Most of these stressors are related to environmental conditions such as hygiene, housing, and ambient temperature (1).

The Brix refractometer was a poor predictor for weight at 24 hours and ADG as indicated by a very weak positive correlation. Even though in this study the Brix refractometer was not able to predict some production measures, it is used with success in other areas of the pig industry. It is used to determine IgG levels in sow colostrum which can help determine if her piglets will achieve adequate transfer of passive immunity, assuming they consumed at least 200 g (6). Hasan et al. (2016) categorised colostrum quality based on IgG content as poor, borderline, adequate or very good, which allows producers to determine if any interventions need to occur, such as fostering, to ensure the piglets receive passive immunity. Hasan et al. (2016) developed this classification for Brix results of colostrum IgG quality (at 0-3 hours) based on a similar proposal by Cash (1999) for equine colostrum. Colostrum IgG peaks shortly after farrowing at approximately 64 mg/mL and will decrease to approximately 10 mg/mL at the end of colostrogenesis (6). The cut-off points for each category were

based around these values and were further developed using a summary from 12 studies by Hurley (2015). The 50 mg/mL was chosen as the cut-off point for “adequate” at early colostrogenesis (0-3 hours) as it is at the lower range of the peak IgG expected at this time. “Poor” was classified as <14.5 mg/mL as this is similar to the IgG expected at the end of colostrogenesis which is unexpectedly low for this time. “Borderline” was classified as 43.8 mg/mL which is slightly less than the average IgG content expected at this time. “Very good” is >78.6 mg/mL which is higher than the expected averages.

The main limitation with this colostrum categorisation is how highly variable colostrum quality is due to the multitude of factors that affect it (sow nutrition (1, 7), maternal stress (2), parity (2), longer farrowing (1, 2, 7)). Lastly, colostrum IgG and piglet serum IgG are positively correlated so it is important to know if the sow has adequate colostrum IgG, but if the pig’s ability to nurse is affected (low BW at birth, poor vitality), the piglet will still be at risk of FPT regardless of the sow’s colostrum quality. There are limited studies on the relationship between piglet serum IgG or Brix % and survival but a few studies found that piglets who died before weaning had lower serum IgG than their littermates (7, 8). There was also one study that investigated the long term (up to 6 weeks of age) effects of colostrum intake on production measures such as growth (8). It was found that piglets that consumed >290 g of colostrum gained approximately 2 kg more live weight by 6 weeks of age than those that consumed less. This study also showed a positive correlation between pre-weaning growth and plasma IgG concentrations at weaning but it is unclear if the increase in growth was due to the passive immunity acquired from the IgG or if the IgG was just a marker for higher colostrum intake (8).

Overall, the progeny from sows performed better than progeny from gilts, except in weight at 24 hours which was the same for both. In sows, there were more born alive, the piglets weighed more at weaning, and they grew faster. The increased performance of the sow's progeny over the gilts may be due to higher concentrations of other components of colostrum such as fat, protein, carbohydrate, vitamins, and minerals as the current study did not find a significant difference in Brix % (marker for Ig content) between parity groups. Colostrum yield may also play a role in these differences but the results are contradictory. Hasan et al. (2019) did not find a relationship between parity and colostrum yield, but Devillers et al. (2007) found 2nd and 3rd parities to have a higher colostrum yield and Decaluwe et al. (2013) found 1st and 3rd parities to have a higher colostrum yield. These different results can most likely be attributed to the fact that colostrum yield is multifactorial which is affected by variables such as nutrition (1, 7), stress (such as from longer farrowing) (2), litter size, and the environment (2).

A limitation to the present study was that it was performed on an Australian gilt multiplier farm which has a high health status. The pre-weaning mortality rate for the selected farm is historically low, at approximately 4%. In the current study, 9.79% of the experiment piglets were counted as mortalities, but this also included piglets that were not fit for weaning. Furthermore, 94% of the piglets had a Brix % ≥ 7.9 suggesting an adequate transfer of passive immunity. Studies that validate Brix % values and survival in the pre-weaning period are needed, but they need to be done on commercial farms where there is a higher rate of FPT. In addition to a low mortality rate and FPT rate, a significant portion of samples (14.1%) had to be excluded from the study due

to lack of enough blood to obtain a reading on the Brix refractometer. This puts into question the accuracy and practicality of the Brix method in a large, busy farrowing house in a commercial setting. The difficulty collecting blood from these piglets was most likely due to their young age, weight, and method of collection of blood. Tail docking wound was chosen over venepuncture to reduce double handling and stress on the piglets. However, some of the piglets bled very little and some not at all from the tail docking wound. To minimise the time spent holding the piglets, effort was made to collect blood from the tail side of the docking wound. However, this approach yielded samples with low PCV and Brix % indicating sample dilution, possibly from intracellular or interstitial fluid released from tissue trauma. Another limitation was the limited amount of records kept during the lactation period. Only mortalities were recorded instead of the reason for mortality or treatment records. A comprehensive monitoring sheet was developed for this study but the farm did not have sufficient staffing available to complete it during the trial period.

Conclusion

In conclusion, the Brix refractometer was not a reliable predictor of piglet mortality when using serum approximately 24 hours postpartum. However, there is a weak positive relationship between Brix % and ADG as well as weight at 24 hours which suggests Brix % can be used to help farm staff predict which piglets are more likely to thrive. Future studies will be required to confirm this as well as determine the accuracy of Brix % when used on farms with higher litter sizes and problems with adequate transfer of passive immunity.

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