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ANIMAL PRODUCTION

Performance of piglets according to colostrum intake and serum immunoglobulin concentration determined by the immunocrit method

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ABSTRACT: *Colostrum is the main source of immunoglobulins for piglets and several studies have shown that colostrum intake by piglets have a significant influence on their immunity as well as their future performance. The aim of this study was to ascertain the correlation between colostrum intake in the first 24 hours and the serum immunoglobulin concentration determined by immunocrit (IR), determine if the birth order of piglets interfered in colostrum intake and the IR, and measure their weight gain up to 42 days of age. One hundred and three piglets were included in the study and divided into two groups according to colostrum intake in the first 24 hours: ≥230 g and <230 g. Piglets with an intake of ≥230 g colostrum at birth had a mean weight of 1.412 ± 0.156 kg (mean ± SEM) while animals with a colostrum intake <230 g weighed 1.317 ± 0.162 kg (P>0.05). The mean IR between colostrum intake groups was 0.075 (<230 g) and 0.096 (≥230 g) (p<0.05). The IR differed between the order of birth of the piglets; where piglets born up to ≤7 had an IR=0.096 while those born from >7 presented with an IR=0.079 (p<0.05). Piglets that consumed more colostrum (≥230 g) in the first 24 hours after birth had a greater immunocrit and greater weight gain from 7 to 42 days of age (p<0.05). We concluded that the order of birth does not affect the colostrum intake, but the IR has a negative correlation (CORR= -0.3101; p<0,05) with the order of birth. Piglets with intake of more than 230 g of colostrum show greater weight gain up to 42 days of age.*
Key words: *newborn, birth order, immunocrit, weight gain.*

Desempenho de leitões de acordo com o consumo de colostro e sua concentração sérica de imunoglobulinas determinada através da técnica de imunócrito

RESUMO: *O colostro é uma das principais fontes de imunoglobulinas para leitões neonatos e vários estudos têm demonstrado que a quantidade ingerida pelo leitão tem influência na sua condição imunológica, bem como no seu desempenho futuro. O trabalho teve como objetivo correlacionar o consumo de colostro com a concentração sérica de imunoglobulinas nas primeiras 24 horas, determinada pelo imunócrito (IR), determinar se a ordem de nascimento interfere no consumo de colostro e IR, e o desempenho de ganho de peso até os 42 dias de vida do leitão. Foram utilizados 103 leitões categorizados em dois grupos conforme consumo de colostro nas primeiras 24 horas de vida em ≥230g e <230g. Leitões que ingeriram ≥230g de colostro ao nascimento apresentaram peso médio de 1,412±0,156 kg (média±EPM), enquanto que animais que tiveram um consumo de colostro <230g o peso foi 1,317±0,162 kg (P>0,05). O IR médio entre os grupos de ingestão de colostro foi de 0,075 (<230g) e 0,096 (≥230g) de colostro (P<0,05). O IR diferiu entre os grupos de ordem de nascimento dos leitões, onde o grupo de leitões nascidos, até ≤7, apresentaram IR 0,096, enquanto os nascidos, a partir >7, apresentaram IR 0,079 (p<0,05). Leitões que consumiram mais colostro (≥230 g) nas primeiras 24 horas de vida tiveram um maior imunócrito e maior ganho de peso dos 7 aos 42 dias de vida (p<0,05). Conclui-se que a ordem de nascimento não prejudica o consumo de colostro, porém a taxa de IR possui uma correlação negativa (CORR= -0,3101; p<0,05) em relação à ordem de nascimento. Leitões que consomem uma quantidade superior a 230 g de colostro apresentam maior ganho de peso até os 42 dias de vida.*
Palavras chave: *neonatos, ordem de nascimento, imunócrito, ganho de peso.*

INTRODUCTION

Genetic improvement in pig breeding has allowed the development of hyperprolific females and with that, the birth of large litters, which make weight variation at birth constant (FERRARI et al., 2014). Thus, it is observed that some piglets do not consume enough colostrum to ensure their

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survival or are already born with low viability and low weight, which worsens their survival conditions (DEVILLERS et al., 2011).

Because piglets are born agammaglobulinemic (SALMON et al., 2009), they rely exclusively on colostrum intake to ensure adequate passive immunity through the absorption of immunoglobulins (Ig) from the mother (ROOKE & BLAND, 2002). In addition to being a source of IgG, IgA, and lymphocytes, colostrum plays a role in thermoregulation and intestinal development (QUESNEL, 2011). However, piglets need to consume 200 g to 250 g of colostrum (DEVILLERS et al., 2011; QUESNEL et al., 2012) to achieve a serum Ig concentration of 10 mg/mL after colostrum intake (NIELSEN et al., 2004). Thus, the determination of serum Ig concentration in piglets in the first 24 hours of life helps to estimate the quantity and quality of the ingested colostrum. The immunocrit ratio (IR) method is a simple, fast, and low-cost method used to indirectly estimate serum Ig concentration in piglets, and also evaluate the quality of colostrum intake (VALLET et al.; 2013).

Passive antibodies are an important source of immunity for piglets during the first four to six weeks of life, thus, ensuring an adequate intake of colostrum during the first 24 hours of life is essential (PETERS et al., 2016). According to LE DIVIDICH et al. (2005), the mortality of piglets in the early postnatal days is partly due to low colostrum intake and; consequently, low immunity, and nutrient intake.

The aim of the present study was to correlate the piglet colostrum intake with serum Ig concentration, determine if birth order affects colostrum intake and IR, and assess weight gain performance up to 42 days of life.

MATERIALS AND METHODS

The study was conducted at a piglet farm located in the extreme west of Santa Catarina (-27,141475, -53,705887) from June to September 2018. One hundred and three neonatal piglets from Camborough 25® (Agroceres PIC®) sows were used (15±4.08 piglets/sow), of birth orders from first to sixth.

During the gestation period, the sows received a diet based on corn and soybean meal (3.214 Mcal ME/kg^{-1}, 12.4% CP, and 0.60% digestible lysine), supplied once a day in an average amount of 2.2 kg until the moment of transfer to the maternity pen. During the lactation period, the sows were given a corn and soybean meal diet (3.300 Mcal EM kg^{-1}, 18.70% CP, and 1.10% digestible lysine), *ad libitum*, from the fourth day of lactation until weaning (21 days). The health program included vaccination for neonatal colibacillosis and progressive atrophic rhinitis at 90 days of gestation.

To estimate colostrum intake, the piglets were weighed on a precision scale (1 g) at birth (P0) and at 24 hours after birth (P1). During this period, they remained with their biological mothers and after that they were cross fostered with other sows. At 21 days of age, they were weaned and housed in the nursery in collective pens (1.85 × 2.50 m–4.62 m^2), at a density of 0.30 m^2/piglet. Nutritional management during the nursery phase was carried out manually with feed given in the form of meal *ad libitum*. The following three-phase nutritional protocol was used: Pre-initial I (3.617 Mcal EM kg^{-1}, 21.85% CP, 1.46% digestible lysine, and 18.00% lactose, 1.0 kg/animal), Pre-initial II (3.602 Mcal ME/kg^{-1}, 21.39% CP, 1.42% digestible lysine, and 12.80% lactose, 4.0 kg/animal), and Initial I (3.474 Mcal ME/kg^{-1}, 20.06% CP, and 1.30% digestible lysine, *ad libitum* until the end of the nursery period).

All piglets were weighed with a digital scale with precision of up to 1g at 7 days (P7), 14 days (P14), 21 days (P21) of life and with a digital scale with precision of up to 10 g at 28 days (P28), 35 days (P35), and 42 days (P42) of life. During the lactation and nursery periods, the piglets were handed over to the management established in the farm.

The colostrum intake of each piglet in the first 24 hours (P1) after birth was estimated according to an equation described by DEVILLERS et al. (2004): CI = -217.4 + 0.217 × t + 1861019 × BW/t + BWB × (54.80 - 1861019/t) × (0.9985 - 3.7×10^{-4}×tFS+ 6.1×10^{-7}× tFS2),where: CI = amount of colostrum consumed (g); *t* = time between birth and weighing (min); *tFS* = time between birth and first suckling (min); BW = current weight (kg); BWB = weight at birth (kg). However, DEVILLERS et al. (2007) makes an assumption in the formula, an interval of 30 minutes between birth and the first colostrum feed.

For the purpose of data analysis, after P1 the piglets were divided into two groups, according to colostrum intake: <230 g-piglets with low colostrum intake (intake below 230 g) and 230 g-piglets with high colostrum intake (intake equal to or above 230 g).

Definition of high and low colostrum intake was based on a mean value reported in literature, which is between 200 g and 250 g of colostrum in the first 24 hours of life (DEVILLERS et al. 2007; DEVILLERS et al. 2011; QUESNEL et al., 2012; FERRARI et al., 2014).

At P1, a blood sample was collected from all piglets through cranial vena cava puncture (Vacutainer®, Labor Import, São Paulo, Brazil) for the estimation of Ig concentration using the immunocrit method. The latter was standardized as an indirect measure of serum Ig concentration, with the reference values reported by VALLET et al. (2013).

Samples were transported to the laboratory where they were centrifuged (Presvac DCL-16-RV, Brazil) for 5 minutes at 8,000 RPM to obtain 1 mL of serum. A 50 µL aliquot of serum and 50 µL of ammonium sulfate 40% were added to an Eppendorf tube and the material was homogenized by vortex mixing. This mixture was subsequently transferred to microcapillary hematocrit tubes (7.5 mm). All samples were processed in duplicated and the capillary tubes were filled to ¾ of their capacity, with one of the capillary ends being heat sealed. The microcapillary samples were immediately centrifuged (Microspin Model 1000, Brazil) at 12,000 RPM for 5 minutes to sediment the precipitated proteins. A millimeter ruler was used to measure the volume of the microcapillaries. The IR was obtained by dividing the height of the precipitate by the height of the entire sample in the microcapillary.

The data of the variable weight were evaluated using a linear mixed model, with repeated measures over time, through the MIXED procedure (CODY, 2018) with a significance level of 5%, with sow used in the model as random effect, fitting the following statistical model: Yijklm = µ + GCCi + DIAj + (GCC*DIA)ij + MATRIZk+ e(i)l + εijklm, where Yijklm = observation (weight, in kg); µ = overall mean of all observations; GCCi = colostrum intake group (<230g and ≥230g); DIAj = weighing day; (GCC*DIA)ij = effect of the interaction colostrum intake group and weighing day; MATRIZ k = random effect of the sow; and (i) l = non-observable random error associated with each experimental unit, assuming NID (0, σ_e^2), εijklm = non-observable random error associated with each observation, where~ NID (0, σ_ε^2).

Simple correlation analysis was performed between the variables and simple linear regressionwas subsequently applied to analyze: group < 230g and group ≥ 230 g colostrum intake, piglet immunocrit, birth order, colostrum intake, and weight gain from 24 hours to 42 days of age. For the analyses, the MIXED, CORR, and REG procedures of the SAS University Edition statistical software (CODY, 2018) were used.

RESULTS AND DISCUSSION

The mean weight of piglets at birth was 1.338±0.361 kg (0.728–2.182 kg). At 24 hours after birth, the piglets were weighed again to estimate colostrum intake according to DEVILERS et al. (2004). Fifty-five (53.4%) piglets with a colostrum intake ≥230 g had a mean weight of 1.574±0.156 kg and 48 (46.6%) piglets with a colostrum intake <230 ghad a mean weight of 1.271±0.163 kg. Weight gain performance of the two groups of colostrum intake over the 42 days of life is shown in table 1.

The mean number of piglets born per parturition was 15.0±4.08 (8-21 piglets) and the mean duration of parturition was 3 hours and 44 minutes (1 hour and 10 minutes to 7 hours and 20 minutes).

The mean weight at birth of piglets did not influence colostrum intake (CORR = -0.0828; P>0.05) (Figure 1). These results are contradictory to those of DEVILLERS et al. (2007) and QUESNEL et al. (2011) who observed that heavier piglets at birth consumed more colostrum. Therefore, it is important to evaluate the piglet's vitality in addition to its weight.

Table 1 - Weight of piglets (mean± SEM) at different ages according to the colostrum intake group (< 230g and ≥230g) (Tukey-Kramer).

Weight Day	------------------------------Colostrum Intake----------------------------		p
	≥ 230 g	<230 g	
0	1.412±0.156	1.317±0.162	0.4304
1	1.574±0.156	1.271±0.163	0.0989
7	2.780±0.159	2.401±0.166	0.0450
14	4.450±0.161	3.874±0.169	0.0029
21	5.673±0.166	5.069±0.170	0.0024
28	5.900±0.167	5.411±0.170	0.0143
35	7.495±0.170	6.621±0.174	<0.0001
42	9.205±0.170	8.145±0.176	<0.0001

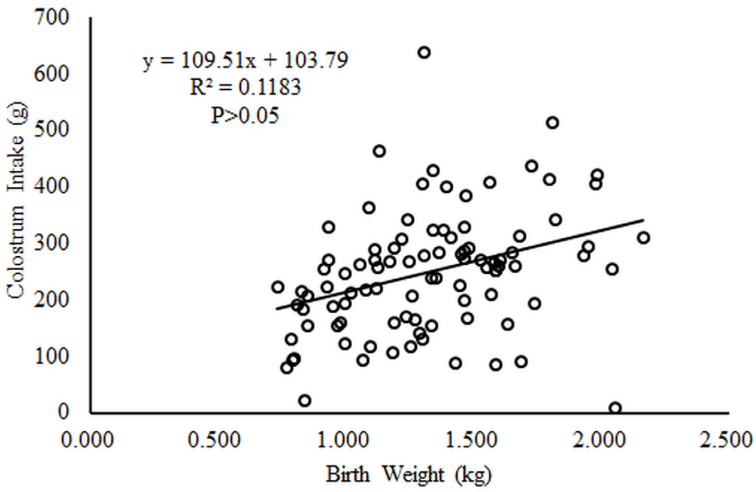

Figure 1 - Colostrum intake in the first 24 hours of life in relation to birth weight (CORR = -0.0828; P>0.05).

Active piglets with good vitality can stimulate the mammary complex and consume an adequate amount of colostrum (QUESNEL, 2011). Similarly, a positive correlation (CORR = 0.9846; p<0.05) was observed between colostrum intake and the mean weight gain of piglets in the first 24 hours of life, 0.112±0.102 kg (≥230 g) and -0.028±0.95 kg (<230 g).

In an experiment performed by FERRARI et al.(2014), it was observed that piglets weighing between 1.1 to 1.7 kg had a large variation in colostrum intake, ranging from 0 g to 547.7 g/piglet, with a low correlation between piglet weight gain and colostrum intake in the first 24 hours of life. Similarly, colostrum intake varies considerably between piglets (30% between litters and 15%–110% within-litter) (LE DIVIDICH et al*.,* 2005). Therefore, piglet vitality at birth, and not necessarily its weight, determines its ability to ingest colostrum (QUESNEL, 2011).

To evaluate the effect of piglet birth order on colostrum intake and IR, the piglets were categorized into two groups according to birth order in the litter (≤7 and >7). There was no effect of birth order on colostrum intake (P>0.05). Similarly, DEVILLERS et al. (2007) reported that piglet birth order did not influence the volume of consumed colostrum, which indicated that piglets born near the end of the parturition are not at a disadvantage compared to piglets born at the beginning of the parturition with regard to colostrum intake. However, the quality of the consumed colostrum may be a limiting factor because the IR was different (CORR = -0.3101; p<0.05) between the group with birth orders first to 7th and the others (Figure 2). This was demonstrated by the regression equation with a coefficient of determination of 0.6944 (p<0.05).

SILVEIRA et al. (2015) showed that piglets born after the 9th piglet were more susceptible to death, representing 71.41% of total pre-weaning mortality. Of these deaths, 64.27% were attributed to weakness, starvation, and underdevelopment. Piglets born later during parturition consume colostrum with lower Ig concentration (ROOKE & BLAND,2002; BLAND et al., 2003; DEVILLERS et al., 2011) making them more prone to mortality because they have lower passive immunity. In the present study, the concentration of Ig in colostrum over time was not evaluated, so it is inferred from the results that there may be a relationship between colostrum quality and the time of its' consumption.

According to DEVILLERS et al. (2004), colostrum is produced between 12 and 48 hours after the onset of labor. However, there is a significant change in its composition in the first 12 hours and the duration of labor may affect colostrum quality with regard to Ig concentration (KLOBASA et al., 1987).

Results obtained in the present study are consistent with those reported by PETERS et al. (2016), showing a negative effect of piglet birth order on IR

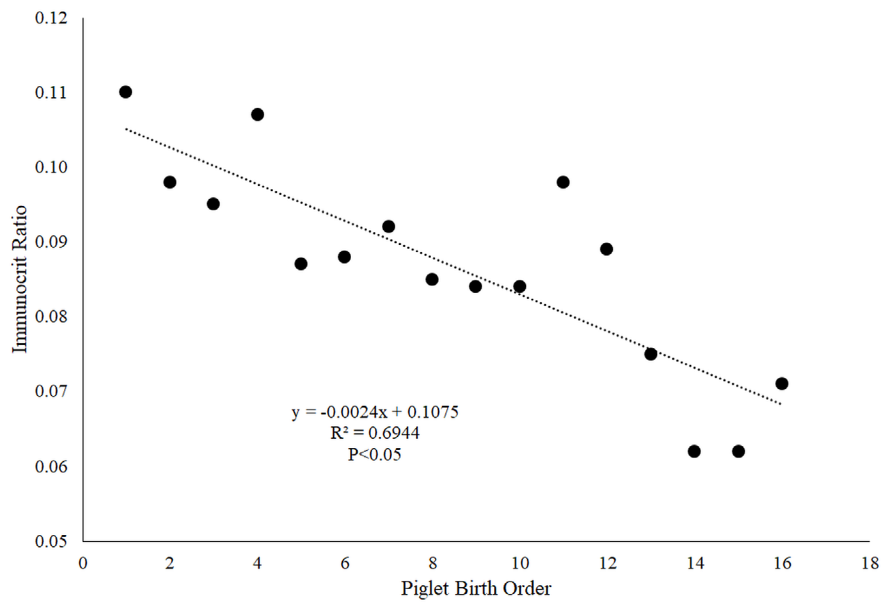

Figure 2 - Mean immunocrit ratio of piglets 24 hours after birth in relation to the birth order within the litter (CORR = -0.3101; p<0.05).

(CORR = -0.3101, P<0.05) (Figure 2). This is in line with the observation that Ig concentrations were lower in piglets whose access to the udder was delayed after delivery (LE DIVIDICH et al., 2004). This data indicated that there is a drop in colostrum quality over time and that even piglets that consume more than 200 g of colostrum may have a lower IR than piglets that consume colostrum at the beginning of parturition.

The evaluation of the IR of piglets considering the time of birth (minutes after the onset of parturition) showed that from 200 minutes onward (Figure 3) the piglets had lower IR and this could be related to the delayed access to the udder as well as the available Ig levels in the colostrum. Therefore, piglets born in prolonged parturitions may be a risk group with regard to transfer of maternal immunity. According to DEVILLERS et al. (2011), piglets born after four or more hours of labor had an IR of 0.015 (5.1 mg/mL) to 0.043 (14.65 mg/mL), which indicates that piglets born from prolonged parturitions have a higher risk of ingesting colostrum with low IgG concentration, which impairs passive immunity. However, low colostrum intake can still provide adequate immunity to piglets, provided they consume colostrum immediately after birth (QUESNEL; FARMER and DEVILLERS, 2012).

Similarly, the dispute of piglets for access to the mammary complex is another risk factor that may compromise colostrum intake. One management alternative to minimize this may be split suckling, which reduces the competition between piglets within the same litter. Such strategy consists in dividing the litter into two halves, with one half having access to the udder while the other half is in the farrowing house, and alternating the groups (PETERS et al., 2016). According to ALONSO et al. (2012), the technique of split suckling resulted in an increase in serum IgG concentration (p<0.05) in piglets from primiparous sows (52.61 mg/mL) relative to piglets of birth orders from 2 to 4 (39.29 mg/mL) and 5 (≥38.63 mg/mL).

In the present study, the mean IR in the two groups of colostrum intake was 0.07538 (<230 g) and 0.09642 (≥230 g) (p<0.05). In a study by VALLET et al. (2013), an IR of 0.125 was obtained in situations of high survival of piglets in the lactation. PETERS et al. (2016) used a mean IR value of 0.098±0.026 as reference in a commercial production system. However, in the present study, a relationship between weight at birth and IR was not demonstrated (CORR = 0.0393; P>0.05) (Figure 4), because low-weight piglets also had high IRs. The data obtained

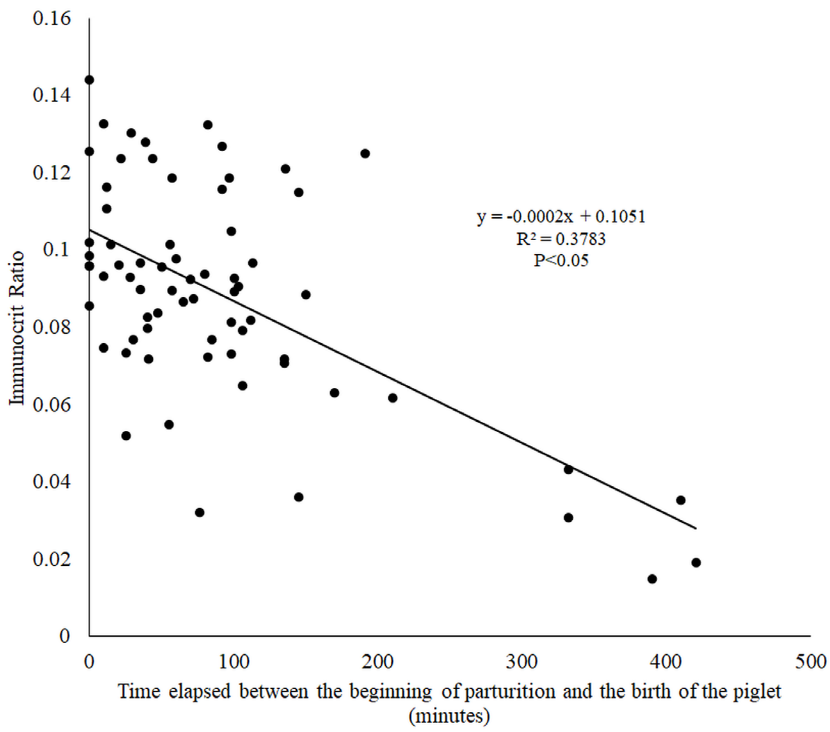

Figure 3 - Immunocrit ratio in relation to the time elapsed between the beginning of parturition and the birth of the piglet (CORR = -0.6166; p<0.05).

showed that weight at birth did not influence the piglets' ability to ingest colostrum. PETERS et al. (2016) reported that in 66% of the assessed production units the highest IRs were obtained in piglets with higher birth weights. According to LE DIVIDICH et al. (2005), heavier piglets at birth were more competitive at the time of feeding than lighter piglets from the same litter.

According to DEVILLERS et al. (2012), a higher IR in the group of piglets with higher colostrum intake may be associated with a higher colostrum concentration of Ig and; consequently, a higher colostrum quality. Similarly, QUESNEL et al. (2012) stated that a relatively low intake of colostrum provides immunity to piglets provided they consume colostrum immediately after birth.

DEVILLERS et al. (2011) stated that serum IgG levels in piglets at 24 hours of age are directly correlated with colostrum intake. However, 8.69% of piglets in the present study had low colostrum intake (73.46±154.45 mL) and a high IR (0.115±0.015), a fact that may be associated with early colostrum intake; i.e., immediately after birth and at the beginning of parturition (Figure 5). In addition, 15.9% of piglets had high colostrum intake (360.06±207.44 mL) and a low IR (0.064±0.014).

The IR differed between the two groups formed by order of birth ($p<0.05$), with the group of piglets of birth order from 1 to 7 having a mean IR of 0.096±0.023 and piglets born after the 7th piglet had a mean IR of 0.080±0.032 ($P<0.05$). These results corroborated the findings of the study conducted by LE DIVIDICH et al. (2004) in which IgG concentrations of the first two and last two piglets born from 15 deliveries with a total mean number of 13±3.6 piglets were determined at 48 hours of age and it was observed that the first born piglets had IgG levels 51% higher ($P<0.01$) than those of the piglets that were born last.

Simple linear regression analysis was used for the variable weight of the piglet from birth to 42 days of life. For the group categorized as having a colostrum intake <230 g, the equation for the slope was: $y=1.2489+0.1571x$ (r^2=0.8647; $P<0.05$). For the ≥230 g colostrum intake group, the following equation was obtained: $y=1.527+0.1767x$ (r^2=0.8623;

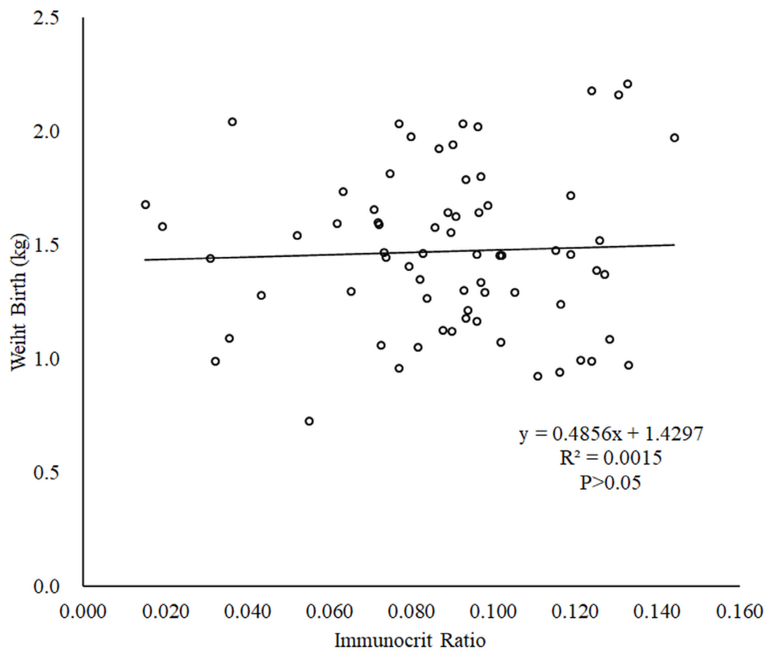

Figure 4 - Relationship between immunocrit ratios and birth weight in 24-hour-old piglets (CORR = 0.0393; P>0.05).

P<0.05). Piglets that consumed more colostrum in the first 24 hours of life had a greater weight from day 7 (P<0.05) and this difference was observed until day 42 (Figure 6). The data indicated that the amount of consumed colostrum is related to improved piglet performance throughout its development. The effect of the adoptive sow after the piglets were cross-fostered on their weight at 21

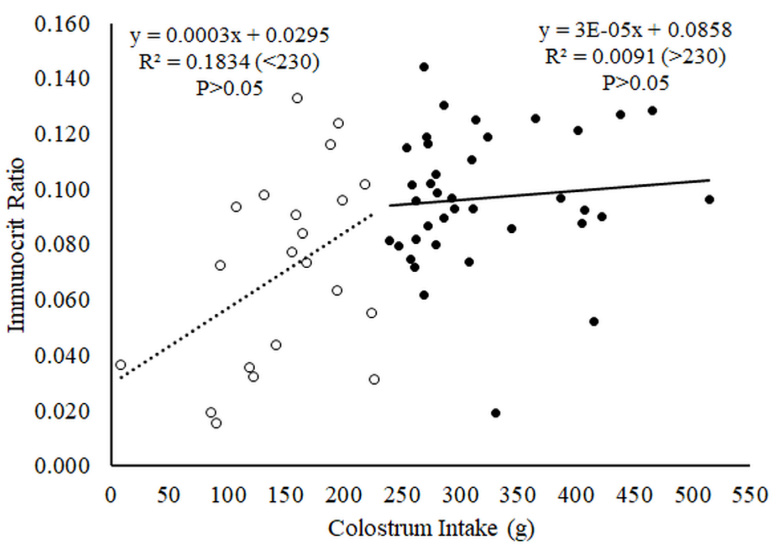

Figure 5 - Immunocrit of piglets at 24 hours after birth in relation to colostrum intake by piglets in the high intake category (≥230g). The white dots indicate piglets that consumed < 230 g of colostrum (CORR = -0.1113; P>0.05). The black dots indicate piglets that consumed ≥230 g of colostrum (CORR= -0.0888; P>0.05).

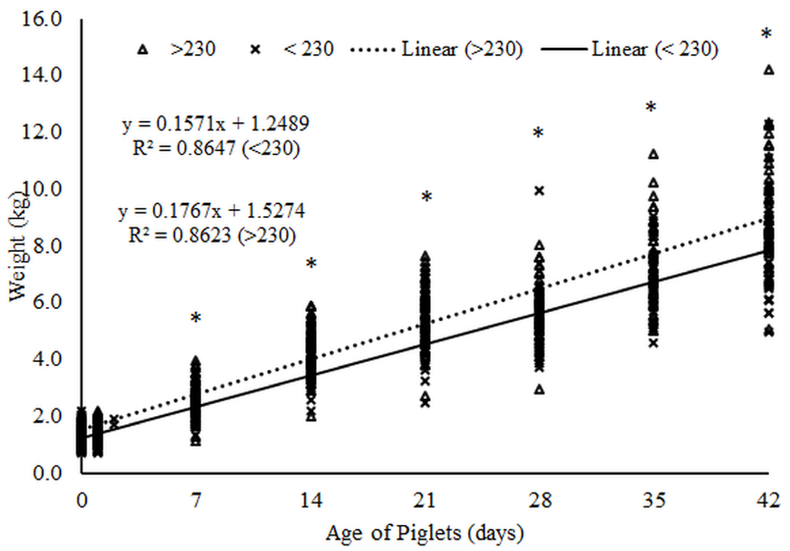

Figure 6 - Weekly weight of piglets from birth to 42 days of life in the high (≥230g) and low (<230g) colostrum intake categories. * indicates difference (p<0.05) according to the Tukey-Kramer test. The solid line and X indicate the group of piglets with colostrum intake <230 g, the dotted line and △indicate the group of piglets with colostrum intake ≥230 g.

days was not evident (P>0.05). This is possibly due to the criteria used in this particular management, in which the number of piglets in each sow was determined by the number of viable teats, which allowed the piglets having full access to the udder during the lactation phase.

DEVILLERS et al. (2011) evaluated the long-term effect of colostrum intake. At 21 days, the piglets were divided into three groups according to colostrum intake (<290 g; 290 to 440 g and >440 g). The group that ingested between 290 gand 440 g of colostrum had a higher weight at 42 days of life (12.34±0.13 kg) (P<0.05) than the group that consumed less than 290 g of colostrum (10.45±0.17 kg).

Similar results were obtained by FERRARI et al. (2014) when colostrum intake was higher than 250 g (P<0.05), thereby corroborating the findings of QUESNEL et al. *(*2012) in which an amount of 250 g of colostrum was essential for good performance. In contrast, studies by MOREIRA et al. (2017) evaluating the performance of piglets that ingested 120 mL and 200 mL of colostrum with or without energy supplementation showed that there was no improvement in weight gain between the groups (P>0.05) until the weaning period. These results obtained by other authors may be due to additional factors that may influence lactation performance, such as milk production, health factors, management, and nutrition (LE DIVIDICH et al., 2005; QUESNEL et al*.,* 2012).

CONCLUSION

The ability of piglets to ingest colostrum was not impaired by order of birth, but the same was not true with regard to the IR, which indicated that birth order, 8th and above consumed lower quality colostrum. Piglets that consumed ≥230g of colostrum had a higher concentration of Ig, as determined by the immunocrit method, and a higher weight at 42 days of age, compared to the group that consumed <230 g.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The methodology used in this study was approved by the Ethics Committee on the Use of Animals of the Universidade do Oeste de Santa Catarina (CEUA-UNOESC) under protocol number 52-2018.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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AUTHORS' CONTRIBUTIONS

PEB, TRBK, GK and AMP conceived and designed experiments, carried out the descriptive analyses, prepared the draft of the manuscript and approved of the final version. ADK, AB, AB and CAG prepared the draft and critically revised the manuscript.

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