**Original Article**

# The relationship between colostrum quality, passive transfer of immunity and birth and weaning weight in neonatal calves

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**Abstract**

Calves are born almost agammaglobulinemic and thus need an adequate amount of good quality colostrum to avoid failure of ~~passive~~ transfer of passive immunity. The quality of colostrum is influenced by several factors such as the volume of colostrum produced, collection time, the concentration of immunoglobulins, breed, the age of dam, and mastitis events in the previous lactation. In this study, we evaluated the relationship between colostrum quality, serum total protein (TP), birth, and weaning weight of the calf.

Seventy-three calves born in the same farm were included in the study. Three liters of colostrum were administered to the calves as soon as the calf could drink while the other 3 L was administered 8 hours later. Immediately after birth, and at 60 days of age, each calf was weighed. Colostrum was evaluated after calving by a qualified operator using an optical Brix refractometer. Twenty-four hours after birth, 10 mL of blood was collected from each calf in order to evaluate the ~~absorption of immunoglobulins~~ serum TP using a digital refractometer. The relationship between colostrum quality, serum TP, birth and weaning weight was analyzed using a mixed linear model.

Colostrum quality increased with parity; serum TP increased in association with an increase in the Brix percentage ~~quality~~ of the colostrum administered to the calves. Data indicated that heavier calves had a lower TP at 24 hours of age than lighter calves under the same colostrum transfer protocol of 6 liters of quality colostrum in 12 hours. Serum TP (g/dL) decreased continuously in all calves from 8.267 by 0.032 x kg of birth weight.

The birth weight of the calves could influence the quantity of colostrum necessary to achieve the same level of TP, so heavy calves should be fed more. Further studies would be necessary in order to evaluate the mechanism of IgG absorption in the gut of calves with different body weight. ~~In addition, the volume of heavy calves is likely the reason for this difference. Consequently, in order to achieve the same level of mass action passive activity, heavy calves should be fed more.~~

*Keywords:* Calf, Colostral Immunity, Serum Total Protein, Calf Weight

## Introduction

The ~~syndesmochorial~~ synepitheliochorial placenta of cows does not allow the transfer of immunoglobulins from the dam to the calf during gestation ~~throughout the intrauterine life~~, so calves are essentially agammaglobulinemic when they are born (Wooding, 1992; Weaver et al., 2000). ~~A good colostrum management and a~~ Prompt colostrum administration helps protect calves from diseases during the neonatal period (McGrath et al., 2016). Colostrum contains a wide spectrum of important immune and nutritional components, and Immunoglobulins G isotype (IgG) represent more than 85% of total Immunoglobulin (Ig) in colostrum. Usually, colostrum quality refers to the quantity of IgG present in the first milk (Godden, 2008). ~~The colostrum quality is influenced by the volume of colostrum produced, the collection time, the concentration of immunoglobulins, bacteria levels, breed and age of dam, nutrition in the preparturient period, season of calving, mastitis events in the previous lactation, preparturient vaccination of the dam and dry period length (Godden, 2008).~~ The administration of high-quality colostrum reduces preweaning morbidity (Donovan et al., 1998), mortality (Robison et al., 1988) and economic losses (Dewell et al., 2006). It also stimulates the calves to grow and increases milk production and longevity in dairy cows (Godden, 2008; Atkinson et al., 2017).

The main important risk factors for failure of ~~passive~~ transfer of passive (FPT) ~~of~~ immunity in calves include feeding calves with poor quality colostrum or with an inadequate volume of colostrum in the first 24 h of life, as well as feeding calves with colostrum contaminated by bacteria (Johnson et al., 2007; Godden, 2008). A simple method to estimate the quantity of IgG in the colostrum is to use a refractometer, and the break point for a high-quality colostrum is 21% Brix (Quigley et al., 2013). ~~The serum total protein (TP) was assessed by a refractometer for field FPT diagnosis in calves, with a test end point >5.5 g/dL (Weaver et al., 2000). The administration of high-quality colostrum reduces preweaning morbidity (Donovan et al., 1998), mortality (Robison et al., 1988) and economic losses (Dewell et al., 2006). It also stimulates the calves to grow, and increases milk production and longevity in dairy cows (Godden, 2008; Atkinson et al., 2017).~~

On-farm protocols usually recommend that an appropriate absorption of IgG in calves can be achieved by administering a minimum of 4 L of high quality colostrum, defined as a colostrum with a concentration of >50g of IgG/L (Godden, 2008). The evaluation of serum total protein (TP) at least 24-hours after the first colostrum intake is considered an indirect measure of serum IgG concentration (Weaver et al., 2000). Serum TP was generally assessed on farm by using a refractometer. In calves, values of TP lower than 5.5 g/dL are associated with FPT diagnosis (Weaver et al., 2000). ~~However,~~ In foals and piglets, the optimal amount of colostrum to be administrated in order to achieve a proper transfer of passive immunity has been assessed according to the body weight of the newborn (Giguère and Polkes, 2005; Devillers et al., 2011). Currently, in calves there are ~~currently~~ no standardized protocols concerning the amount and timing of colostrum administration during the first 24h after the birth, related to the calf birth weight. ~~Although wrong timing and quality of colostrum administration are recognized as the main causes of FPT, other factors could represent a risk for FPT in calves. Stress during birth such as dystocia, along with the environment and individual handling of the animals can negatively affect the dam and her offspring and may lead to a decrease in Ig within colostrum secreted by the dam and absorbed by her calf (Godden, 2008; Murray and Leslie, 2013).~~ Gaspers and colleagues (2014) suggested a negative correlation between serum IgG and birth weight in beef calves, supposing that heavier calves may have less IgG concentration than lighter calves within the first 24 hours after birth. A better understanding of the correlation between colostrum and birth weight in Holstein and crossbred calves may be useful in order to set a standardized protocol for artificial reared calves. The aim of the present study was to evaluate the relationship between colostrum quality, serum TP (24 h after the administration of colostrum), birth weight and weaning weight of the calf.

## Materials and Methods

The present study was approved by the Institutional Animal Care and Use Committee (OPBA, Pisa, prot. n. 0023045/2018) and was conducted at a dairy farm “Centro di Ricerche Agro-Ambientali E. Avanzi” (CIRAA) owned by the University of Pisa, where nearly 120 animals were maintained in free-stall conditions. All the procedures were in compliance with the 2010/63/EU directive regarding the protection of animals in scientific experiments.

Inclusion criteria were no assistance at ~~easy~~ calving and the complete ingestion ~~assumption~~ of 6 L of dam colostrum in the first 24 h of life (Godden, 2008). During the study period (January 2018-January 2019), the population of calves was made up of a total of 79 animals, all the same age, born from 79 Italian Friesian cows. Seventy-three out of 79 calves (92.4%) met the inclusion criteria and were included in this study. Details concerning calves included in the present study are reported in table 1. ~~Forty-six/73 (63%) cows underwent artificial insemination (AI) with Italian Friesian semen, while 27/73 (37%) cows underwent AI with Limousine semen. This reproductive management was made in order to obtain rearing calves for the farm. It also meant that crossbred calves could be obtained which are in higher demand for the beef market. Thirty-~~

~~five/73 (48%) were Italian Friesian female calves, 11/73 (15%) Italian Friesian male calves, 17/73 (23%) were crossbred females, and 10/73 (14%) were crossbred males.~~ All the calves were reared under the same management protocol. ~~Immediately~~ Within 30 minutes after calving, the calf was removed from the dam, weighed with a scale (ID 3000, Tru-Test Limited, USA), and identified. Calves were then housed in a single straw-bedded pen (2.5x2 m) that allow contact between the calves, in accordance with European Legislation (2008/119/CE).

~~Colostrum was evaluated b~~ Between 30 minutes and 1.5 hours after calving, colostrum was collected from the four udder quarters by complete milking and a 10 mL sample was collected from the pool in a sterile milk tube. The sample was immediately evaluated by putting a drop of colostrum on the daylight plate of ~~using~~ an optical Brix refractometer (Atago brix N1, Japan) with a range of 0 to 32% Brix. ~~A total of 10 mL of colostrum was collected from the dam in a sterile milk probe, and immediately evaluated by putting a drop of colostrum on the Brix refractometer.~~ ~~The colostrum quality~~ Colostrum was classified as high quality if colostrum was higher than 21% Brix scale, according to the literature (Quigley et al., 2013). A total of 3 L of the dam's colostrum was administered with a nipple bucket as soon as the calf could drink (between 30 minutes and 2 hours after birth). Further 3 L were administered within the next eight hours (Godden, 2008). ~~Three days after calving, the dam was milked at 05.00 and 17.00.~~ Thus, a total of 6 L of thei~~r own~~ dam's ~~colostrum~~ transition milk was administered twice a day.

Twenty-four hours after birth, 10 mL of blood was collected from the jugular vein from each calf in order to assess serum TP as an indirect measure of serum IgG concentration. ~~the passive transfer of the immunoglobulins.~~ ~~Samples were collected by jugular venipuncture in red-top Vacutainer tubes (10-mL BD Vacutainer glass serum tube, silicone-coated; Becton Dickinson and Co., Franklin Lakes, NJ) and refrigerated until evaluation. All samples were processed within three hours of collection. The sample was centrifuged (Legend RT, Sorvall; ThermoFisher Scientific Inc., Waltham, MA) at 1.565 × g for 15 minutes in order to collect the serum.~~ Serum TP was measured using a ~~different~~ refractometer designed specifically for this purpose~~. In fact, a temperature-compensating digital refractometer~~ (AR200; Reichert Analytical Instruments, Reichert Inc., Depew, NY) ~~was used to assess the state of passive transfer in each calf~~ (Morrill et al., 2013; Deelen et al., 2014; Thornhill et al., 2015). ~~The FPT status~~ FPT was defined as a serum TP score below 5.5 g/dL (Weaver et al., 2000).

On the fourth day after birth, all calves received 3 L of whole milk at 39°C twice a day. From the third day after birth, fresh and clean water was provided to each calf *ad libitum*. Grass hay was available for all the calves after the first week of life. All the feeding procedures were conducted by a skilled operator who ~~also~~ used a nipple bucket. In case of diarrhea no change in feeding management was adopted~~. Thus,~~ but calves also received one tablet of Effydral® (Italy Zoetis Ltd.) ~~(sodium chloride 2.34 g, potassium chloride 1.12 g, sodium bicarbonate 6.72 g, citric acid anhydrous 3.84 g, lactose monohydrate 32.44 g, glycine 2.25 g)~~ in 2 L of warm water every 24h. The calves were moved to a ~~collective~~ group pen after the third week of life. A pellet starter feed was administered to the calves from the third week of life in order to gradually stimulate the rumen, along with the above-mentioned milk meal. ~~Each calf~~ The female Italian Friesian calves were ~~was~~ then weaned abruptly at 60 days of life.

Male Italian Friesian calves (n=11) and all the crossbred calves (n=27) were sold for fattening at 30 days of life. All the female Italian Friesian calves ~~(male and female)~~ were kept as replacement heifers, thus it was possible to weighed them again at weaning (n=34). ~~One/35 of the female Friesian calves died before weaning.~~

Data concerning parity, mastitis events in the previous lactation and the estimate production in the current lactation of each cow were recorded using software for dairy farm management (Afifarm™, Afimilk®, Israel).

The relationship between colostrum quality, as above defined (IgG concentration estimated by refractometer), and birth weight was analyzed using the following mixed linear model (SAS Institute, 1999):

$$y_{ijkzfq} = \mu + W_i + M_j + Pr_k + Pa_q + A_f + \varepsilon_{ijkzfq}$$

where

$y_{ijkzfq}$ = quality of colostrum (% Brix);

$W_i$ = linear effect of the i-th calf weight at birth (73 levels);

$M_j$ = fixed effect of the j-th mastitis events number in the previous lactation of the dam (0, 1, ≥2);

$Pr_k$ = fixed effect of the k-th milk production level (≤5000, 5000-7000, ≥7000 L per lactation);

$Pa_q$ = fixed effect of the q-th parity level (primiparous, secondiparous and pluriparous);

$A_f$ = random effect of the f-th cow (73 levels);

$\varepsilon_{ijkzwqf}$ = random residual

Interactions between fixed factors were tested and removed by the model because not significant.

Serum TP data of all enrolled calves were analyzed with the following mixed linear model (SAS Institute, 1999):

$y_{ijkzfq} = \mu + b1\ x_{ij} + b2\ z_{ij} + B_k + S_z + B_k \times S_z + A_f(B_k) + \varepsilon_{ijkzfq}$

where

$y_{ijkzfq}$ = serum TP (g/dL);

*b1* = covariate of calf weight (x) on the variable;

*b2* = covariate of % Brix colostrum level (z) on the variable;

$B_k$ = fixed effect of k-th breed (Holstein Friesian and Crossbreed);

$S_z$ = fixed effect of the z-th sex (male and female);

$A_f$ = random effect of the f-th calf (73 levels), nested within breed;

$\varepsilon_{ijkzwqf}$ = random residual

Data of serum TP of female Italian Friesian calves were analyzed using the following mixed linear model (SAS Institute, 1999):

$y_{ijk} = \mu + b1\ x_{ij} + b2\ z_{ij} + A_k + \varepsilon_{ijk}$

where

$y_{ijk}$ = serum TP (g/dL);

*b1* = covariate of calf weaning weight (x) on the variable;

*b2* = covariate of % Brix colostrum level (z) on the variable;

$A_f$ = random effect of the f-th calf (34 levels);

$\varepsilon_{ijkzwqf}$ = random residual

The results on the effects were presented as least squares means±standard errors. The linear contrasts were tested in the first model by the t-test with Tukey's adjustment within each parity level. Differences between means were declared significant at $P \leq 0.05$.

The correlation between weaning weight of female calves and TP serum concentration was also evaluated by correlation analysis (SAS Institute, 1999).

## Results

All the calves enrolled in the present study were included. One female calf died at 45 days of life due to severe pneumonia. Thus, only data concerning birth weight, colostrum quality and serum TP were included for this animal.

The average ~~quality~~ Brix percentage of colostrum administered to the calves was 24.8±0.03%~~ Brix~~, ranging from 18 to 31%. ~~Thus, no colostrum had a Brix score of less than 18%.~~ Four/73 (5.5%) cows showed a sufficient quality of colostrum (between 18% and 20% on the Brix scale), while 69/73 (94.5%) cows showed high quality colostrum (between 21% and 31% on the Brix scale) (Quigley et al., 2013). The average serum TP was 7±0.72 g/dL, with a range between 5 to 8.6 g/dL. Only 1/73 (1.4%) calves had serum TP less than 5.5 g/dL.

The overall mean birth weight was 40.68±6.66 Kg. For the Italian Friesian calves, the mean birth weight was 38.19±6.61 Kg and 40.68±6.69 Kg for female and males, respectively. The mean birth weight for crossbred calves was 40.56±6.77 Kg and 42.02±6.33 Kg, for female and males, respectively. The mean weaning weight, evaluated for the 34 Italian Friesian female calves, was 73.38±10.52 Kg.

Cows included in this study were 29/73 (40%) primiparous, 23/73 (31%) secondiparous, and 21/73 (29%) multiparous. The mean parity was 2.11±1.21. The number of episodes of mastitis in the previous lactation was 0 for 26/73 (35.6%) cows, 1 for 19/73 (26%) cows, 2 for 24/73 (32.9%) cows, and ≥3 for 4/73 (5.5%) cows. The production measured in the current lactation was 6255±1485 L for primiparous, 6434±1523 L for secondiparous, and 7297±1481 L for multiparous.

The Brix percentage of colostrum was significantly affected by the parity ($P<0.05$) (Figure 1), whereas the number of mastitis events in the previous lactation and the milk yield production did not significantly affect the Brix percentage of colostrum. Serum TP was significantly positively correlated with the colostrum quality expressed as Brix percentage value ($P<0.05$, $R^2=0.19$) (Figure 2), and to the birth weight of the calves ($P<0.01$, $R^2=0.24$) (Figure 3). Serum TP (g/dL) decreased continuously in all calves from 8.267 by

0.032*birth weight. Serum TP was not correlated with the weaning weight of the Holstein female calves (P=0.676; $R^2$ = 0.086).

**Discussion**

~~Despite the large number of studies in the literature, FPT still is still a significant worldwide management problem that may lead to increased mortality in calves (Godden, 2008; Beam et al., 2009; Raboisson et al., 2016; Atkinson et al., 2017). An evaluation of colostrum provides a simple first step in the prevention of FPT in calves and provides a mean to predict the risk of FPT. The estimation of serum TP is an excellent test for herd monitoring and is easily performed by practitioners. Appropriate serum TP levels in neonatal calves after colostrum administration is associated with improved health, increased weight gain, reduced risk of being culled, and increased milk production at the first lactation (Godden 2008; Furman-Fratczak et al. 2011).~~ The purpose of this study was to evaluate the relationship between weight of calves at birth and weaning, colostrum quality and serum TP level.

Digital and optical refractometers can be used to evaluate the IgG concentration of colostrum, that is considered a pivotal parameter of the colostrum quality (Bielmann et al., 2010; Morrill et al., 2012; Quigley et al., 2013). In the present study, the average Brix percentage of the colostrum produced was higher than the value (21% Brix) suggested by the literature as cut-off point for the assessment of the immunological quality of colostrum (Quigley et al., 2013). Thus, most of the cows included in the study produced high quality colostrum as regards the IgG concentration estimated by refractometric analysis. Only four cows produced a colostrum ~~slightly~~ below 21% Brix (ranging between 18-20% Brix). However, calves fed with this colostrum did not show FPT, and their serum TP was higher than 6 g/dL.

Several factors can influence the colostrum IgG content. In this study, colostrum IgG content expressed as Brix percentage increased with increasing parity. In the literature there are contrasting results about the effect of parity. IgG concentration of colostrum has been related to parity in Holstein Friesian cows (Tyler et al., 1999; Moore et al., 2005; Gulliksen et al., 2008), but not in crossbreed cows (Coleman et al., 2015; Hang et al., 2017). This inconsistency might be related to differences in the populations, genetics and management of the cows.

~~Several studies have reported a relationship between serum TP and IgG in calves (Weaver et al., 2000; Godden, 2008). Refractometry provides an approximation of the serum immunoglobulin concentration,~~

~~because immunoglobulins constitute a large proportion of the protein in neonatal calf serum (Calloway et al., 2002).~~

In the present study, serum TP in calves increased alongside the increase in the Brix value of the colostrum that was administered. This result is in line with previous studies performed in dairy calves (Jaster, 2005; Deelen et al., 2014), which showed that dairy producers can successfully monitor their colostrum management and improve the overall success of passive transfer using a Brix refractometer to estimate IgG concentration of colostrum and calf serum (Deelen et al., 2014).

Serum protein levels below 5.5 g/dL provide reasonable predictive values for FPT (Godden, 2008). In the present study, on average, serum TP concentrations were over the serum cut-off point for FPT diagnosis (Weaver et al., 2000). Only in one case, serum TPs were below the cut-off of FPT despite the high-quality colostrum received by the calf. Since all calves were born from easy calving, FPT caused by hypoxia or respiratory acidosis secondary to dystocia can be ruled out (Murray and Leslie, 2013).

No significant relationship between serum TP and the weaning weight of female calves were found in the present study. This result confirmed what reported by Furman-Fratczak et al. (2011) who found no significant differences in growth rate between calves fed colostrum with different IgG levels during the first six months of life.

Several studies have discussed the appropriate amount of colostrum to administer to calves (Weaver et al., 2000; Godden, 2008). It appears that achieving the same level of passive transfer in all calves is likely to be in the economic interest of the farmer. Yet, FPT is not the only problem that can occur in neonatal calves. High serum TP levels show high protection from other neonatal diseases such as pneumonia, diarrhea, etc. (Furman-Fratczak et al., 2011). Thus, achieving an optimal transfer of passive immunity in all newborn calves is the key point in a dairy farm.

Although all the calves included in this study presented a serum TP level over the cut-off point for FTP, serum TP concentrations were higher in calves with a lower birth weight compared to those with a higher birth weight. This result confirmed a previous study performed on beef calves (Angus breed), in which the authors found that heavier calves showed less IgG concentration ~~absorbed less IgG~~ than lighter calves within the first 24 hours after birth (Gaspers et al., 2014). To the best of our knowledge, there are no studies that have evaluated serum TP concentrations and birth weight in dairy and crossbred calves~~.~~ Since the amount of colostrum administered to the calves was the same, differences in the serum TP concentrations according to the birth weight could be related to differences in the colostrum requirements. In equines, the administration of a total of 1 to 2 L of colostrum high in IgG concentration is recommended for a 45 kg foal while in piglets the minimum colostrum intake of 200 g/d is recommended to achieve a good transfer of passive immunity (Giguère and Polkes, 2005; Devillers et al., 2011). The feeding requirements of newborn calves are dependent on their birth weight. Thus, to obtain a more uniform serum TP level (the simplest measure of passive transfer), the level of quality of colostrum delivered to the calf must be proportional to the birth weight. This could improve the on-farm protocol for the management of colostrum administration in the first 24h after calving.~~,~~ ~~and would thus reduce gastrointestinal or respiratory tract diseases during the pre-weaning phase~~.

## Conclusions

Heavier calves demonstrated lower TP at 24 hours of age than lighter calves under the same colostrum transfer protocol of 6 liters of quality colostrum in 12 hours. The birth weight of the calves could influence the quantity of colostrum necessary to achieve the same level of TP, so heavy calves should be fed more. Further studies are necessary in order to evaluate the mechanism of IgG absorption in the gut of calves with different body weight. ~~The volume of heavier calves is likely the reason for this difference. Consequently, to achieve the same level of mass action passive activity, heavy calves should be fed more. We plan to further investigate this aspect in the future.~~


## Conflict of interest

None of the authors have any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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**Figure legends**

Fig. 1. Colostrum quality evaluated by Brix scale in first, second and third or more parity cows. Legend: a,b significant difference ($P<0.05$).

Fig. 2. Effect of the colostrum quality covariate on total proteins ($P=0.042$). $R^2 = 0.19$.

Fig. 3. Effect of the birth weight covariate on total proteins ($P<0.01$). $R^2 = 0.24$.

Table 1. Description of 73 calves included.

| | Male | Female |
|---|---|---|
| Italian Friesian calves | 11/73 (15%) | 35/73 (48%) |
| Crossbreed calves (Italian Friesian x Limousine) | 10/73 (14%) | 17/73 (23%) |