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Absorption of colostral immunoglobulin G by Icelandic dairy calves

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ABSTRACT

Serum samples were taken from 11 Icelandic dairy calves, at birth and 24 hours later, and 11 colostrum samples were taken from their mothers with the concentration of IgG measured by ELISA. The amount of colostrum ingested by each calf was also measured. The results showed that the mean concentration of IgG in cow colostrum at birth was 11.54 mg ml^{-1}. The mean concentration in the serum of calves that had ingested known quantities of colostrum was 8.02 mg ml^{-1} 24 hours postpartum. Both values were lower than described in other cattle breeds. Significant correlation existed between the amount of colostral IgG consumed and serum concentration of IgG in the calves 24 hours postpartum. The mean apparent efficiency of absorption (AEA) was 43%, which is high compared to other studies. Although the study group was small, the results give an indication of the ability of Icelandic calves to absorb colostral IgG.

Keywords: Bovine colostrum, immunoglobulin G (IgG), passive transfer of immunity, apparent efficiency of absorption (AEA).

YFIRLIT

Upptaka íslenskra kálfa á ónæmisglóbúlíni G úr broddi

Sermissýnum var safnað úr 11 kálfum við burð og sólarhring síðar, auk þess sem broddsýni voru tekin úr mæðrum þeirra. Styrkur IgG var mældur í öllum þessum sýnum með elísuaðferð, auk þess að magn brodds sem hver kálfur drakk var skráð. Meðalstyrkur IgG í broddi var 11,54 mg IgG ml^{-1} og í sermi sólarhringsgamalla kálfa 8,02 mg IgG ml^{-1}, hvort tveggja undir viðmiðum sem notuð eru fyrir erlend ræktunarkyn. Fylgni fannst milli heildarmagns IgG í broddinum sem kálfarnir drukku og styrks IgG í sermi þeirra sólarhring eftir burð. Meðalgildi áætlaðs frásogs á IgG var 43% sem er hátt miðað við erlendar rannsóknir. Þó rannsóknin sé gerð á fáum einstaklingum gefa niðurstöðurnar nokkra hugmynd um getu íslenskra kálfa til þess að taka upp IgG úr broddi.

INTRODUCTION

Immunoglobulin G (IgG) can be found in most body fluids and is important when fighting bacterial or viral infections. In cattle, IgG cannot penetrate through the placenta, so maternal IgG must be passed via colostrum and is absorbed through the calf's intestinal wall (Simister 2003; Pross & Grattendick 2007). This absorption is called **passive transfer of immunity** and gives the offspring its first protection against infectious diseases (Furman-Fratczak et al. 2011). Calves that fail to absorb sufficient levels of IgG are more susceptible to disease and mortality than those with higher levels of IgG in serum (Rajala & Castrén 1995). Adequate passive transfer has taken place when a calf's IgG level in serum has reached at least 10 mg ml^{-1} 24-48 hours after birth (Gay 1983). Even though passive transfer of immunity is important for the calf and its survival, calves with low IgG concentrations have survived infections and diseases, indicating that other factors are also involved in protection against some diseases (Dewell et al. 2006). For the calf to obtain a serum IgG concentration of 10 mg IgG ml^{-1} from colostrum, a minimum reference value of 50 g L^{-1} in colostrum has been calculated (Halleran et al. 2017).

The Icelandic cattle breed is the only dairy breed in Iceland and is most related to the northern Scandinavian breeds (Gautason et al. 2019; Lien et al. 1999). In previous studies, colostral Ig concentration has been shown to vary between cattle breeds (Muller & Ellinger 1981), so it is relevant to study passive immunity of Icelandic calves. Preliminary studies have already implied that the IgG level in colostrum of Icelandic cattle is lower than the published reference value for good quality colostrum (Jóna Kristín Vagnsdóttir, 2018). Young calves in Iceland are prone to bacterial and coccidial diarrhoea, however viral diarrhoea and respiratory diseases are very rare in Icelandic calves, in contrast to calves in many other countries (Virtala et al. 1999). Therefore, although Icelandic bovine colostrum may be of suboptimal IgG concentration, Icelandic calves seem to receive sufficient levels of IgG to fend off existing pathogens in their environment.

The aim of this study was to measure the concentration of IgG in calf serum at birth and at 24 hours postpartum, to determine whether calves receive sufficient IgG after birth for immunological protection, as defined by studies on other breeds. The aim was also to measure the IgG in the colostrum of mothers to determine whether there is a correlation between the concentrations of IgG in colostrum and calf serum at 24 hours.

MATERIALS AND METHODS

Animals and ethical approval

All samples were collected from Icelandic cattle at Hvanneyri dairy farm, Agricultural University of Iceland. Paired serum samples were taken from 11 calves at birth and approximately 24 hours later. In addition, 11 colostrum samples from their mothers were collected immediately after birth. The samples were taken during the period of 23 January until 13 March 2019.

Calves were taken away from their dams at birth. They were kept on dry hay with other calve in a pen of approx. 43 m^2 (ca 8.5m x 5m). Calves were given 1.5-2.9 L of colostrum from their mothers at each feeding, 2-3 times during the first 24 hours, totalling on average 5.3 L. The first colostrum feeding, averaging 1.7 L, was given within one hour of birth. Eight of the calves were weighed at birth.

The study was carried out under licence no. 2018-11-02 according to law no. 55/2013 on animal welfare and regulation 460/2017 on the protection of animals used for scientific purposes.

Serum samples

Blood samples were taken from calves by jugular vein puncture at birth (0 hours) and again 24 hours later, using 9 ml BD Vacutainer tubes containing serum clot activator. Samples were tilted a few times then kept upright at 4°C until centrifuged within 24 hours at 1932 x g for 10 minutes at 4°C; 1.5 ml of serum was taken from the top and kept frozen at -70°C until analysed.

Colostrum samples
Colostrum samples from the first milking postpartum were taken. Samples of 45-50 ml were stored in falcon tubes at -20°C until they were moved to the laboratory, where they were thawed in a hotbox, their IgG concentration measured with ELISA and two small samples (1.9 ml) were stored at -70°C.

ELISA
Bovine IgG ELISA DuoSet (R&D Systems, Bio-Techne) was used for all IgG measurements according to the manufacturer's instructions. All wells on a 96-well Maxisorp plate (Nunc) were incubated overnight at room temperature with 150 µl of goat anti-bovine IgG, at 0.2 µg ml^{-1} in phosphate buffered saline (PBS, pH 7.4). After incubation, the plate was washed four times with washing solution (PBS with 0.05% Tween 20) and then the plate was incubated with 300 µl of PBS containing 5% Tween-20 (Elisa buffer) for one hour at room temperature, followed by a wash as before. Recombinant bovine IgG, serially diluted in Elisa buffer, was used as standard in the range of 93.8 to 6000 pg ml^{-1}. The serum samples were measured in four two-fold serial dilutions, starting at 1/5,000 for serum samples and 1/800,000 for samples taken 24 h after birth. The colostrum samples were measured in eight two-fold dilutions starting at 1/8,000,000. Samples and standard were measured in duplicate, with 50 µl per well. Biotinylated goat anti-bovine IgG was diluted 1:2,000 in Elisa buffer and 100 µl added immediately into all wells and the plate was put on a shaker at 50 rpm for two hours at room temperature. Following a wash as before, Streptavidin-HRP was diluted 1:40 in Elisa buffer and 150 µl pipetted into all wells and incubated for 20 minutes in the dark at room temperature, followed by a further wash as before. Then 150 µl of substrate (TMB One, Kem-En-Tec) was pipetted into all wells and incubated in the dark at room temperature, until a dark blue colour had developed in the highest standard when 50 µl of stop solution (0.18 mM H_2SO_4) were added. The absorbance (OD) of the wells was read at 450 nm with an Original Multiskan EX reader (Thermo Electron). A seven-point standard curve was constructed and the concentration of IgG in the samples read therefrom.

Apparent efficiency of absorption
The apparent efficiency of absorption (AEA) of IgG can be calculated using the following formula (Conneely et al. 2014):

$$AEA = ((Serum\ IgG\ (g\ L^{-1}) * Plasma\ volume\ (L)) / IgG\ intake\ (g) * 100$$

This requires the knowledge of the plasma volume of the calf, which is not known for Icelandic calves. The plasma volume of Jersey and Holstein calves has been defined as 9-10% of the birth weight of the calf (Quigley et al. 1998). In this study, 9% of birth weight was used.

Statistics and processing
The mean ± standard deviation (SD) was calculated for the samples and statistical significance was set at $p < 0.05$. The graphs and simple linear regression were done with GraphPad Prism 2019.

RESULTS

Calves and cows
Seven bull-calves and four heifer-calves were included in the study group (Table 1). Calves were fed on an average 5.3 litres of colostrum during the first 24 hours, and the average weight of the eight calves that were weighed was 35.8 kg. Parity of dams ranged from first to sixth calf.

IgG concentration
On average, IgG concentration in calf serum went from 0.009 to 8.02 mg ml^{-1} during the first 24 hours (Table 2). Colostrum fed to the calves contained on average 11.54 mg ml^{-1} IgG, with a range from 4.65 to 18.67 mg ml^{-1}. Figure 1 shows the correlation between the IgG concentration in the colostrum consumed and in the serum of calves 24 hours after first colostrum feeding ($R^2 = 0.3930$, $p = 0.039$). During the first 24 hours

Table 1. Data on the 11 calves included in the study, their date of birth, gender, birthweight, parity of dam and total amount of colostrum fed during the first 24 hours.

Sample no.	Date of birth	Calf gender	Weight at birth (kg)	Parity of dam	Amount of colostrum fed (L)
001	28.01.19	male	-	2	5.0
002	29.01.19	female	37.0	3	5.0
003	31.01.19	male	-	1	4.0
004	08.02.19	female	31.0	1	6.0
005	11.02.19	male	42.0	2	6.3
006	12.02.19	female	26.0	1	5.5
007	15.02.19	male	32.0	3	6.0
008	23.02.19	female	39.0	6	5.1
009	04.03.19	male	-	5	5.6
010	12.03.19	male	35.5	5	5.0
011	13.03.19	male	36.0	1	4.5
		Average	35.8	Average	5.3

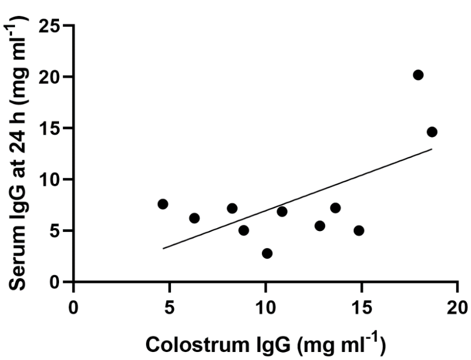

Figure 1. Correlation between IgG concentration in colostrum consumed by Icelandic calves, and IgG concentration in their serum 24 hours postpartum ($R^2 = 0.3930$, $p = 0.039$).

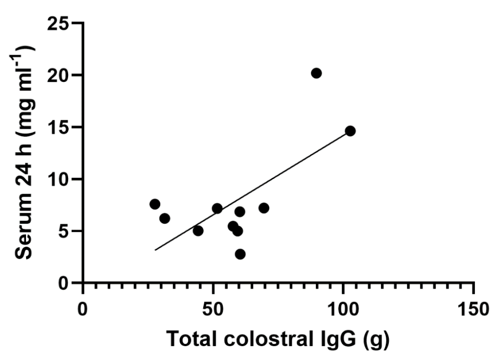

Figure 2. Correlation between the total amount of colostral IgG consumed by Icelandic calves and IgG concentration in their serum 24 hours postpartum ($R^2 = 0.4645$, $p = 0.021$).

postpartum, calves received in total 27.7-102.7 g IgG, corresponding to 0.09-0.39% of their birthweight (for the eight calves weighed). Figure 2 shows the correlation between the total amount of IgG received and the resulting IgG concentration in serum ($R^2 = 0.465$, $p = 0.021$). Figure 3 shows the correlation between the ratio of IgG amount to birthweight and the resulting IgG concentration in serum ($R^2 = 0.500$, $p = 0.0498$). No significant correlation was found between IgG concentration in serum and either the ratio of total of colostrum fed per kg birthweight for the eight calves weighed ($R^2 = 0.2294$, $p = 0.230$) or the birthweight for the eight calves ($R^2 = 0.2102$, $p = 0.253$).

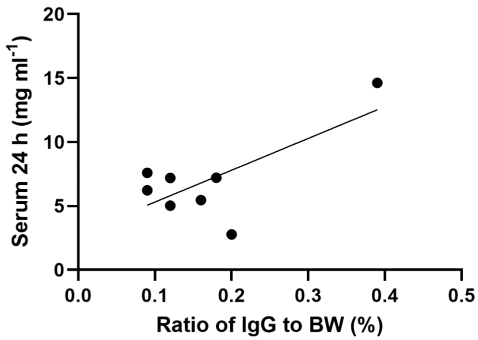

Figure 3. Correlation between the ratio of IgG consumed and birthweight of Icelandic calves and their serum concentration of IgG 24 hours postpartum ($R^2 = 0.500$, $p = 0.0498$). Birthweight was only registered for eight calves.

Table 2. Concentration of IgG in serum samples taken from calves at birth (0h) and 24 hours after first colostrum feeding, as well as in the colostrum they were fed.

	Serum IgG		Colostrum IgG
Sample no.	0h (mg ml^{-1})	24h (mg ml^{-1})	(mg ml^{-1})
001	0.007	20.20	17.95
002	0.011	6.22	6.29
003	0.008	5.02	14.86
004	0.013	2.78	10.08
005	0.009	7.19	8.26
006	0.008	14.63	18.67
007	0.012	7.59	4.65
008	0.005	7.22	13.64
009	0.007	6.87	10.86
010	0.014	5.03	8.86
011	0.009	5.46	12.83
Mean	0.009	8.02	11.54
SD	0.003	5.00	4.52

Apparent efficiency of absorption

The eight calves for which birthweight was available had a mean estimated blood volume of 3.1 litres and consumed on average 55.7 g of IgG via colostrum, leading to a mean AEA of 43% (Table 3).

Table 3. Apparent efficiency of absorption (AEA) of IgG in eight Icelandic dairy calves, as calculated using serum IgG 24 hours after birth, estimated blood volume and total colostral IgG consumed.

Sample no.	Serum IgG 24h (mg ml^{-1})	Estimated blood volume (L)	Total IgG consumed (g)	Apparent efficiency of absorption (%)
002	6.22	3.33	31.46	65.8
004	2.78	2.79	60.49	12.8
005	7.19	3.78	51.62	52.6
006	14.63	2.34	102.69	33.3
007	7.59	2.88	27.67	79.0
008	7.22	3.51	69.56	36.4
010	5.03	3.20	44.30	36.3
011	5.46	3.24	57.74	30.7
Mean	7.0	3.1	55.7	43.4
SD	3.5	0.5	23.7	21.2

DISCUSSION

This is the first study reporting the concentration of IgG in the serum of Icelandic dairy calves. Interestingly, there was already a minute concentration of IgG in serum samples taken at birth. This is not surprising, as traces of IgG can be found in newborn calves, up to a concentration of 1 mg ml^{-1} (Conneely et al. 2014). However, this level of IgG does not provide enough protection for the calf against microorganisms in its surroundings (Barrington and Parish 2001). The average concentration of IgG in serum samples taken at 24 hours postpartum was 8.02 mg IgG ml^{-1} ± 5.0, ranging between 2.78 and 20.20 mg IgG ml^{-1}. This is compatible with research done on Holstein calves both in Europe and the United States (Furman-Fratczak et al. 2011; Sakai et al. 2012), indicating that Icelandic calves take up sufficient IgG compared to other breeds although only two of the eleven calves reached the value of successful passive transfer of immunity. Numerous factors can increase the risk for failure of passive transfer of immunity (FTP), such as bacterial contamination of colostrum (Poulsen et al. 2010) and calving difficulties leading to poor vitality of the calf (Furman-Fratczak et al. 2011). Poor vitality of the calf not only negatively influences the amount of colostrum the calf can consume, but also the absorption of IgG through the intestinal mucosa, as reviewed by Weaver et al. (2000).

The average colostral IgG concentration observed in the Icelandic cows was considerably lower than in Holstein (79.48 mg ml^{-1}) and Jersey (72.9 mg ml^{-1}) cows (Furman-Fratczak et al. 2011; Morrill et al. 2015). Low median concentration (35 g L^{-1}) and variation (5-129 mg ml^{-1}) are known in Red Norwegian cows (Johnsen et al. 2019) and low concentrations (22 mg ml^{-1}) with wide range were described by Erhard et al. (1999), although without information on breed. The average colostrum IgG concentration in the present study was, therefore, only a little over half of the lowest previously reported concentration, ranging from 4.65 to 17.95 mg IgG ml^{-1}. Such variation is not unique, as considerable individual variation has been reported in Jersey cows (13-154 mg ml^{-1} in Morrill et al. 2015), even if the mean concentration was a very acceptable 72,9 mg ml^{-1}.

As there were only 11 calves and their dams in this study, conclusions must be drawn with care. However, the results show a significant influence of the absolute amount of IgG fed to each calf on the IgG serum concentration achieved. The results therefore give an indication of the ability of Icelandic calves to absorb a large part of the IgG ingested via colostrum. Birthweight was regrettably not registered for three of the calves, and, therefore, it was not possible to calculate the AEA for each calf. The AEA calculated with IgG intake measured by ELISA was widely distributed, ranging from 12.8% to 79.0%, but the average of 43.4% is high compared to the 21-28% recorded for Holstein and Jersey calves (Jones et al. 2004; Swan et al. 2007). Halleran et al. (2017) found a wide range of AEA values in Holstein calves and suggested that feeding larger volumes of colostrum and stricter genetic selection could increase the IgG concentration in serum. The study was conducted on 100 calves in 5 herds and the average AEA was 28%; however, the larger sample size and different analytical methods might affect the comparability of these studies.

As mentioned before, colostrum samples contained less than 50 mg IgG ml^{-1}, suggesting that the colostrum from these cows might not provide their calves with enough IgG to withstand disease. However, with low morbidity of neonatal calves in the herd, the relatively low concentration of IgG in their serum seems not to have a major effect on their health. The results rather suggest that the low risk of infections, due to the lack of harmful microorganisms in the environment, leads to lower IgG concentrations (Bush & Staley 2010). The relatively low IgG serum concentration in Icelandic calves seems to be sufficient for the infection pressure in their environment, although the concentration of other Ig types such as IgA, might also have a relatively bigger role than in other breeds (Ahmann et al. 2021). The cows in this study were kept in a freestall, as are most Icelandic dairy cows (73% of all cows in 2019), and milked automatically, as is the case for 40% of Icelandic dairy farms (Landssamband kúabænda 2020). According to Icelandic regulation, antibiotics are only used on an individual basis and based on a diagnosis by a veterinarian, and vaccines or immunomodulators are not used on the study farm (Reglugerð um heimildir dýralækna til að ávísa lyfjum nr. 539/2000).

Calves were given their first feeding of colostrum within one hour of birth. It is important to feed calves the appropriate amount of colostrum as soon after birth as possible. However, they should not be overfed in the first feeding and instead additional smaller feeds should be given during the first 24 hours (Chigerwe et al. 2008). In their study, Conneely et al. (2014) found that calves receiving colostrum corresponding to 10% of their bodyweight had a slightly lower serum concentration and AEA than calves receiving 8.5% of their bodyweight. The calves received the whole amount in one feed, which is less optimal with such quantities. In our study, calves had an average AEA of 43% and consumed colostrum corresponding to 13-21% of their birthweight during the first 24 hours, distributed over two to three feedings (3-8% at a time). Conneely et al. (2014) concluded that calves fed colostrum within two hours postpartum had a higher AEA and, after that, a higher colostral IgG concentration was needed to ensure similar serum concentrations. This indicates the importance of both the timing and amount of feeding provided to calves during their first hours of life.

Our results show a weak positive association between the IgG concentration in colostrum fed to calves and the concentration in their serum 24 hours after first feeding. A stronger correlation was seen when the total amount of IgG was calculated, verifying the high efficiency of absorption, and validating the method of smaller, more frequent feedings. The study design involved feeding the calves varying amounts of colostrum, thus following routine practice on the farm, instead of feeding a standard amount of pooled colostrum. Erhard et al. (1999) concluded that the absorption of IgG relies on other factors and not only colostrum quality. In a study involving colostrum supplement, serum IgG in calves fed a medium- or low-quality colostrum plus colostrum supplement was higher than for calves fed high-quality colostrum (McCoy & Hurley 1997). Other studies found that IgG in colostrum was absorbed more efficiently than IgG contained in a colostrum supplement (Morin et al. 1997). The absorption of IgG clearly relies on more factors than the amount of IgG available in the colostrum, importantly birthweight of the calf (Shivley et al. 2018), although birthweight was not shown to be a causal factor in this study.

In conclusion, our study showed that feeding calves on average 5.3 litres of colostrum in two to three feedings over 24 hours is highly effective. The average concentration in calf serum was considerably under the reference value for adequate transfer of immunity as defined in other breeds. This was also true of the mean colostrum IgG concentration, which was lower than the defined reference value for quality colostrum in other breeds. However, the average apparent efficiency of IgG absorption was high, enabling the calves to utilise a large proportion of the available colostral IgG. The absolute amount of colostral IgG fed to calves during the first 24 hours postpartum correlated well with their serum concentration of IgG. For future studies, it would be important to increase the sample size considerably, to include more herds, and to collect the birthweight and birth records for all calves studied.

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