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Study of the Effects of Modified Colostrum Feeding Method on Passive Transfer Success in New-born Calves and Comparison with the Classical Method

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ABSTRACT In this study, it was aimed to evaluate a new colostrum feeding protocol in terms of passive transfer success. In the study, 70 calves each from two different farms with similar characteristics except colostrum feeding protocols were used. According to the modified method, new-born calves were given as much colostrum as the calf could drink, once every 3 hours, a total of 5 times in the first 12 hours. After 12 hours, as in the classical method, 2.5 liters of colostrum was given to the calves in the morning and evening, and then the calves were fed with milk. The amount of colostrum consumed by the calves fed with the modified method at each meal was recorded, and the blood IgG levels were measured using the ELISA method. In the first 5 feedings made in the modified method, the calves drank an average of 5.51 L colostrum in the first 12-hour period, 2.52 L in the first feeding, 0.86 L in the second feeding, 0.52 L in the third feeding, 0.98 L in the fourth feeding, and 0.63 L in the fifth feeding, respectively. While the mean blood IgG level of the calves in the modified colostral feeding group was 37.33 mg/ml, it was measured as 31.04 mg/ml in the classical colostral feeding group. As a result, with this difference made in the colostrum feeding method, the blood IgG levels of the calves reached a significantly higher level compared to the classical method.

Keywords: *Calf, Colostrum, Cow, IgG, Passive immune transfer.*

ÖZ

Yeni Doğan Buzağılarda Modifiye Kolostrum Besleme Yönteminin Pasif Transfer Başarısına Etkilerinin Araştırılması ve Klasik Metot ile Karşılaştırılması

Bu çalışmada, yeni bir kolostrum besleme protokolünün pasif transfer başarısı yönünden değerlendirilmesi amaçlanmıştır. Çalışmada kolostrum besleme protokolleri dışındaki özellikleri benzer olan iki farklı çiftlikten 70' er buzağı kullanılmıştır. Modifiye metotta, yenidoğan buzağılara her 3 saatte bir, ilk 12 saatte toplam 5 kez buzağının içebildiği kadar kolostrum içirilmiştir. 12 saat sonraki beslemelerde klasik metotta olduğu gibi sabah ve akşam 2.5 litre kolostrum ve devamında sütle besleme yapılmıştır. Modifiye metotla beslenen buzağıların her öğünde içtikleri kolostrum miktarları kaydedilmiş, kan IgG seviyeleri ise ELISA metodu kullanılarak ölçülmüştür. Modifiye metotta yapılan ilk 5 beslemede sırasıyla; birinci beslemede 2.52 L, ikinci beslemede 0.86 L, üçüncü beslemede 0.52 L, dördüncü beslemede 0.98 L ve beşinci beslemede 0.63 L olmak üzere ilk 12 saatlik periyotta toplamda ortalama olarak 5.51 L kolostrum içmişlerdir. Modifiye kolostral besleme grubundaki buzağıların ortalama kan IgG seviyesi 37.33 mg/ml olarak ölçülürken, klasik kolostral besleme grubunda ise 31.04 mg/ml olarak ölçülmüştür. Sonuç olarak kolostrum besleme yönteminde yapılan bu farklılık ile klasik metoda göre buzağıların kan IgG seviyelerinin önemli ölçüde yüksek seviyeye ulaşmıştır.

Anahtar Kelimeler: *Buzağı, İnek, IgG, Kolostrum, Pasif immun transfer.*

INTRODUCTION

In a successful colostral passive immune transfer, the calf's serum IgG concentration is expected to rise above 10 g/L between 32-48 hours (Godson et al. 2003). In order for passive immunity to be successful, it is recommended to pay attention to the conditions called 3Q (Quality, Quantity, Quickly) in colostrum intake (Azkur and Aksoy 2018). The success of passive transfer achieved through colostrum can be affected by various factors, including the quality and quantity of colostrum used in feeding, the method of feeding, and the timing of feeding (Kara and Ceylan 2021).

The quality of colostrum is determined mainly by the level of IgG, which constitutes 85% of colostral Ig, and more specifically, of colostral Ig alone (Godden 2008). The quality of colostrum should be assessed after each birth, ensuring that only quality colostrums are used for feeding and cryopreservation (Kaygısız and Köse 2007). The

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timing of colostrum administration is of great importance in the success of passive transfer. Since intestinal epithelial cells do not mature in the first few hours of life in new-born calves and have a vesicular/vacuole structure, Ig's and other macromolecules are absorbed with maximum efficiency within the first 4 hours without changing (Chigerwe et al. 2009). While it decreases to 12 hours due to structural and chemical changes, it decreases to a minimum in 24 hours (Quigley et al. 2002; Godson et al. 2003; Gökçe and Erdoğan 2013). Another reason for the rapid delivery of colostrum is that the quality of colostrum decreases over time. Structural differences separating colostrum from milk disappear and it turns into normal milk formation (Quigley et al. 2013). In colostrum collected 6 hours, 10 and 14 hours after birth, compared to colostrum collected at 0 hours, there was a 17%, 27% and 33% decrease in Ig levels, respectively (Moore et al. 2005). At the same time, while Ig absorption capacity is close to 100% immediately after birth, it decreases to 50% after 6 hours, to 33% after 8 hours, and comes to a standstill after 24 hours (Cortese 2009). The method of administration of colostrum is important in terms of passage to the intestines and the amount of administration. Calf can be fed by colostrum directly from its mother, or it can be given with a bottle or through esophageal tube (Gökçe and Erdoğan 2013). Natural absorption is known to cause passive transfer failure. Calves cannot get enough colostrum and the first sucking period is delayed for various reasons, such as weak maternal instinct and structural defects in the udder (Weaver et al. 2000; Godson et al. 2003; Bilal 2007). The esophageal tube is a great convenience for those who do not want to suck, but it prolongs the passage to the intestines as it prevents the formation of the sulcus esophagicus (Lateur-Rowet and Breukink 1983). In bottle feeding, the sucking reflex is stimulated and colostrum reaches the abomasum directly thanks to the sulcus oesophagicus, the first sucking time is shortened and the amount of delivery can be determined exactly (Godden et al. 2009a).

Situations, where colostral passive immune transfer fails, are called passive transfer failure (PTF) (Şentürk 2012). The reference value in passive transfer failure is that the IgG concentration in the blood serum of the calf remains below 10 mg/ml in the 24-48 hours postpartum (Godden 2008; Çakıroğlu et al. 2010). PTF is not a disease, it is a condition that predisposes calves to the development of diseases (Weaver et al. 2000). PTF causes an increase in morbidity and mortality rates in the first 6 months of age, especially in the neonatal period. It is a management problem that cannot be ignored in terms of farm productivity and profitability, as it can lead to many consequences such as decreased feed efficiency and daily live weight gains, prolongation of the first calving age, decreased milk yield in the first two lactations and accordingly increased cattle removal from the herd. (Weaver et al. 2000; Godden 2008).

Every stage of herd management in professional enterprises is carried out within the framework of pre-formed protocols. However, it is not possible for veterinarians to follow every practice in large-scale farms, which can only be managed with a large number of workers. For this reason, in the creation of preferred protocols for herd management, the protocol should be applicable and auditable as well as being intended to provide maximum benefit. In addition, it should not be forgotten that the risks posed by the applications and the extra stress load may have adverse effects on health and passive transfer success. In this study, it was aimed to investigate new colostrum feeding strategies in order to minimize the cases of passive transfer failure due to delay in emptying of the abomasum due to human error, extra stress during calf forcing and colostrum feeding processes and excessive fullness in large-scale farms.

MATERIAL AND METHODS

This study was carried out with the permission of the Kırıkkale University Animal Experiments Local Ethics Committee at the meeting numbered 2021/01 on 21.01.2021, decision numbered 01.

Animal Material

The material of the study consisted of cows fed in two different dairy farms in Ankara. The classical feeding and modified feeding groups that are the subject of the study are also the routine feeding protocols of different farms. The calves included in the study from the first farm formed the “classical colostrum feeding group”. In the classical colostrum feeding group, calves were given 10% of their body weight in colostrum within the first 4 hours after birth, and then 2.5 L milk was fed twice a day in the morning and evening. Bottle feeding was normally used, and calves that did not receive the targeted amount of colostrum were fed with an esophageal tube. In the modified colostrum feeding group, colostrum was given as much as the calf could drink voluntarily at each feeding. In the second farm, a different colostrum feeding protocol was created and a "modified feeding" trial was carried out. According to this protocol, calves were fed with colostrum within the first 1 hour of birth, and in the following period, colostrum was given every 3 hours (at 3, 6, 9, and 12 hours) in the first 12 hours, a total of 5 times and in quantities that the calf could drink. The aforementioned modified feeding protocol is a protocol created by the farm against passive transfer failures due to worker errors and aiming the calf to receive maximum colostrum voluntarily. In both groups, 2.5 L (1 bottle) of milk was fed twice daily, in the morning and evening, after the 12th hour. In both groups, calves who could not stand alone were weak, had no sucking reflex, had a difficult birth, and were born by cesarean were excluded from the study.

Work on both farms started on the same date. It was aimed to minimize the effect of climatic conditions on the work with the study that started simultaneously in the farms located in the same geographical region. Both farms were large farms with more than 500 dairy cows. The dry period and new-born protocols of these farms are similar. Holstein cows were used in both farms included in our study. In both farms, animals are taken to the dry period 2 months before birth. In order to protect newborns from calf diarrhea, cows are vaccinated against calf diarrhea agents twice during the dry period. Cows are taken to the birth chambers 1 week before the birth. After birth, the mother is allowed to dry the calf by licking it, then they are fed for 24 hours in a separate area in the delivery room. After 24 hours, the calves are separated and placed in the calf huts. Colostrum feedings in both farms, keeping records and notifying the veterinarians of the calves to be administered esophageal tubes were made by the workers working in the delivery room. 70 calves from both farms were included in the study.

Collection of samples and data

Blood samples were collected from V. Jugularis using an 18-gauge needle (Beybi®, Istanbul, Turkey) in the 32-48 hour age range of the calves and were taken into red-capped serum tubes (Ayset®, Adana, Turkey). The

collected blood was centrifuged at 3000 rpm for 10 minutes to obtain serum and stored in – 20 °C freezers. The amount of colostrum taken by the calves during the first 12 hours in the farm where the modified colostrum was fed was recorded by the delivery room personnel. In the case of using esophageal tube in the farm where conventional feeding was done, the calves were recorded.

The amount of colostrum used in the feeding of the calves fed with the classical method was not recorded, and the amount to be given to the farm personnel was informed and no intervention was made afterward.

IgG Analysis

IgG concentrations of serum samples were determined using a commercial ELISA kit (Bovine IgG ELISA Kit, Biox, Belgium). Analyzes were performed in Diagen Diagnostic laboratories according to the application principles of the commercial kit.

Statistical Analysis

Statistical analyses were performed with SPSS (Version 17.0; Chicago, IL). For continuous data normality distribution and variance homogeneity assumptions, Kolmogorov Smirnov test was performed. Descriptive statistics were calculated as mean and standard deviation minimum and maximum. Independent samples t-test (Indipendent Samples Test) was applied to the parameters to analyze the difference between the groups since the data were in accordance with the normal distribution. $p<0.05$ was accepted as the limit of significance.

Before statistical analysis, normality analyses were performed to see if the distribution of the data was normal, and the Independent Sample T-Test was applied to the data showing normal distribution.

RESULTS

Within the study, two different colostral feeding methods were compared. The first feeding after birth was taken as the 1st feeding, and feeding was made every 3 hours after the birth, forming the 2nd, 3rd, 4th, and 5th feedings. In the first 12-hour period, the modified colostrum feeding group was fed 5 times, while the classical colostrum feeding group was fed 2 times, at the 0th and 12th hours. In the modified colostrum feeding group, the amount of colostrum that the calf can drink voluntarily was given at each feeding; They drank an average of 5.51 L colostrum in the first 12-hour period, 2.52 L in the first feeding, 0.86 L in the second feeding, 0.52 L in the third feeding, 0.98 L in the fourth feeding, and 0.63 L in the fifth feeding (Table 1).

Within the study, 70 calves from both groups were evaluated. While the mean blood IgG level of the calves in the modified colostral feeding group was 37.33 mg/ml, it was measured as 31.04 mg/ml in the classical colostral feeding group. With this difference made in the colostrum feeding method, the blood IgG levels of the calves reached a significantly higher level compared to the classical method ($p<0.05$) (Table 2).

It was observed that blood IgG levels of all calves in both groups were above 10 mg/ml, which is the threshold value for passive transfer failure (PTF).

Table 1: Average amount of colostrum consumed in the first 12 hours in calves in the modified colostrum feeding (MCF) group.

Group	1. Feeding	2. Feeding	3. Feeding	4. Feeding	5. Feeding	Total
MCF	2.52	0.86	0.52	0.98	0.63	5.51

Table 2: Comparison of IgG levels between groups.

	Group	N	Mean	SD	SE	p
Serum IgG	MCF	70	37.33	13.68	1.63	**0.003**
	CCF	70	31.04	10.71	1.28	

*MCF: Modified colostral feeding, CCF: Classical colostral feeding.

DISCUSSION AND CONCLUSION

Successful immunity is provided by colostrum in calves born with agammaglobulinemic (Dewell et al. 2006; Godden 2008; Gökçe and Erdoğan 2013). In a successful colostral passive immune transfer, the calf's serum IgG concentration is expected to rise above 10 g/L between 32-48 hours (Godson et al. 2003). In this study, the serum IgG concentration of all calves in the modified colostrum feeding and classical colostrum feeding groups was well above 10g/L and it was determined that a successful passive transfer was achieved. This is an indication that the newborn protocols on both farms were successful.

In order for passive immunity to be successful, it is recommended that care should be taken to ensure the Quality, Quantity and Quickly conditions, which are called the 3Q formula, in colostrum intake (Azkur and Aksoy 2018). It is known that in the first 4 hours of life, Ig's are absorbed unchanged in relation to the vesicular/vacuolated structure of the intestines (Chigerwe et al. 2009). It has been determined that the absorption of Ig decreases over time due to the development of the intestines, gaining digestive properties, increased protease activity and increased acidity in the abomasum. For this reason, while the absorption of Ig is at its maximum level in the first 4 hours, it decreases in the 12th hour (Quigley 2007). In a study, it was reported that the intake of equal amounts of colostrum at 1, 2, 3 and 4 hours did not cause a change in the serum IgG ratio (Chigerwe et al. 2009). It was determined that it decreased to a minimum level in the first 24 hours (Quigley 2002; Godson et al. 2003; Gökçe and Erdoğan 2013). In another study, the amount of IgG needed by the calf for successful passive transfer was found to be 150-200gr at the 2nd hour, 164-226g at the 6th hour, and 185-309gr at the 12th hour after birth (Chigerwe et al. 2008). In this study, serum IgG ratio was measured as 37.33 mg/L in the modified model. In the classical colostrum feeding method, the average IgG value was measured as 31.04 mg/L (Table 2). When the models were compared, the ability of the modified model to transfer more IgG to the serum was found to be significantly higher than the classical model ($p<0.05$).

Frequent feeding during periods of higher intestinal absorption in modified feeding with a significantly higher serum IgG ratio is thought to be effective.

There is an accepted view that increasing the amount of colostrum to be given to the calf will also increase the total amount of Ig reaching the intestines, regardless of its quality, and will affect the success of its passive transfer. (Gökçe and Erdoğan 2013). However, it has been reported that the increase of colostrum above a certain rate will cause a negative effect, and the amount given after a certain amount of colostrum has a negative correlation with the absorption ability (Conneely et al. 2014). It is thought that reaching saturation is effective in the transport of macromolecules (Stott et al. 1979). In a study, when the colostrum IgG concentration was above 20g/L, it was observed that 1 liter of colostrum increased the serum IgG ratio more than the equivalent 2L colostrum in terms of IgG (Stott and Fellah 1983). In the study by Conneely et al. 7%, 8.5% and 10% of live weight of colostrum was given to all groups by tube within the first 2 hours after birth. It was determined that the IgG concentration measured at the 24th hour in calves was significantly higher in those who ingested 8.5% of the live weight colostrum. In this study, calves in the modified group received an average of 6.3% of their weight in colostrum within the first hour. The second feeding was given after 3 hours, that is, within 4 hours of birth. With the second feeding, an average of 8.45% of the total body weight of colostrum was given within the first 4 hours. In the first 4 hours, calves fed with the modified method received 8.45% of their weight, and an estimated 10% of their weight in the classical method, and the IgG level was found to be higher in the group that received 8.45% colostrum in line with Conneely 2014.

In studies on colostrum quality, it has been determined that the Ig concentration in colostrum is at its highest level after birth and begins to decrease gradually after birth (Baumrucker et al. 2010; Conneely et al. 2013). In a study, it was reported that the IgG concentration in colostrum decreased by 3.7% every hour after birth (Morin et al. 2010). In another study, it was reported that delaying colostrum expression for 6, 10, and 14 hours after delivery caused a decrease in the amount of IgG in colostrum by 17%, 27%, and 33%, respectively (Moore et al. 2005). In the present study, the calves fed with the modified model were fed every 3 hours, but the mothers were not milked every 3 hours, the colostrum obtained at the first milking was pooled, and the first milking colostrum of each newly born mother was included in the feeding. For this reason, although the feedings were done every 3 hours, in fact, during the first 12 hours, the first milking was fed with colostrums with the highest IgG level in five feedings. Therefore, it was not affected by the colostrum quality, which is expected to decrease with the next hour.

In addition to the rate, quantity and quality of colostrum delivery, the methods of delivery of colostrum are also of great importance in the success of passive transfer. The method of administration directly affects the success of passive immune transfer, as it determines the time of colostrum reaching the intestines and the amount of colostrum consumed (Godden 2008). Colostrum can be given to the calf directly from mother by sucking the udder, bottle or with an esophageal tube (Gökçe and Erdoğan 2013). It is not recommended for the calf to take colostrum from its mother by natural sucking method, as it causes a high rate of passive immune transfer failure (Weaver et al. 2000). In studies, it has been determined that passive transfer failure is seen at high rates such as 22.3-61% in the natural breastfeeding method (Besser et al. 1991; Filteau et al. 2003; Trotz-Williams et al. 2008; Morrill et al. 2012; Quigley et al. 2013). Although feeding using the esophageal tube is seen as a convenience in the feeding of calves that do not want to suckle, milk goes to the rumen because it does not stimulate the closure of the sulcus esophagus. It will be delayed by 2-4 hours for the milk that goes to the rumen first to pass into the intestines (Lateur-Rowet and Breukink 1983). In one study, 1.5L colostrum substituted calves fed with an esophageal tube and bottle showed an increase of 27.6% and 26% in serum IgG concentration and colostrum absorption ability in bottle fed calves, respectively (Godden et al. 2009b). In addition, it has been stated that the possibility of adverse events such as esophageal and pharyngeal trauma, aspiration pneumonia and stress is high during esophageal tube applications (Chigerwe et al. 2012). Various studies have been conducted on whether hyperadrenalemia caused by various stresses in newborns may suppress colostral immunoglobulin absorption or terminate intestinal permeability (Stott 1980). While colostrum consumption can be reduced by 74% in the first 12 hours in calves with fetal stress (Vermorel et al. 1989), it has been found that severe acidosis reduces colostrum uptake by 52% and serum IgG concentration by 35% (Vermorel et al. 1989; Drewry et al. 1999). Heat stress in calves has also been associated with low serum IgG concentrations through reduced calf viability or reduced intestinal permeability (Stott et al. 1976; Stott 1980; Donovan et al. 1986). In this study, it was thought that feeding with an esophageal tube may also create a stress factor. In addition, due to the use of dirty environmental equipment, it can colonize my digestive system and cause the closure of the intestines (Quigley et al. 1998). Although the use of esophageal tube was not required in any of the calves fed with the classical method in the studies, the stress factor was partially reduced, but forcing the calf to receive the targeted amount in the first feeding is a source of stress on the calf. Considering that stress and increase in cortisol levels negatively affect Ig absorption, it can be thought that modified feeding reduces stress factors. In this study, it is thought that calves fed with the modified method are effective in significantly ($p<0.05$) high serum IgG concentrations.

The biggest advantages of giving colostrum with a bottle are; since the sucking reflex is stimulated, colostrum can be directly transferred to the abomasum by forming the sulcus esophagicus as in natural sucking, the amount of colostrum given can be controlled, the calf is not waited for colostrum sucking, it can be applied as soon as it is born (Godden et al. 2009a). In the first 4 hours of maximum absorption, a calf with a birth weight of 43 kg should receive 100 g of IgG in order to achieve a sufficient level of serum IgG by successful passive immune transfer. In order to achieve this value, an average of 4 liters of colostrum with a concentration of 25 g/L should be taken, while it would be sufficient to take 2 liters from a colostrum containing 50 g/L IgG and only 1 liter of colostrum with a concentration of 100 g/L IgG (Gökçe and Erdoğan 2013). It has been reported that the second application within the first 12 hours following the administration of colostrum in newborn calves causes an increase in the serum IgG ratio (Morin et al. 1997). In one study, it was shared that ≥3 liters of colostrum in the first 4 hours and 1 more liter of colostrum within 12 hours would be sufficient for a successful passive transfer (Chigerwe et al. 2009).

A decrease in the absorption efficiency of IgG is seen when calves are fed large volumes of colostrum in a single feed

(Mokhber-Dezfooli et al. 2012). This is due to mechanical swelling of the abomasum and other anterior stomachs, resulting in a reduction in emptying of the abomasum (Mokhber-Dezfooli et al. 2012). Abomasal emptying rate is a factor affecting colostral IgG absorption (Mokhber-Dezfooli et al. 2012). Slower abomasum emptying may result in decreased absorption of colostral components (Sakai et al. 2012). Chigerwe et al. (2008) reported that calves fed 4 L colostrum had a reduced serum IgG concentration at 48 h compared to calves fed 3 L colostrum. Jaster et al. (2005) found that the serum IgG concentration at the 48th hour was 38.6 g/L and 45.6 g/L, respectively, in two groups given 4 liters of colostrum immediately after birth, 2 liters of colostrum immediately after birth, and 2 liters of colostrum at the 12th hour. In this study, 4 liters of colostrum in the classical model was drunk with the help of a bottle. It has been understood that the amount of colostrum given in the classical method delays the emptying of the abomasum and prolongs the transit time to the intestines due to the administration of more colostrum than the volume of the abomasum. For this reason, it is thought that the serum IgG ratio in the modified method is higher.

In the classical colostrum feeding method, which is effectively applied today, calves given 10% of their weight in colostrum make their next feeding 12 hours later. Colostrum can be affected by factors such as the quality and quantity of colostrum used in feeding, the method of administration and the time of administration for passive immune transfer success. This study was conducted on two different farms with similar newborn protocols except for the colostrum feeding method. As a result of the study, it was seen that giving as much milk as the calf can drink every 3 hours caused the serum IgG concentration to be significantly higher ($p<0.05$) compared to the classical method. Considering the results of this study, it was seen that the quality of colostrum milked for the second feeding in the classical method in the classical method decreased after 12 hours, the calf's colostrum absorption decreased, the attempts to make the calf drink colostrum involuntarily in the first feeding disrupted the absorption by causing stress, and giving a high amount of colostrum at one time delayed the emptying of the abomasum. However, it should be noted that this study is not an experimental study, the amount of colostrum used in each feeding is not recorded in the classical method, and the colostrum quality and total IgG mass given are not measured. In addition, the modified feeding method can be applied practically in large-scale farms with a large number of animals, where fresh colostrum can be reached continuously, who employ personnel whose sole duty is the care and feeding of calves, and its practical application in smaller-scale farms such as extra personnel, labor and continuous colostrum thawing. It should be noted that it is not suitable as it will require processes. This method is designed to minimize worker errors, eliminate extra stress on calves and ensure constant control of calves in farms with a large number of animals and difficult to control new-born protocols. More precise results can be obtained by conducting an experimentally controlled trial of this method, which has been tested as a field study.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Idea / Concept: EK, HK
Supervision / Consultancy: EK, HK
Data Collection and / or Processing: EK, HK
Analysis and / or Interpretation: EK, HK
Writing the Article: EK, HK
Critical Review: EK, HK

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