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A STUDY ON PASSIVE IMMUNITY TRANSFER IN BEEF CATTLE CALVES UNDER EXTENSIVE SYSTEMS

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PERPUSTAKAAN UMIVERSITI MALAYSIA SABAH

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF AGRICULTURE SCIENCE WITH HONOURS

> LIVESTOCK PRODUCTION PROGRAMME SCHOOL OF SUSTAINABLE AGRICULTURE UNIVERSITI MALAYSIA SABAH 2010



DECLARATION

I hereby declare that this dissertation is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that no part of this dissertation has been previously or currently submitted for a degree at this or any other university.

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ABSTRACT

The increasing demand for beef supply requires greater production of live marketed calves. The common calf diseases such as diarrhea, septicemia and pneumonia which contribute to high morbidity and mortality of the preweaning calves became the greatest challenge to the livestock industry in Malaysia. Passive immunity transferred from dam through colostrum is vital to protect the calf from disease infection in the first few weeks of its life. This study was conducted on Brahman calves born between April and July 2009 and between November and December 2009 at the Livestock Breeding Centre, Tawau, Sabah. The objectives of this study were to quantify the occurrence of common calf diseases and mortality and to evaluate the relationship of sex, birth weight, age and weight at sampling on serum TP of the calves at about one week until nine months of age. Refractometer method was used to determine the passive immunity status of the calves by measuring the serum total protein (TP). A failure in passive transfer (FPT) was indicated by serum TP concentration lower than 5.0 g/dl, the normal serum TP value in calves range from 5-6 g/dl and adequate when the serum TP concentration over 6.0 g/dl. It was observed that majority of the calves (75.9%) in this study were suffering from hypoproteinemia. Birth weight (21-25 kg) was a significant factor that influenced serum TP level and it was more significant in female calves. Age (6-8 days) of the calves either male or female was significant factor affecting serum TP. However, the common calf diseases and mortality cases observed were very low. Refractometer can be used to indicate the status of passive immunity status of calves born at a farm. Morbidity and mortality of calves with failure of passive transfer can be reduced through good management practices.



KAJIAN MENGENAI PEMINDAHAN IMUNITI PASIF DALAM ANAK LEMBU PEDAGING DI BAWAH SISTEM LEPAS BEBAS

ABSTRAK

Permintaan terhadap daging lembu yang semakin meningkat memerlukan pengeluaran yang lebih tinggi terhadap anak lembu yang boleh dijual. Penyakit yang biasa dihidapi anak lembu seperti cirit-birit, septisemia dan radang paru-paru yang boleh menyumbang kepada kemorbidan dan kematian anak lembu sebelum cerai susu menjadi cabaran yang hebat kepada industri ternakan di Malaysia. Pemindahan imuniti pasif daripada induk lembu melalui kolostrum adalah penting untuk melindungi anak lembu daripada jangkitan penyakit pada minggu-minggu terawal dalam hidupnya. Kajian ini dijalankan pada anak lembu Brahman yang lahir dalam tempoh di antara April dan Julai 2009 dan di antara November dan Disember 2009 di Pusat Pembiakan Ternakan, Tawau, Sabah. Objektf kajian ini adalah untuk mengkuantitikan kemunculan penyakit-penyakit yang biasa dihadapi anak lembu dan kematian dan menilai hubungan di antara jantina, berat lahir, umur dan berat semasa persampelan terhadap jumlah protein di dalam serum anak lembu pada umur lebih kurang seminggu sehingga sembilan bulan. Kaedah refraktometer telah digunakan untuk menentukan tahap imuniti pasif anak lembu dengan mengukur jumlah protein di dalam serum. Kegagalan dalam pemindahan imuniti pasif ditunjukkan melalui kepekatan jumlah protein di dalam serum yang kurang daripada 5.0 g/dl, normal pada 5-6 g/dl dan mencukupi apabila kepekatan jumlah protein di dalam serum melebihi 6.0 g/dl. Kebanyakan anak lembu (75.9%) di dalam kajian ini menghadapi masalah hipoproteinemia. Berat lahir (21-25 kg) adalah faktor yang signifikan dalam mempengaruhi jumlah protein di dalam serum dan ianya lebih signifikan pada anak lembu betina. Umur (6-8 hari) anak lembu sama ada jantan atau betina menjadi faktor signifikan dalam mempengaruhi jumlah protein di dalam serum. vang Walaubagaimanapun, didapati bahawa penyakit yang biasa dihidapi anak lembu dan kes kematian adalah sangat rendah. Refraktometer boleh digunakan untuk menunjukkan tahap imuniti pasif anak lembu di dalam ladang tersebut. Kemorbidan dan kematian anak lembu yang disebabkan oleh kegagalan pemindahan imuniti pasif boleh dikurangkan melalui pengurusan ladang yang bagus.



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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

χ² °C	Chi-square Degree celcius
<	Less than
>	More than
P	Significance value
AEA	Apparent efficiency of absorption
BCS	Body condition score
BW	Birth weight
d	Days
E	East
FPT	Failure passive transfer
g/dl	Gram per deciliter
ha	Hectare
h	Hours
IC	
Ig	Immune complexes
-	Immunoglobulin
IgA	Immunoglobulin A
IgD	Immunoglobulin D
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgG1	Immunoglobulin G1
IgG2	Immunoglobulin G2
IgM	Immunoglobulin M
Inc	Incorporation
kg	Kilogram
Ltd	Limited
MT	Metric tons
mg/dl	Miligram per deciliter
ml	Mililiter
mm	Milimeter
N	North
®	Registered
rpm	Revolutions per minute
SPSS	Statistical Package for the Social Sciences
TP	Total protein



CHAPTER 1

INTRODUCTION

1.1 Introduction

In 1980, more than 80% of the beef herd in Peninsular Malaysia was in the hands of smallholders (Samuel and Kwan, 1984). Most of them practiced traditional subsistence farming. They rear only a small number of cattle and used as a 'bank' or sold when they required immediate cash. Integration in oil palm plantation is another system commercialized today, in beef cattle industry in Malaysia. In 1980, 11,000 metric tons of the domestic beef production constituted about 50% of the beef supply while the remaining 50% was derived from imports (Samuel and Kwan, 1984). Nowadays, the largely dominated local beef production has decreased and highly dependent on imported beef. Demand for beef in the year 2000 which was about 100,000 MT, was expected to increase to around 170,000 MT by the year 2010 (Eusof and Abas, 2002) and 300,000 MT by the year 2020 (Eusof and Yaakub, 2009). Presently, 75% of the market share in Malaysia is accounted for by boneless imported beef, only about 15% from local beef and the rest is derived from imported live animals (Eusof and Abas, 2002). The increasing human population demands a higher beef supply. The average Malavsian per capita beef consumption rapidly increased and was expected to be 8.4 kg by this year (Azlan et al., 2009).

Government plays an important role in enhancing the growth of the beef industry. Importation of potential breeds of cattle from other countries such as Australia for breeding programmes was an initiative by the government to improve productivity of local beef cattle. Material inputs including livestock were also supplied to help smallholders through many schemes and development plans. However, slow growth rates and poor management practices contributes to the low productivity of beef production in Malaysia. In addition, disease problems have resulted in high levels of mortality among the calves. Antibody levels play important roles in reducing calf mortality and the feeding of colostrums after birth is very important and crucial for the transfer of passive immunity from dam to calf.

The calves were born without their own immunoglobulins (Ig). Antibody levels are developed in newborn calves through passive immunity passed from the dam through colostrums. Passive immunity gives temporary protection by transfer of certain immune substances like IgG, IgA and IgM from resistant individuals (Selk, 1998). Measurement of total protein by refractometer provides indirect assessment of the immunological status of the calves by measuring total protein instead of specific amount of immunoglobulin. Resistance to disease was greatly dependent on antibodies or immunoglobulins. Newborn calves can have a successful passive immunity transfer through natural suckling with the presence of the dam (McBeath et al., 1971; Rajala and Castren, 1995). The level of immunity in the first few weeks of life will determine the health status of the calf and the degree of resistance to disease infections. Failure to absorb the initial immune substance from colostrum may predispose the calf to disease, death or minimal future productivity (Quigley and Drewry, 1998). Sick calves will have lower weight gain and increase the cost of production. Calves that survive infectious and noninfectious diseases may cause continued economic loss because they may exhibit permanent stunting, unthriftiness and decreased productivity.

1.2 Justification

No reported research had been done to study the passive immunity in beef cattle in Malaysia especially on cattle reared under extensive system. Therefore, this study will examine the status of passive immunity among calves reared under extensive system. Losses due to low immunity level also can be reduced through improvement of the farm management practice.



1.3 Objectives

The objectives of this study are:-

- 1. To quantify the occurrence of common calf diseases and mortality
- 2. To evaluate the relationship between sex, birth weight, age and weight at sampling on serum total protein level



CHAPTER 2

LITERATURE REVIEW

2.1 Immune System Development

The existence of immunity to infectious disease was well recognized since 1900's (Tizard, 2009). Immunity can be referred to as reactions by an animal's body to foreign substances such as microbes and various macromolecules, independent of a physiological or pathological result of the reaction (Abbas *et al.*, 1991). Multiple defense mechanisms are needed to ensure the body is free from disease; physical barriers that exclude invaders, innate immunity that provides rapid initial protection and, acquired immunity that provide prolonged effective immunity. A pathogen is an organism that can cause disease and vary greatly in their ability to overcome the immune defense of an individual (Tizard, 2009).

Immune system development of the calves starts since post-conception; the fetal thymus is by 40 days, the bone marrow and spleen by 55 days, lymph nodes at 60 days and peripheral blood lymphocytes are seen by day 45 (Tizard, 2009). Uterus provides a sterile environment for the fetus (Stormont, 1972) before it is born into an environment where it is immediately exposed to various pathogenic microorganisms. The development of acquired immunity depends on antigenic stimulation (Natalija *et al.*, 2006) and it takes some time to become fully functional. Thus, newborn calves are most susceptible to infection for the first few weeks of life (Quigley and Drewry, 1998; Suh *et al.*, 2003).



2.2 Passive Immunity

Passive immunization requires that antibodies be produced in a donor animal by active immunization and then given to the susceptible animals for immediate protection (Tizard, 2009). Passive immunity is transferred from dam to calf through ingestion of colostrums which is vital to the health and survival of neonates until they are capable to develop their own immunoglobulins (Ig). Calves are born essentially agammaglobulinemic, since transplacental transfer of immunity from the dam does not occur (LeBlanc, 1986). The half-life of the antibodies passively acquired from the dam average between 1 and 2 weeks (Stormont, 1972). Endogenous antibody production in the calf began at 8-16 days old for IgG1, IgG2 and IgM, whereas for IgA at around 64 days (Husband *et al.*, 1972). Passive transfer of immunoglobulin through colostrum is essential for temporary protection of the calf and will determine the performance of calf during its life.

Maternal antibodies are not reachable by the fetus due to the syndesmochorial structure of ruminant placenta in which the chorionic epithelium is in direct contact with uterine tissues (Tizard, 2009). Newborn are highly dependent on antibodies transferred through colostrums for immediate protection since transplacental passage of Ig molecules is totally prevented. This is supported in work done by Natalija *et al.* (2006) who reported that, immediately after birth calves had no immune complexes (IC) in their sera. Nonimmunoglobulin proteins in plasma at 0 hour of age may be used for glucogenesis and protein synthesis in the body before ingestion of colostrums (Quigley *et al.*, 2002). According to Jezek and Klinkon (2004), the bovine epiteliochorial placenta is impermeable for protein macromolecules which cause calves to be born without Ig. However, Stormont (1972) stated that, fetus can make their own antibodies but sterile environment in the uterus totally prevent them from exposure to foreign antigens.

2.3 Contents of Colostrum

Colostrum is the first milk produced by a cow following calving (Fitzpatrick and Katherine, 2006). A newborn calf does not have the protective levels of immune bodies before ingestion of colostrum (Howe, 1924). Immunoglobulin is concentrated in colostrum from about five week's prepartum, probably in response to rising estrogen

concentration in the dam (LeBlanc, 1986; Selk, 1998) and reaching maximum levels approximately three weeks later (LeBlanc, 1986). These immune substances are then transported to the lumen of the mammary gland and released into colostrum for ingestion of the neonates. Colostrum contains higher amounts of proteins, fat, vitamins such as A and E, minerals, cytokines and trypsin inhibitor which helps protects immunoglobulin from digestion in the calf's gut compared to milk. Bovine colostrum contains about 1 million lymphocyte/ml and may survive up to 36 hours in the intestine of newborn calves (Tizard, 2009). It is extremely important that calves receive at least 2 litres of colostrums within the first six hours of birth (Fitzpatrick and Katherine, 2006) for immediate protection against disease.

The major Ig in colostrums is IgG, but there are also significant amounts of IgM and IgA as shown in Table 2.1. All of the IgG, most of the IgM, and about half of the IgA in bovine colostrums are derived from the cow's blood (LeBlanc, 1986; Tizard, 2009). The amount of IgG transferred through a mother's colostrum represents its immunological experiences due to antigen exposure. The amount of immunoglobulins in colostrums depletes rapidly following calving due to hormonal changes and degradation proteins (Husband *et al.*, 1972). As lactation progresses colostrum will gradually change to milk.

		In	Immunoglobulin (mg/dl)			
Species	Fluid —	IgA	IgM	IgG		
Cow	Colostrum	100-700	300-1300	2400-8000		
CON	Milk	10-50	10-20	50-750		
Ewe	Colostrum	100-700	400-1200	4000-6000		
LWE	Milk	5-12	0-7	60-100		
Horse	Colostrum	500-1500	100-350	1500-5000		
HUISC	Milk	50-100	5-10	20-50		

Table 2.1	Colostral and	milk immunoglobulin	levels in	domestic animals
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Source: Tizard, 2009

2.4 Immunoglobulin Classes

Antibody molecules are globulin proteins that have a compact, globular form called immunoglobulins (Ig) (Tortora *et al.*, 2007). They are present in highest concentrations and can be easily obtained from blood serum (Tizard, 2009). Immunoglobulins consist of five classes: IgG, IgM, IgA, IgE, and IgD. The characteristics of each of these classes are shown in Table 2.2. Each class has a different role in the immune response and acts in a specific environment.

Property	Immunoglobulin class					
rioperty	IgM	IgG	IgA	IgE	IqD	
Molecular weight Subunits Largely synthesized in;	900,000 5 Spleen and lymph nodes	180,000 1 Spleen and lymph nodes	360,000 2 Intestinal and respiratory tracts	200,000 1 Intestinal and respiratory tracts	180,000 1 Spleen and lymph nodes	

Table 2.2 Major immunoglobulin classes in domestic mammals

Source: Tizard, 2009

Immunoglobulin G (IgG) consists about 80% (highest concentration) of all antibodies in serum (Tortora *et al.*, 2007). It is the smallest of the immunoglobulin molecules and mainly responsible for systemic defense by crossing the blood vessel and enter tissue fluid. Suh *et al.* (2003) indicate that mean serum IgG level of beef calves was approximately twice that of dairy calves until 14 days of age. They found that, at 24 hours post partum, the mean serum IgG level reached the peak in both group of calves but after 24 hours, the levels fell gradually, but not significantly until 14 days after birth.

Immunoglobulin M (IgM) has the second highest concentration of all antibodies in serum. It is a very large immunoglobulin and rarely enters tissue fluids even at sites of acute inflammation (Tizard, 2009). Suh *et al.* (2003) shows that the level of IgM was significantly higher in beef calves than in dairy calves at 24 hours and four days after birth. In beef calves, it fell sharply until six days after birth but thereafter, it remained steady until 14 days after birth.

The third highest concentration in most mammals is immunoglobulin A (IgA). It is responsible for the defense of the intestinal and respiratory tracts (Tizard, 2009). It is the only major class of Ig which is not readily absorbed via the gut (Stormont, 1972). Secretory component binds to IgA dimers and protects it from digestion by intestinal proteases (Tizard, 2009). That is why IgA is more resistant to the action of digestive enzymes where it is retained in the gut and carry out protective action rather than being absorbed into the blood stream of the newborn. Suh *et al.* (2003) showed that mean serum IgA level was significantly higher in beef calves than in dairy calves at 24 hours, four and six days after birth. In beef calves, it fell sharply at four days after birth and remained steady until 14 days after birth.



Immunoglobulin E (IgE) is in extremely low concentrations in serum. It is responsible for immunity to parasitic worms and for allergies. It has the shortest halflife (two to three days) compared to the other Ig and is easily destroyed by mild heat treatment (Tizard, 2009).

Immunoglobulin D (IgD) is found in the blood in very little amount. Compared to the other Ig classes, IgD is evolutionary labile and shows many variations in structure (Tizard, 2009). Its function is not well-defined (Tortora *et al.*, 2007).

2.5 Factors Affecting Serum Total Protein

2.5.1 Increased total protein concentration

There are many factors that contribute to the increase in serum total protein concentration. Ingestion of colostrum may cause temporary high level of globulin concentrations. According to Morag (1989), relative water deficiency due to excessive venous stasis during collection of blood sample may cause fluid and small molecules to leave the plasma, leading to high protein concentration. Disease like cirrhosis of the liver and autoimmune disease also can increase globulin fractions. Hemolyzed blood samples, hemoconcentration and use of plasma will give falsely high readings (LeBlanc, 1986).

2.5.2 Decreased total protein concentration

According to Morag (1989), relative water excess usually produced iatrogenically will cause the sample falsely diluted. Hypoalbuminemia or excessive protein losses can be caused by many factors such as glomerulonephritis, intestinal protein loss usually due to heavy parasite burdens and hemorrhage. Decreased protein synthesis can be caused by many factors such as failure to ingest adequate amount of colostrum, malabsorption, lack of protein in the diet, liver failure, and viral infections.

2.5.3 Sex

According to Quigley and Drewry (1998), sex of the calf may influence apparent efficiency of absorption (AEA). They state that heifer calves generally have higher serum IgG concentrations than bull calves. However, they are not clear whether gender of the calf may be related more to blood volume than to AEA. Besides that, there are also possibilities that the larger size of bull calves may influence the metabolic state of the calves and affecting Ig absorption. However, Vann *et al.* (1995) reported that there are no effects of calf gender on AEA in *Bos indicus* or *Bos taurus* calves. Norman *et al.* (1981) stated that sex of offspring does not affect IgG1 concentration in serum of the calves and this is in agreement with Filteau *et al.* (2003). Eventhough higher proportion of failure passive transfer (FPT) among male beef calves was reported by Filteau *et al.* (2003), it might be due to dystocia, since the report included a greater number of complicated deliveries among males in this study.

2.5.4 Birth weight

Inadequate protein intake by dams before parturition may affect birth weight of calves which is followed by higher risk of mortality and morbidity (Quigley *et al.*, 2002). However, according to Norman *et al.* (1981) birth weight was not a significant factor influencing variation in immunoglobulin concentration in serum of the calves. They stated that, very small and excessively heavy animals may be less vigorous at birth and less capable of obtaining colostrum in the first few hours after birth.

2.5.5 Age

Many studies observed the levels of Ig in the first few weeks of life. According to Norman *et al.* (1981), the concentration of IgG1 in calves serum is slightly higher at 24 hr than 36 hr since the capacity of absorption ceases at about 26 hr postparturition and by 36 hr, various metabolic processes have taken place. Intestinal epithelial cells lose their ability to absorb after about 24 hr because of the maturation of the cells and the development of the intracellular digestive apparatus (Quigley and Drewry, 1998). Within 24 hr to 7 d of age, mean serum Ig concentration of calves was not related to age in days at sampling (Filteau *et al.*, 2003). Age of the dam also may affect calf serum IgG1 concentration where calves from younger cows especially heifer had lower serum Ig concentrations than calves from older cows with five or more lactations (Norman *et al.*, 1981).



2.5.6 Amount of Ig Present in Feeding Colostrums

Amount of Ig fed is determined by the concentration of Ig present in the colostrum and the volume fed. Concentration of Ig in colostrum varies but effective colostral Ig transfer can be achieved with natural suckling (McBeath *et al.*, 1971; Rajala and Castren, 1995). A very low level of immunoglobulin detected in serum of unsuckled animals (Tizard, 2009). Calf breed and age of dam had significant influence on concentrations of IgG1 (Muggli *et al.*, 1984). According to Selk (1998), calving difficulty also may influence Ig concentrations in blood of one-day old calves. IgG1 will decrease from 2401 mg/dl to 1918.5 mg/dl as calving difficulty score increase from 1 (unassisted) to 3 (assisted after at least one hour of labour, difficult pull). Reduction in IgM also results as it decreases from 194.8 mg/dl to 135.6 mg/dl as calving difficulty score increase from 1 to 3.

Body condition score (BCS) of both, cows and heifers at calving also influence concentration of immunoglobulin (Selk, 1998). IgG1 will increase from 1998.1 mg/dl to 2348.9 mg/dl as BCS increase from 3 (very thin) to 6 (good). The same happens for IgM as it increases from 194.8 mg/dl to 304.1 mg/dl when BCS increase from 3 to 6.

2.5.7 Efficiency of Absorption of Ig

The ability to absorb Ig in calves is completely lost by 24-36 hours after birth (Suh *et al.*, 2003). According to Vann *et al.* (1995) the efficiency of absorption of total immunoglobulin at 6 and 12 hour were not affected by sex of calf, breed type, or any interaction. Variation in IgG absorption and protein was unrelated to birth weight but probably related to differences in metabolic state of the animals (Quigley *et al.*, 2002). Husband *et al.* (1972) stated that ratios for absorption efficiency of Ig decreased in the following order: IgM > IgA > IgG2 > IgG1. Decreasing rate of Ig absorption is varies; IgG can be absorbed for 27 hours, IgA for 22 hours and IgM for only 16 hours (LeBlanc, 1986). Timing of colostrum feeding is important because the efficiency of Ig absorption decreases from the time of birth due to intestinal closure. Total immunoglobulin absorption decreases from 66% to 6% as time of feeding colostrum (hours after birth) increase from 6 hours to 24 hours (Selk, 1998).



Several studies have reported different results on the effects of maternal prepartum nutrition on newborn calves. Blecha *et al.* (1981) reported that maternal crude protein consumption were positively correlated with absorption of IgG1 and IgG2 by the calf at 12, 18, 24 and 36 hr after birth. However, whether it would be sufficient to lower resistance to disease is not known. This is also supported by Burton *et al.* (1984) who observed depressed serum immunoglobulin concentrations when calves were born to dams that had been restricted in protein intake during their last trimester. In contrast, Hough *et al.* (1990) reported that serum IgG concentration was not affected by prepartum maternal nutrient treatment, suggesting that the calves ability to absorb colostrally derived IgG was not altered by maternal nutrient restriction.

Poor teat conformation or poor mothering ability will cause the calf to receive colostrum at a much later hour. LeBlanc (1986) reported that efficiency of immunoglobulin absorption will increase as the calf is allowed to remain with its dam for the first 24 hours. Selk (1998) also reported that 70% more Ig is absorbed by mothered calves from a standard feed than non-mothered calves.

2.6 Common Diseases in Newborn Calves

The phagocytic and bactericidal capacity of neutrophils declines near birth as steroid levels increase (Tizard, 2009). Colostral immunoglobulin can give partial protection to newborn calves. In one survey reported by Selk (1998), death from pneumonia was reduced in calves that had high serum levels of colostral immunoglobulin. During the first several weeks of calf life, immunoglobulins in the gut play an important role in protection against intestinal disease (Suh *et al.*, 2003). Protection against enteric disease is obtained through the continuous intake of IgA or IgG1 from milk (Tizard, 2009). Continuous feeding of colostrum after the gut has closed may be beneficial as it gives protection against rotaviral diarrhea (Selk, 1998). During the first two weeks of life, septicemic and enteric diseases are commonly seen in calves and at highest risk of death during the first week of life (Suh *et al.*, 2003). Survey reports by USDA beef researchers and ranchers show that diseases including scours and pneumonia cause significant numbers of young calf deaths (Selk, 1998).

Colostral transfer of immunity is essential for the survival of young animals, but it may also cause disease. Hemolytic disease occurs naturally as the motion becomes immunized against the red cells of her fetus, antibodies transferred through colostrum may cause massive erythrocyte destruction in the newborn animal (Tizard, 2009). Newborn calves, didn't show symptoms of the disease until they have ingested colostrum where passive immunity passed through the gut (Stormont, 1972).

2.7 Refractometer Method

Measurement of total protein by refractometer is suitable for herd monitoring and provides a reasonably accurate assessment of passive transfer status (Weaver *et al.*, 2008). It is a rapid and simple test (Reid and Martinez, 1975; LeBlanc, 1986) where single blood sample can be analyzed within four minutes (Reid and Martinez, 1975). It provides only indirect assessment of the immunological status of the calves by measuring total protein instead of specific amount of Ig. The refractometer is based on the principle that the refractive index of a solution measured depends on its concentration (Reid and Martinez, 1975; Pratt, 1997). Hemolyzed blood samples, hemoconcentration and use of plasma will give artificially high reading using this method (LeBlanc, 1986).

2.8 Level of Immunoglobulins

Immunoglobulin-deficient calves can be indicated by low total protein level since immunoglobulin fractions make up approximately one-third of the total protein (McBeath *et al.*, 1971). Indirect assessment of serum immunoglobulins by using refractometer provides useful information on immunological status of the calves (Reid and Martinez, 1975). Calves with serum TP level less than 5 g/dl have minimum absorption of colostrum, 5-6 g/dl are marginal, whereas calves with TP concentration more than 6.0 g/dl is considered to have obtained adequate amount of colostrum (LeBlanc, 1986). Mortality rate decreased as serum TP increased from 4.0 to 5.0 g/dl, a small improvement from 5.0 to 6.0 g/dl and no improvement in mortality rate as TP level over 6.0 g/dl (Donovan *et al.*, 1998).



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