Colostrum:
Properties, Functions, and Importance.

The Relationship Between the Immunoglobulin Concentration in Holstein Colostrum and the Total Serum Protein in Holstein Heifer Calves.

Honors Thesis
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PASS WITH DISTINCTION
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Colostrum: Properties, Functions, and Importance

Neonatal mammals require milk for growth and survival, but colostrum is of particular importance for newborns. Colostrum is the first milk provided by the mammalian mother to its offspring. The ability of the offspring to suckle this milk within a few hours after its birth determines which newborns are strong and able to survive and which newborns will not be able to live without further assistance (5, 13, 14). Many studies have been done on the subject of colostrum consumption to understand the relationships and processes of the first milk to the health of the neonatal mammal. It has been determined that different mammals, even within species such as dairy cows and beef cows, have different colostrum qualities and compositions (4, 25). In this paper, many aspects of dairy cow colostrum will be discussed: how the colostrum differs from mid-lactation milk and the importance of the differences, the health effects of colostrum quality on neonatal calves, testing techniques, feeding styles, absorption, and both the external and internal influences on colostrum quality and colostrum absorption in the calf.

Holstein cow colostrum differs from mid-lactation milk in a variety of ways. Colostrum has on average, 6.7% more fat and 2.3% less lactose than mid-lactation milk (14). Calves are born without stored energy reserves and need the colostrum immediately for energy. The higher lipid content of colostrum allows the newborn calf to rapidly gain strength. Vitamins and minerals (except iron) are in higher percentages in colostrum than mid-lactation milk to provide the first necessary co-factors for future digestion, absorption, and utilization of nutrients (3, 7, 11, and 14). However, it is the protein content at 14% that is the most valued component of the milk to help prevent morbidity or mortality. The protein in the colostrum not only consists of the mid-lactation protein casein at 4.8%, which is needed for muscle growth, protein synthesis, and energy, but also, immunoglobulins (Ig) at 6.0% (14). Immunoglobulins (Ig) are the most important proteins when it comes to colostrum composition. Immunoglobulins are macromolecule proteins that can be found in five varieties, four of which are found in dairy cows. These four proteins are IgE, IgA, IgM, and IgG (many types of IgG have been discovered) in varying amounts and with different antibody functions (15). Immunoglobulin proteins are absorbed through the small intestine into the lymphatic system and further into the blood become antibodies needed to protect the calf's health against environmental pathogens (5, 7, and 14).

In adult ruminants, nutrients are absorbed after full digestion breaks the feedstuffs down to simple molecules. Yet, as colostrum is different in composition, it is also absorbed differently from mid-lactation milk and dry feedstuffs. The method for absorption of Ig proteins is unique.
The entire protein molecule, without digestion destroying its form or capabilities, passes through the cells on the mucosal epithelial membrane of the small intestine by pinocytosis and passive transfer into the lymphatic system. Later, the Ig are transferred through the thoracic duct into the bloodstream where they become active antibodies (8).

The time sequence in which immunoglobulins are absorbed can be examined in two different ways. The timing of immunoglobulin absorption is dependent upon how soon the colostrum is fed before closure occurs. This idea requires data about the concentration of large protein macromolecules from the first colostrum (colostrum from the first milking), the quantity of the colostrum, and the time at which the newborn dairy calf consumes the colostrum (7, 9, 13 and 22). The second method which to observe the efficiency of Ig absorption can be by the order the immunoglobulin(s) through the intestinal epithelial. This concept can be related to the concentration of Ig in the milk and the time of colostrum consumption as well (8, 10 and 23).

The period of time during which Ig absorption through the small intestine occurs and the efficiency of the absorption are difficult to determine exactly because of the many external and internal influences in the calf's surroundings. Indirect measurements (i.e. serum samples to be used to measure Ig in the blood), special circumstances with environment and calving, and the colostrum quality all effect how quickly the immunoglobulins in the milk are transported into the lymphatic system and further into the bloodstream. The epithelial mucosal cells in the small intestine can only absorb Ig for a limited period of time before closure occurs. It is essential to feed colostrum for Ig transfer as soon as possible, though for a short period of time, prolonging the absorption of Ig is possible by completely withholding colostrum and only feeding glucose for energy (9, 21). Studies disagree about the time at which closure is complete. Closure rates vary between 0 - 24 hours to 0 - 48 hours depending upon the study. Yet, it is agreed that the Ig concentration found in the blood serum of tested calves was significantly higher if the colostrum was fed before 6 - 8 hours of age then that fed by 12 hours of age. A distinct, linear decrease in absorption can be observed (7-10, 22 and 23). In a 1982 study performed in Canada, it was determined that calves fed 2 liters of colostrum before 6 hours had a 65.8% absorption of Ig into the blood serum and at 12 hours that absorption had decreased to 46.9%. By 36 hours of age, absorption had dropped to as low as 6.7%. This is a linear decrease in absorption efficiency from 2 - 20 hours of age after birth with an average absorption efficiency of 44% between 2 - 7 hours after birth (10). Another study indicated however, that if a ruminant does not receive colostrum immediately, but rather only ionic molecules and glucose, that the closure of the intestinal mucosal cells could be delayed for a few hours (9). A different study had data that
indicated that the rate of absorption was rapid for the first four hours after ingesting colostrum, but the passive transmission of macromolecules decreased quickly after the initial activation of pinocytosis was started and the finite number transfers utilized (10). This suggests that though the closure may occur after a particular point in early life, stimulation by protein transfer is also important for signaling pinocytosis. At the same time, the rate of Ig transfer is less than that of the calves fed before 12 hours of age and has been shown to be only 44.3% efficient at the first feeding (9).

The order of which the immunoglobulins are absorbed has also been studied. The pinocytosis of the four immunoglobulins, IgE, IgA, IgM, and the many IgGs, can be compared by the concentration of the macromolecules in the colostrum, the amount of absorption that occurs to the blood (indirectly measured through serum samples), and the time at which they are imbibed by the calf. IgG is the most prominent immunoglobulin, ranging from 70 - 80% of the Ig concentration in colostrum. IgM has the second highest concentration levels at around 20%, followed by IgA, and lastly IgE. IgG is absorbed the first and has the highest rate of absorption efficiency due to the concentration level in the colostrum. IgG can be absorbed through the epithelial cells from 21 – 27 hours if the calf is fed immediately after birth and if colostrum is not fed for 12 hours, IgG can be absorbed up to 33 hours at a lower rate of efficiency (8, 9, 10 and 12). Immunoglobulin M is an important antibody to prevent infections from occurring. With 20% of the Ig protein concentration in the colostrum, IgM has the second highest absorption rate, but less time to be absorbed. Immunoglobulin M only has 16 - 26 hours for absorption if fed immediately after parturition or 31 hours if the colostrum is not fed until 12 hours after birth. Immunoglobulin A has even lower concentrations of colostrum protein than IgM and less IgA is transferred for this reason. However, with less concentration to stimulate the epithelial cells with, there is more time for absorption. Immunoglobulin A has 17 - 24 hours to be transferred to the lymphatic system if fed immediately, or 32 hours if colostrum is withheld for 12 hours (8, 10). Immunoglobulin E is found in such small amounts that it is mentioned only because it is "found in very low levels (15, pg. 25)" but it is still important for calf health. The amount of the immunoglobulin transferred and the time period in which it is carried to the lymphatic system are yet to be researched (15).

The mechanism of immunoglobulin absorption in the small intestine is closely related to the rate of passive transfer. As the cells the epithelial mucosal layer of the lumen start to close between 0 - 48 hours of neonate age, the rate of absorption of all immunoglobulins is decreased linearly. The concentration of the Ig and the closure time for each allows for different levels of
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the immunoglobulins to be absorbed. Even though many studies argue about the true time of closure, there is a general agreement that adequate quantities of quality colostrum should be provided for the neonate before 6 - 12 hours of life (5, 7-10, 12-17, and 22-25).

The quality of colostrum and the effects of adequate immunoglobulin absorption through efficient passive transfer of all macromolecule varieties in the small intestine, have dramatic results on calves' health. Without the absorption of the Ig into the lymphatic system and later, the blood for antibodies, calf health has a greater tendency for sickness and death. Without adequate immunoglobulin absorption or TP, Holstein heifer calves will be more susceptible to disease and environmental pathogens such as septicemia, enteric-toxemia or enteritis, salmonellosis, pneumonia, diarrhea, colisepticemia, other escherichia coli infections, and many more (1, 5 and 15).

Higher quality colostrum indicates that the density of the immunoglobulins within the milk is large enough for calves to receive the required 100 g of Ig. The suggested amount of colostrum is on average, 2 liters. These numbers provide adequate immunity to protect against environmental pathogens and fewer sick and dead calves are seen (1). With a high concentration of immunoglobulins, the activation of pinocytosis and intestinal cells allows for rapid and efficient transport of adequate Ig across the intestinal mucosal layer into the lymphatic system. With a low concentration of Ig, fewer immunoglobulins reach the intestinal brush border for pinocytosis transport. The lowered number of macromolecules reaching the small intestine and the fewer immunoglobulins being transported once absorption is activated makes adequate TP is difficult to achieve (9, 10, 13, 23 and 24).

When one dairy lost over 50% of new heifers calves and the rest of the calves grew slowly and were frequently ill, it was determined through colostrum testing that the immunoglobulin levels were low. Low immunoglobulin levels do not provide enough macromolecules for absorption and the results can be economically devastating. There are two scenarios that happen to calves without adequate immunity. First, the calves will grow slower and be unproductive 'runts' or after the first week of life, the calves will lose appetite and eat poorly, grow longish hair on the face and head and the eyes will appear to sink into the head. They will become weak and unable to get up and/or stand and finally die through starvation or dehydration. For adequate amount of colostral immunoglobulins to be absorbed, the quality and quantity of colostrum should be monitored as the colostrum quality varies from cow to cow (16).

Feeding styles have been researched to determine which method of colostrum feeding provides the best chance for calf survival. Several feeding methods are available and can be
divided into two styles. The first style is natural suckling. The calf stays with its mother for two days until the cow has stopped producing colostrum. This method has worked for thousands of years with animals in the wild, however with the new genetically improved animals, this often does not allow for the calf to receive large amounts of immunoglobulins (1). Dairy cows have been bred to increase milk production and start producing milk as soon the oxytocin from parturition reaches the mammary gland for milk letdown (19). This increases the milk quantity and decreases quality as the density of the immunoglobulins in the colostrum decreases (5). Calves can only ingest so much milk and if allowed to stay with their mother, they typically do not drink enough colostrum to provide the necessary immunoglobulins. Failure of passive transfer with suckling has been recorded as high as 61.4% (1).

The other methods of feeding neonatal calves are artificial, with a bottle, bucket, or an esophageal tube feeder (1, 5, 9). Here the colostrum quality can be tested for, the amount of milk fed is known, and the cow is separated easier from her offspring. Bottle-feeding is the most natural way to feed as the calf, if healthy, is inclined to instinctively suck on anything when it is hungry. This allows for the use of the esophageal groove so that the milk passes to the omasum and further to the abomasum. Bottle feeding takes a long time when trying to get a calf to ingest the required amount of colostrum and even longer if the calf does not want to drink and failure of passive transfer can be around 19.3%. Bucket feeding takes time with training, but also allows for the sucking instinct to close the esophageal groove for the colostrum to reach the abomasum. However, the work required may not be worth natural feeding style (1, 9). The quickest way to artificially feed multiple dairy calves is to use the esophageal tube feeder. The long tube is slid down the esophagus to the reticulum and then the colostrum is either gravity forced or fed down the tube. This is quick, easy, and insures the adequate and necessary amount of colostrum is ingested. However, it also places colostrum into the rumen, which smaller at birth in ratio to an adult bovine (<16% of entire stomach), and will place >50% of the milk in the abomasum and small intestine for absorption. In a few cases, colostrum in the rumen will ferment and cause problems with diarrhea or by denaturing the protein and destroying the special properties before absorption can occur. Yet, it is the best way to prevent low antibody count in the calf serum and has a failure of passive transfer rate of 10.8% (1, 11).

Testing is done for two main reasons when colostrum is considered. There are tests to examine the density of the immunoglobulins in the colostrum and there are the tests to determine if the immunoglobulins were absorbed into the bloodstream of the neonate. Testing colostrum is an important first step in assuring the calf's future health. If the density of Ig in the milk is too
low (< 1.056), inefficient absorption will occur and the amount of total serum protein (TP) will
equate in the blood to prevent illness, morbidity, or mortality (1, 3, 5, 7, 17 and 22-24). There are two main tests for determining the density of Ig in dairy colostrum. Though other tests
do exist, they are used more frequently on equine colostrum (4).

The most accurate test is Radial Immunodiffusion Technique (RID) and the second less
accurate form of testing is known as either a Colostrometer or a Hydrometer. Radial
Immunodiffusion Technique is an agar gel precipitation quantification method for soluble
proteins. The results are most accurate when used on blood taken from calves younger than a
week of age and older than 24 hours (27).

The Colostrometer is less complicated and less accurate due to environmental and human
error. A slender tube, weighted at the bottom with a sized piece of metal in a bulb-like end, the
Colostrometer is divided into three sections and colored for ease of use. The first or top part of
the meter is for low density or poor quality colostrum, the middle section for good colostrum,
and the bottom for high-density colostrum or excellent. The range at which the sections are
placed and the colors vary with producer. The instrument is dropped into a narrow cylinder
filled with colostrum, which is older than one hour and less than three hours of age. At one hour,
the colostrum contains too much air from the milking machine and the reading will be low due to
the decreased density. After three hours, the colostrum starts to separate and must be stirred to
mix all the nutrients together. Mixing the colostrum and then testing for Ig concentration will get
comparable results to the 1-3 hour old colostrum but test results may be influenced if the milk is
not mixed completely. The colostrum should be between 72 - 76 degrees Fahrenheit for the
proper density as well. Below 72 degrees Fahrenheit, the colostrum density reading will be
higher than the actual density and poor colostrum will appear adequate or good. A temperature
above 76 degrees Fahrenheit will cause the colostrum to have a lower density reading that is also
misleading and could cause the disposal of adequate or good colostrum. From this information,
the extent of human error can be understandably large. Environmental problems such as the
humidity, air temperature, and thermometer inaccuracies must also be taken into effect when
using this particular test. Easy to use on the farm, errors could lead to calf death with poor
colostrum quality being delivered. In a study performed by Pritchett, it was determined that the
correlation coefficient of the Colostrometer to RID had an accuracy of negative .469. This
indicates that the colostrum is more likely to be a false high rather than a false low and calves
would receive more poor quality colostrum due to inaccurate reading (16).
There are a great number of tests to determine the absorbed Ig density in the calves' blood, or more correctly, tests for the failure of Ig absorption in the calves' blood. Total serum solids and plasma fibrinogen determinations can be measured with refractometer. Albumin and globulin, total serum proteins, glutaraldehyde coagulation, sodium sulfite precipitation, and Enzyme Linked Immunodiffusion Assays (ELISA) are all tests performed by test kits. The zinc turbidity test has established testing methods. However, none of these tests are as accurate as the RID or TP tests (4). When these tests were examined for accuracy, the comparison was done with RID (92 - 98% accuracy) or by TP using only the IgG concentration (4, 27).

Radial Immunodiffusion Technique is used as a comparison due to the accuracy of the readings but takes time, laboratory equipment, and laboratory skills to perform. Total serum protein is often used as well as a comparison and uses a refractometer to determine the IgG density. The amount of light refraction passing through the serum is proportional to the amount of dissolved solids, most of which are protein and thus provides a good measure of TP in the blood serum (18).

Some methods are more readily adapted to farm use while others need more specific conditions. Zinc sulfate turbidity has an accuracy of 63.2% to 68.86% when testing for failure of passive transfer and can be tested with established methods. One zinc sulfate turbidity assay that can be used on the farm utilizes distilled water from a sealed package, 0.1-mL aliquot of blood serum and 6 mL of zinc sulfate solution in a 13 x 100-mm borosilicate test tube. Allowed to incubate for an hour at 23 degrees C, the reaction is determined by the clarity of the solution. A positive reaction or adequate Ig concentration causes the solute to be translucent and a newspaper cannot be read behind the tube. A negative reaction or low Ig concentration in the calf blood serum remains clear and the reading of the newspaper behind the tube is possible (18).

Sodium sulfite turbidity has an accuracy of 86.3% when determining that the IgG concentration is inadequate, but the accuracy decreases as the concentration of the IgG increases in the blood serum. The highest concentration in a particular study was read accurately 37.07% of the time (4). Purchased test kits can be used on location to measure colostrum density. One particular assay uses solutions of 14%, 16% and 18% sodium sulfate solutions in distilled water. 0.1 mL serum is placed in 3 borosilicate tests tubes with 1.9 mL of each solution and incubated for 15 minutes at 23 degrees C. When held up against newspaper, a negative or 0 reading was determined when all test tubes were free from turbidity. A +1 was determined when the 18% solution was translucent and the 14% and 16% solution tubes were clear. Both the 16% and the 18% solution tubes being translucent and the 14% solution being clear determined +2. +3 was
read when all solution test tubes were translucent and a newspaper could not be read behind the tubes (18).

Glutaraldehyde coagulation tests (GCT) have mixed results. Tennant states that the Glutaraldehyde Coagulation Test "appears to have practical application in preventive medical programs designed to identify calves with hypogammaglobulinemia (26, pg. 848)" and can be accurate in reading inadequate IgG concentrations in blood serum. However, it is questionable about the accuracy of the GCT kits when Tyler determined that serum IgG concentration was associated with time and the strength of this association was minimal (28). This particular test requires a specific amount of whole blood being placed into the test kit assay tube and mixed with the glutaraldehyde reagent. After mixing the two fluids, if the blood coagulates there is adequate protein in the blood as 80% of the Ig protein in blood is IgG. When the blood does not coagulate, then the protein concentration is low and the passive transfer was not great enough to prevent morbidity and mortality (2, 28).

Other tests such as the TP and the albumin concentration tests can be determined from chemistry analyzer tests and globulin concentrations calculated by subtracting albumin from the TP. Following the manufacturer's instructions, ELISA test-kits can be accurately used as well (4).

Testing is a good way to determine the quality of the colostrum being fed, but improving the colostrum provided from the cows in the field may be necessary if all the colostrum qualities are coming in low or with inadequate Ig concentrations. There are many external and internal influences that can effect the quality of the colostrum provided by the dam to the offspring (3, 5 and 17). External factors include time of calving to the first milking, possibly dry period length and lactation number, and feed quality. Internal factors can be seen through vaccinations, nutrition, and general health with stress (3, 17).

For the dam, many external factors can decrease the colostrum quality. The length of time that it requires between calving and the first milking affects the density of the immunoglobulins in the colostrum. Immunoglobulin G in colostrum is transferred across the mammary membrane similar to the absorption process in the calf's small intestine with pinocytosis for a limited amount of time (21). Immunoglobulins M and A are produced in the mammary gland (15). After parturition, waiting eight hours to be milked can decrease the colostrum Ig density. The colostrum density starts to decline as the udder drips and the milk production increases. Dry length period has not been correlated with colostrum quality but good nutrition during the dry period has definite influences upon the content (3). With severely poor
nutrition over time (whether low energy or nutrient deficient), the quantity of the colostrum can be decreased and quality lowered as well. It was observed that cows that gave birth to their calves after poor forage during the dry period had less weight and body condition and gave poorer quality colostrum (17). However, it has been shown that a moderate 15% decrease in feed quantity had no effect upon either the colostrum or the birth weight of the calf (3).

Internal factors such as stress from calving, weather patterns and temperatures have some affect upon the dams' colostrum but observations have not determined how much. A cow with twins has higher stress and the colostrum tends to have less concentrated protein content (4). It has been assumed that the larger the lactation number, the more antibodies the cow is likely to harbor and the greater the number of Ig that can be passed onto the colostrum. This is not always true and the colostrum of all fresh cows and heifers needs to be tested before any decisions are made. First time milkers can often have just as good of colostrum as fourth or fifth time milkers if the heifers are in good health (4, 17). The health of the cow at time of parturition can have a significant effect upon the quality of the colostrum. A sick cow with high stress due to late gestation, parturition, and early lactation can be weaker than normal with fighting off the infection and by the maintaining other bodily functions (4, 5 and 21). This decreases both the quantity and the quality of the colostrum produced in the udder. Genetics from mother to daughter have little or no effect upon colostrum quality (4).

It is not all the dam's problem when a calf can not absorb the necessary macromolecule proteins. Calves can have decreased absorption rates because of external stimuli from stress of birth, separation from the mother, disease, and weather. Internal factors often include genetics and the strength at birth to stand, be hungry, suckling instinct, and willingness to eat (3, 5 and 25).

Mothering effects have been proven to increase the TP in the blood in a few studies. Calves left to suckle their dams or left with their dams not suckling, have been shown to benefit from the mothering, however the results have never been produced again with similar experiments (25). Seasonal variations have affects on absorption as well. Failure of passive transfer is higher in winter months as the colostrum quality may decrease due to increased nutritional requirements, farm management practices of pulling the calf immediately away from the dam, and temperature stress preventing inadequate consumption and afterwards, absorption. Internal problems from the calf can involve, tough birthing, temperature stress and physical weakness that can lead to the unwillingness of a newborn to nurse. Two of 11 Holstein calves who failed to receive colostrum due to inappitence had failure of passive transfer (13). Weak
calves from either twins or just significantly weaker calves tend to show lower amounts of TP in the blood than stronger calves with a probability of 0.05 (3).

Colostrum is extremely important for neonatal calves and there are many influences that can affect the quality and quantity of the milk. The dams' health, stress, and seasonal variations with weather all affect the colostrum before it ever reaches the calf. How well the calf absorbs the colostrum is also a factor in maintaining calf health. The quality of the colostrum can be tested before the calf ever receives its nutritional benefits through either natural or artificial feeding. Though this can help insure the calf receives the suggested requirement of 2 liters of colostrum or 100 g of Ig, the calf itself affects the amount of Ig which make their way into the blood stream to form antibodies. The amount of TP can be measured in the blood to determine whether adequate absorption occurred. Yet, all this information is useless if one well-known fact is ignored. Feeding the neonatal calf adequate amounts of colostrum immediately after birth prevents mortality and morbidity.
The Relationship between the Immunoglobulin Concentration in Holstein Colostrum and the Total Serum Protein in Holstein Heifer Calves.

Abstract:
This experiment was designed to determine the effects of Colostrometer tested colostrum on the total serum protein (TP) of Holstein heifer calves. The experiment was performed on a working dairy where 40 – 50% of the heifer calves had been dying because of poor colostrum quality. For three months, May 12, 1999 through August 10, 1999, the heifer calves born were fed 4 L of first colostrum through an esophageal tube feeder within 8 hours of age. The calves were given injections of 1/2 cc BoSe, 1 cc Vitamins A&D complex and 2 cc Nasolgen with the first feeding. Colostrum was tested with a Colostrometer or Hydrometer between 1 and 3 hours of age and between 72 - 76° Fahrenheit for immunoglobulin (Ig) concentration. After day 1 of age and before 7 days of age, 3 cc of blood were drawn from the calves necks' and placed into red topped tubes for TP testing with refractometer techniques. The final results indicated that using colostrum tested to have a higher Ig concentration with the Colostrometer improved the TP of tested calves. However, the data did not show a strong relationship between the two variables as both human error and external factors were involved.

Introduction:
It is known that if neonatal calves do not receive colostrum quickly or in great enough quantities, health problems are seen with disease and/or diarrhea and sometimes have irreversible effects (5, 7, 8, 9, 12, 13, 14, 17 and 22 - 25). Colostrum contains more fat, protein, vitamins and minerals (except iron) than mid-lactation milk and it is this composition that makes colostrum ingestion upon birth so important to neonatal calves (11, 14). The larger volume of fat provides energy required for a newborn calf to survive and the vitamins and minerals are important cofactors for future digestion and nutrient absorption, but it is the different protein components that are the most important constituent in the first milk (11). Colostrum not only contains the protein casein found in mid-lactation milk, but protein macromolecules known as immunoglobulins (Ig) (12, 14 and 15). These immunoglobulins are absorbed without degradation through the small intestinal mucosal cells by pinocytosis in a process that ceases after either a finite number of protein molecules have been transported, or a limited amount of time passes (21). Many studies and experiments have been performed to determine the amount of colostrum required by calves.
for adequate absorption, the concentration of the Ig in the colostrum for adequate absorption, and the time at which colostrum needs to be fed (1, 5, 7–10, 12–18 and 22–25).

Though the numbers vary from trial to trial with wide parameters, a few key ideas have been determined. Colostrum can be fed up to 12 hours before absorption decreases to an inefficient level and it is important that enough Ig are present in the colostrum for adequate absorption. If calves are provided with a larger amount of colostrum soon after birth (natural suckling), the concentration of the macromolecule proteins is less important as a healthy, hungry calf can imbibe several liters of milk before closure occurs (5). Yet, natural suckling does not always work as most dairy cows have colostrum quality of Ig<35 g and calves frequently do not imbibe enough colostrum for adequate amounts of Ig (16). If only a limited amount of colostrum is presented at birth to the calf, then the concentration of the immunoglobulins in the milk becomes very important (13, 22). Calves require 100 g of Ig before the intestinal mucosal cell are unable to perform pinocytosis (5, 13 and 14). The more concentrated the Ig in the first colostrum, the better the absorption rate will be as Ig are transported quicker within the first four hours after feeding and absorption decreases at a linear rate from 2–20 hours (7). Matte et al observed that absorption percentage declined rapidly to 46.9% absorption at 12 hours and only 6.0% absorption at 48 hours. McCoy et al states however, that the epithelial cells are impermeable after 24 hours of age and absorption can not occur by 3 hours. The fact remains however, that when calves are fed within 12 hours after parturition, higher concentrations of Ig in colostrum can increase the TP or antibodies seen in the blood above the safety level of 4.8 g/dL TP (7).

The concentration of Ig in the colostrum is known as colostrum quality (14). The higher the concentration of proteins, the greater the number of immunoglobulins that can be absorbed by the calf's intestinal epithelial cells to increase blood antibody levels. Thus, a high Ig concentration in the colostrum can result with a high TP, though this is not always the case (3). There are several methods of testing colostrum and though some methods have been proven to be accurate, they can be expensive and require specific machines (4). A simpler way to measure colostrum is by using the Colostrometer. However, using a Colostrometer is not as accurate. Human error with colostrum temperature, thermometer reading, impatience, the age of the colostrum, and reading the instrument can all cause inaccurate readings. Most often the Colostrometer is read so that poor colostrum is thought to be good and this can cause a problem with providing calves with adequate immunoglobulins. Environmental factors such as humidity and the area where measurements are being taken may also affect the readings (16).
experiment was designed to determine if Colostrometer tested colostrum could raise the total serum protein to safe levels for good health in Holstein heifer calves.

**Methods and Materials:**

For three months on a working dairy farm, May 12 through August 10, 33 neonate Holstein heifer calves were placed in separated stalls immediately after birth and were fed 4 L of Colostrometer tested colostrum within eight hours of age. Colostrum was tested with a Colostrometer or Hydrometer and divided into two categories. The first category of colostrum was colostrum for heifers and had 50 g of Ig or greater. It was labeled with the cow's number, Ig concentration and date with red duct tape. The colostrum that was tested below 50 Ig concentration was marked with the cow's number, Ig concentration and date in green duct tape and fed to the bull calves (16). The information on the tape was recorded on a spreadsheet as the heifer calves were fed. Colostrum feeding was a random process though all colostrum was fed before seven days of age. At the time of feeding, calves were also given 1/2 cc BoSe, 1 cc Vitamins A&D complex and 2 cc Nasolgen. If the colostrum was available, 2 L more of colostrum was fed to the calves within six hours of the first feeding, but this factor was not constant. Between days 1 and 7, 3 cc of blood was taken by sterile syringe from the neck and placed into red topped tubes for coagulation. The blood was removed from the farm by the local veterinarian for refractometer testing to determine the TP concentration, an indirect measure of the total amount of immunoglobulin absorption that had occurred. Statistical analysis was calculated using SigmaStat Statistical Solutions Version 2.0 and Student's t-test with two variables.

**Colostrum testing with Colostrometer or Hydrometer:** The Hydrometer is a tool used to test the colostrum immunoglobulin density (16). It is a long slender tube with a bulb on the end with a specifically measured amount of metal in the bottom to allow it to sink inside a graduated cylinder filled with the sample colostrum. Inside the slender tube, a rolled up piece of paper has the Ig measurements printed upon it. “Poor” quality colostrum or low Ig density colostrum measurements are on top of the column and are labeled from 0 Ig to 50 Ig with a pink background. The “good” colostrum measurements are located in the middle of the labeled column from 50 Ig to 90 Ig with a green background. “Excellent” colostrum is located on the bottom from 90 Ig to 140 Ig and has a yellow background. The idea of the Hydrometer/Colostrometer is that the greater the Ig density, the more the tool will float upward and thus measure the density of the colostrum. However, particular conditions need to be met before testing the colostrum for accurate results. The colostrum should be older than one hour so
that the air from the milking machines has a chance to dissipate. If the colostrum is older than 3 hours, it will have started to separate and will need stirring before testing so that the different densities have a chance to be measured at one reading. The colostrum needs to be between 72 and 76° F so that the colostrum will not give an incorrect density test. If the colostrum is too cold, then the density will be greater and a chance for bad colostrum to be recorded as good will occur. If the colostrum is at a greater temperature than 76° F then there is the possibility of good colostrum to be read poorly and having it thrown away (16).

**Total Protein:** The test for TP in the blood determines the amount of immunoglobulins that are passively transferred across the calf’s intestinal tract. Total serum protein was tested for by drawing blood from each calf as cleanly as possible and then allowing for the blood to clot in a red topped tube. After clotting, the blood serum was placed on the refractometer instrument, which measures the amount of refraction of light passing through. Since the refraction is proportional to the amount of dissolved solids, most of which are proteins, the readings provide accurate measurements of the TP in the serum (18).

**Results:**

Feeding higher quality colostrum to the heifer calves prevented all but two deaths in the 3-month period, but as the experiment was placed on a working dairy farm, the data is questionable with too many external factors and human influences. Table I shows eight colostrum quality groupings with ranges of 25 g of Ig, the number of calves fed within the colostrum group, and the average total protein in the groups with the high and low TP measurement. The data indicates that the higher the average colostrum quality fed (including the effects of external and internal factors), to the calves, the higher the average TP in the blood. Two calves died of pneumonia and/or respiratory problems. These two calves were fed lower quality colostrum, but had TP measurements well above the minimum 4.8 TP measurement and should have survived (5, 9, 13 and 17).

Using SigmaStat for calculations, the calves randomly fed the higher quality colostrum had a higher total protein (P<0.019) than calves fed the lower quality colostrum though the regression correlation is weak with a calculated 0.0992 (Graph 1). Overall, the total serum protein was improved (Table 2) (20).

Before statistically analyzing the data, a few calculations were necessary. All calves received 4 L of colostrum within 8 hours of birth. However, some calves also received 2 L more colostrum around 6 hours after the initial feeding. As calves absorb most of the Ig within the first four hours after feeding, the second colostrum feeding was cut from the data and not used in
calculations (27). The average Ig concentration of the first 4-L of colostrum was then calculated. After determining the mean of the first colostrum fed, the calves were then divided into two groups. The calves fed the higher quality colostrum with 80 Ig or greater (G1) had 17 TP data points. The calves fed the lower quality colostrum with 79 Ig or lower (G2) had 16 TP data points. Using the TP measurements, the average TP of the two groups was calculated. The first group had a total protein level of 5.776 and a standard deviation of 0.519. The second group had a TP average of 5.350 and a calculated standard deviation of 0.463 (Table 2) (6, 20).

Using the student t test with \( t = 2.484 \) and 31 degrees of freedom, the calculated probability was 0.019 above results happening due to chance. The 95 percent confidence level for the difference of means was 0.0763 to 0.777 (Table 2). Though these results pass the statistical tests, the information needs to be looked at carefully before determining whether the experiment was successful (6, 20).

**Graph 1:**

![Graph 1](image)

**Table 1:**

<table>
<thead>
<tr>
<th>Group Number</th>
<th>Average Colostrum Quality, First Feeding in the group</th>
<th>Number of Feedings</th>
<th>Average Total Protein</th>
<th>High Total Protein</th>
<th>Low Total Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>100 - 124</td>
<td>6</td>
<td>5.5</td>
<td>6.3</td>
<td>4.7</td>
</tr>
<tr>
<td>2</td>
<td>125 - 149</td>
<td>5</td>
<td>5.4</td>
<td>5.8</td>
<td>4.9</td>
</tr>
<tr>
<td>3</td>
<td>150 - 174</td>
<td>5</td>
<td>5.6</td>
<td>6.1</td>
<td>5.4</td>
</tr>
<tr>
<td>4*</td>
<td>175 - 199</td>
<td>7</td>
<td>5.2</td>
<td>6.0</td>
<td>4.8</td>
</tr>
<tr>
<td>5</td>
<td>200 - 124</td>
<td>8</td>
<td>5.8</td>
<td>6.8</td>
<td>5.2</td>
</tr>
<tr>
<td>6</td>
<td>125 - 149</td>
<td>1</td>
<td>6.8</td>
<td>6.8</td>
<td>6.8</td>
</tr>
<tr>
<td>7</td>
<td>250 - 274</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>275 - 299</td>
<td>1</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
</tbody>
</table>

*Death occurred due to respiratory illness.
Table 2: **Student t-test Calculations**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (high)</td>
<td>17</td>
<td>5.776</td>
<td>0.519</td>
<td>0.426</td>
</tr>
<tr>
<td>G2 (low)</td>
<td>16</td>
<td>5.350</td>
<td>0.116</td>
<td></td>
</tr>
</tbody>
</table>

$t = 2.484$ with 31 degrees of freedom. $(P = 0.019)$

95% confidence interval for difference of means: $0.0763 - 0.777$

Power of performed test with alpha $= 0.050$: 0.593.

$-> 0.593$ is below the desired power of 0.800.

**Discussion:**

Studies show that by increasing the quality of colostrum fed to neonatal calves above 100 g of Ig can drastically improve their chances of surviving environmental pathogens. Though, greater concentrations of macromolecules in a predetermined volume of first colostrum will increase the calf’s chances of adequate passive transfer, other factors also effect the absorption (1, 5, 7 – 10, 12 – 18 and 22 – 25).

The low total serum protein in the first heifer calves was increased in later calves with increasing colostrum quality, but the increase, though significantly different $(P < 0.019)$ was low. Calves fed the lower quality colostrum could have had higher total proteins than the calves fed the higher quality colostrum, and some G1 calves did have an average TP above the G2 calves (Table 1). Reasons behind this lower TP with higher fed Ig colostrum can be related to colostrum quality and its quality measuring techniques, the feeding methods and time of feeding in some cases, and the stress upon calves with the efficiency of absorption (3, 16).

In this experiment, the Colostrometer was being tested to determine if the instrument could be used successfully to increase the TP of heifer calves. The Colostrometer is not completely accurate and many human errors and technical errors can be found with its measuring technique. The temperature of the colostrum needs to be measured first and be between 72 - 76° F. An accurate thermometer and patience is required to get a true reading. Often on a working dairy, these two items are in short supply. Human error with reading the thermometer and the Colostrometer can affect the quality of colostrum being fed to calves. A study determined that the Colostrometer was only 26% accurate above 50 mg of globulin/mL and 67% sensitive with lower Ig values (16). So, although the colostrum was thought to have a greater concentration of Ig, the true density is questionable. Yet, as the difference between the two groups was significantly different and the average TP increased with the measured quality and worked well enough to be worth the time and effort.
Calves were fed through an esophageal feeder before 8 hours of age. Colostrum was in short supply for a while when the quality was low and an attempt was being made to increase the colostrum Ig density with cow nutrition, health, and milking times. As the quality increased, the time to feeding decreased. It has been shown that calves not fed colostrum or any protein feed in the early hours after birth retain the ability to absorb adequate amounts of Ig before closure is completed if colostrum is provided within 12 hours (8, 10 and 12). The feeding of the 2 L of colostrum a few hours after the initial feeding could have had some effect upon the results, but not enough to alter the data significantly. The most active absorption and the greatest absorption efficiency occurs for four hours from the first feeding and closure increases linearly from 2 – 20 hours after (7, 27).

Feeding styles may have affected the colostrum absorption however, as the esophageal feeder places the colostrum into the rumen, bypassing the esophageal groove to the abomasum. Yet, this feeding method has been shown to have the highest results in reducing failure of passive transfer and as the rumen is small and undeveloped, most of the milk passes to the abomasum automatically (1).

External environmental factors and internal animal factors also affect the efficiency of colostrum absorption by the calf. Birthing stress, illness, weather, genetics, and multiple births are factors that can all affect the rate of colostrum absorption and the quality of colostrum for absorption. A rough birth or twins stresses the dam at parturition and lower immunoglobulin levels are seen in the colostrum while the calf or calves suffer due to the pressure of parturition and general weakness from placental starvation. Illness has been shown to lower colostrum quality and the calf then suffers from low Ig concentration and weakness from possible intra-uterine infection. Weather and seasons affect both the dam and calf as the mothers produce fewer immunoglobulins in the winter and the calves weaken with cold faster. Genetics, though they have been proven to have little effects upon the dam's colostrum, help determine if the calf will be strong enough and have the drive to suckle and survive (4). With these factors in mind, it is necessary to test both the colostrum and the calf blood for adequate transfer of colostral macromolecule proteins across the calf's small intestine (8, 10, 12, 13, 17 and 22).

The calves at this particular dairy were large and some required assistance at parturition. Many calves had to be pulled from the heifers and a few of the cows. This stress of calving may have lower the colostrum quality early on and late milking after birthing diluted the density of the macromolecule proteins in the colostrum as the cow had milk let-down with the oxytocin
release (19). Previously poor nutrition may have affected the colostrum quality as well by decreasing the cow's immune system with energy losses (4).

On this farm, the increasing colostrum qualities from the dams improved the TP of the calves and decreased the death loss of live births. The use of the Colostrometer to determine the density of immunoglobulins in colostrum to improve the health and TP in the Holstein heifer calves, though affected with outside factors, was successful and proves that the use of this simple tool can be affective in preventing death in replacement heifer calves.

**Conclusion/ Implications:**

This study suggests that by increasing the concentration of immunoglobulins in the colostrum will help improve calf health and place the total serum protein content above the dangerous, low level of 4.8 TP. Though testing the colostrum with a Colostrometer is not the most accurate method of testing colostrum for neonates, as readings are affected not only by human error, but environmental affects as well, the significance in this experiment indicates that the instrument is useful and worth the time and effort it takes. The Colostrometer used with care, is an adequate and inexpensive device to insure calf health.

**Thanks:**

Many thanks are owed to the people who made this project possible. Wilbur Bishop, whose calves were experimented with, Dr. Perry Senfield who tested the blood samples and brought supplies, Sonia Rivera for her help, and Dr. Thomas Besser for his patience and advice.


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Thesis Title            Relationship Between Immunoglobulin Conc. in Holstein Colostrum &
                        Total Serum Protein in Holstein Heifer Calves

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<tr>
<td></td>
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