Effect Of Conventional Or Standard Colostrum Feeding Protocol On Immune Response And Total Serum Proteins Of Newborn Nili Ravi Buffalo Calves

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INTRODUCTION

There are many immunological and biochemical adaptations are being in dams of ruminants followed to fulfill the nutritional requirements of fetus as well as newborn calves for their proper growth and development of defense system in the body. During the phase of fetal life the defense system of mother, protect and give nourishment to the fetus (Lora *et al*. 2018). In ruminants the immunity is being transferred after parturition mostly. The colostrum is a combination of secretions secreted by mammary glands near to the parturition day. The composition of colostrum varies in the sense of protein profile and immunoglobulin present in it (Uma Kant Verma *et al.* 2018). The immunity coming from colostrum i.e. passive immunity transfer PIT) is central to health, welfare and performance of newborn buffalo calf (McGee and Earley 2019).

After the birth the new born calves are protected as well as nourished through colostrum and milk provided by their mothers. This is the survival of bovine species in the environment (Hopker *et al*. 2020). The new born buffalo calves require immunity during fetal as well as after birth. The fetal and post birth life require immunity which is transfer passively by placental and colostrum respectively. Despite of well-known importance of colostrum in adequate quantity in sense of passive immunity transfer for calf health and its survival, the failed PIT remains a widespread problem worldwide in dairy industry (Nevard *et al*. 2020).

The immunoglobulin is described as a major immunity component which is transferred to the calves from their dams only through the colostrum feeding (Sala 2020). The information regarding total protein profile and level of immunoglobulin in the colostrum or milk following first week of parturition as well as the changes in the total protein in calf serum is a chief importance to understand the growth performance of calves in future reared for meat or dairy purposes (Osorio 2020). If we get knowledge about the relationship between colostrum and serum total protein we can easily understand the outcomes of future in sense of farming and about the health of calves with defense against various contagious disease which can be fatal to the calves(Turini *et al*. 2020).

The immunity being transferred from mother to calf through colostrum is referred as passive immunity. The major component is known as Immunoglobulin G content. It is about 75% of total serum immunoglobulin (Pierzynowska *et al*. 2020). The IgG provide protection and act as a major defense against newborn morbidity and mortality till the body produce own antibodies against pathogens. In the absence of **Fetal Passive Immunity Transfer (FPIT)** there are the chances of **calf diarrhea,** which is a major cause of early calf mortality. The newborn Calves with abundant PIT are in fact more resistant to communicable diseases and have higher sick and mortality rates, as well as calf diarrhea and severe respiratory diseases. There is an important epidemiological character of many viruses like infection of corona virus in newly born bovine calves that depends on the protection against diseases depends on the available antibodies of colostrum which are transferred from dam to calf. The antibodies in colostrum not fight or protect against infections but they do contributions in lumen immunity by re-secretion into the lumen of gut (Lotfollahzadeh *et al.* 2020). The **new born diarrhea** is one of the main causes of calf death rates in the early age, thus disturbing the benefit and production competence of buffalo farms (Brunauer *et al*. 2021). In most buffalo farms, the occurrence of calf deaths from birth to wean can increase lethal to more than double if calves are on such conditions (Zhu *et al*. 2021).

TP and IgG content in the first colostrum is higher in buffalo than in crossbred cows. The Individual variation in Ig content of first colostrum may be dependable for difference in propensity of calves to infection. The decrease in Ig content during subsequent milking is quicker in dairy crossbred cows. The chief class of immunoglobulin is IgG in buffalos and cows (Rossi *et al.* 2022). When a calf takes colostrum from dam it is absorbed in the gut. The level Total Protein in serum becomes increased due to absorption and then low due to metabolism of proteins. There is a strong relationship between colostrum IgG and Total protein with serum IgG and Total Protein levels (Pisoni *et al.* 2022).

The Refract-meters are suitable for estimation of colostrum quality and PIT (Akköse *et al*. 2022).It is the simple and easy to handle on-farm usage to monitor the colostrum managing plans in bovines (Manosalva *et al*. 2022). But in Nili Ravi buffalo calf no such relationship was detected through Refract-meters. The aim of this study is to adapt the best method of colostrum feeding to the young ones and the use of Refract-meters for detection of IgG content both in colostrum and serum.

Pakistan is an agriculture country and livestock is a major contributor in Economy as well as in GDP growth rate. There are a huge number of families attached with conventional dairy farming and Buffaloes are of great importance in dairy production especially in Punjab. The milk of buffalo is mostly consumed in our environment (Khan *et al.* 2022). Instead of prime importance of buffalos in Pakistan there is no such study available on Nili Ravi Buffalo newborn calves regarding relationship between colostrum quality (in sense of TP and IgG) and serum TP- IgG which are being transferred through colostrum to the young ones.

There are two methods of colostrum feeding, one is conventional method in which colostrum is fed ad libitum as well as in standard method colostrum is fed at 8.5% of calf body weight
i.e. half in morning and half in evening.

The objective of this study is to determine the best method of colostrum feeding in newly born calves of Nili Ravi Buffalo, it is assumed that the conventional method is not best method of colostrum feeding as compared to standard feeding method, as it is laborious, the quantity of colostrum in each quarter is not quantified as calf gets feeding direct through teat and there are maximum chances of calf diarrhea, if the calf gets extra colostrum feeding.

Therefore we think to adapt international method i.e. standard protocol of colostrum feeding to avoid all above mentioned problems.
To compare both methods this study was conducted for seven days postpartum on the basis of IgG levels of colostrum with calves serum IgG level, TP level in colostrum with TP in serum of the calves, Estimation of glucose level in serum of both calves groups, weight gain and Body Condition Scores of both groups separately, then correlation between IgG level, TP in Colostrum of dam and Serum of calves was checked to determine the best colostrum feeding method in newly born calves of Nili Ravi Buffalo on the basis of IgG level, TP, Glucose and weight gain in calves.

LITERATURE REVIEW

In dams of ruminants there are many immunological and biochemical adaptations are being followed to fulfill the nutritional requirements of fetus as well as newborn calves for their proper growth and development of defense system in the body (Aydogdu and Guzelbektes 2018). During the phase of fetal life the defense system of mother, protect and give nourishment to the fetus. After the birth the new born calves are protected as well as nourished through colostrum and milk provided by their mothers. This is the survival of bovine species in the environment (Uma Kant Verma *et al.*2018). The estimation of Ig contents of colostrum and serum using both on-farm testing and in a more user-friendly format companionable with a laboratory format (M. Dirikic *et al.* 2018*).*

The initial immunity of new born calves depends upon the adequate quantity, timing and quality of colostrum being fed to them. Poor quality of colostrum having less availability of immunoglobulin is contribution to failed or less transfer of passive immunity from mother to calf which leads to high chance of diseases and results in death. There the quality of colostrum and its estimation of levels of immunoglobulin at farm is of prime importance to ensure the transfer of passive immunity (Drikic *et al*. 2018). The bovine's milk is constituted of many components that are immunoglobulin in action and some have antimicrobial effect. The immunoglobulin of bovines especially IgG is under observation since 1973 and many researchers have been conducted on its potential effectiveness on immunity and various infections both in humans and calves. The IgG not only binds with pathogens but also has action on many allergens (Puppel *et al.* 2019). The level of IgG in colostrum/milk can be increased by vaccinating the dams to get hyper-immune. This hyper-immune colostrum is enriched strongly for IgG who recognizes the pathogens against which vaccination was done (Ulfman *et al.* 2018). Main cause of death neonates due to very low level of passive immunity being transferred from dam to calves in early hours of parturition (Souza 2019).

The total protein of colostrum or milk profile changes is most important to know the Dam- newborn PIT in shape of immunoglobulin's IgG. The study of changes in protein profile of colostrum/milk is of paramount importance in understanding the Dam-newborn convey of immunoglobulin proteins. The IgG level helps us to know health status of

mother and newborn(Johnsen *et al.* 2019). The TP and IgG content in the first colostrum is higher in buffalo than in crossbred cows. The Individual variation in Ig content of first colostrum may be dependable for differences in propensity of calves to infection. The decrease in Ig content during subsequent milking is quicker in dairy crossbred cows. The chief class of immunoglobulin is IgG in buffalos and cows (Elsohaby *et al.* 2019). The level of IgG level depends upon three factors, absorption of colostrum and age of calf and birth weight of the calf (Hopker *et al.* 2020).

There is variation in level of IgG of Colostrum and serum, due to estimation method and techniques. The new born buffalo calves require immunity during fetal as well as after birth. The fetal and post birth life require immunity which is transfer passively by placental and colostrum respectively. (Hopker *et al.* 2020).TP is suggested as a dependable indicator to estimate both colostrum quality and PIT in buffalo calves (Souza *et al.* 2020).

The neonates with very low Passive Immune transfer are more prone to contagious diseases and have higher morbidity and mortality rates, having scour and respiratory diseases (Souza *et al.* 2020). The PIT status was a significant source of distinction in growth when buffalo calves get colostrum from mother. By increasing PIT to neonates enhance the growth in early life (Playford and Weiser 2021). There is no production of immunoglobulin in the body of baby calves by their own in early days of their life. So they are fully dependent on passive immunity transferred through colostrum feeding which is a defense against fatal contagious diseases and mortality in early calf's life (Hue *et al.* 2021a).

The prevalence of adequate level of Passive immunity transfer (PIT) is associated with the ample quantity of colostrum being fed as first meal to the newborns as well as accurate time to feed them. The management practices/procedures varies widely farm to farm and have many effects on PIT in calves (Morin *et al.* 2021).

There are various methods to detect the levels of IgG and Total protein in colostrum as well as in serum of the calves. The most economical and rapid method to determine the colostrum total protein which can be used at farm level is Brix Refract meter(Hue *et al.* 2021b). The serum total proteins estimation by Refract meter is a widely used tool to evaluate the failed transfer of immunity (passive), which can be described as low concentration of IgG i.e. less than 10g/dL in serum or serum total proteins less than 5.2g/dL which is measured 24hours of new born calf life(Lopez *et al.* 2021).

There was a strong correlation resulted from Brix measurement and ELISA estimation on serum is an suitable, quick, handy on-farm technique for detection of colostrum IgG and milk total solids and colostrum IgG level (Giammarco *et al.* 2021). The dairy farmers can monitor their colostrum feeding management and success of PIT using refract-meters. The serum has been the commonly used as tissue of sample mostly in newborn calves of 24hours age and less than a week of age. The exact estimation of IgG is generally considered to be the accurate observing of PIT but are of high cost procedures as well as slow and only to measure IgG. The measurement of serum TP with a Refract meter is very quick, economical and accurate test for IgG uptake as the most of serum total proteins in newborn calf is of IgG (Skirving *et al.* 2022).
The immunoglobulin is described as a major immunity component which is transferred to the calves from their dams only through the colostrum feeding. The immunity being transferred from mother to calf through colostrum is referred as passive immunity. In the absence of fetal passive immunity transfer (FPIT) there are the chances of calf diarrhoea which is a major cause of early calf mortality. Therefore the current research was aimed to identify the most effective colostrum feeding method in relation to passive immunity transfer so to evaluate either the control method of colostrum feeding is better than conventional feeding method.

The whole study was conducted in Government Livestock Experimental Farm, Bhuniky tehsil Pattoki District Kasur. The two groups of newly born Nili Ravi Buffalo Calves were arranged i.e. conventional and control group. In conventional group 10 Nili-Ravi buffalo calves were pail fed with colostrum/milk at libitum for seven days where as in controlled group 10 calves were fed with colostrum/ milk at 8.5% of B.W (3.5litre) half in morning and half in evening for seven days. The samples of colostrum/milk and blood were taken from zero days to seven days in the morning. The colostrum TP and IgG levels as well as TP and IgG levels in calves' serum were detected on daily basis to evaluate the PIT and the relationship between colostrum and serum Total protein. The weights of the calves were also recorded initially at birth and finally on day seven to check daily average weight gain. The whole data was statistically evaluated by repeated measure ANOVA followed by Duncan tests. IBM Statistical Package for the Social Sciences (SPSS) version 25. Each test on the data applied using significance level p value < 0.05.

The results showed that the level of IgG was high in the standard group of calves being fed with colostrum at 8.5% of body weight as compared to the calves in conventional group fed at Summary libitum. As well as the other parameters i.e. TP, glucose, weight, wither height, rump height, body length, and chest circumference were also checked and the results showed increased TP, Glucose and body scores in standard calves group.

The designed study suggested adapting the standard method of colostrum feeding to neonates of Nili Ravi Buffalos in response to passive immunity transfer as well as the relationship between the colostrum-serum IgG and TP level following first week of parturition rather to adapt conventional method having low passive immunity transfer (PIT).

STATEMENT OF PROBLEM

In newly-born Nili Ravi Buffalo calves, the method of colostrum feeding following parturition as a controlled method (Standard method) that comprised of feeding it at the rate of 8.5 % of calf body weight is better than the conventional method of colostrum feeding which is *ad libitum*.The current research is, therefore, aimed to: Evaluate the effects of colostrum feeding strategy (controlled vs. conventional) on body weight, body development, levels of IgG and total proteins in both colostrum and serum along with serum concentration of glucose in newly born Nili Ravi buffalo calves during the first week of age.

MATERIALS AND METHODS

3.1 Experimental Station:

The present study was performed at the Government Livestock Experimental Station, Bhunikey Tehsil Pattoki, District Kasur, shown in figure 3.1:

Figure 3.1: Experimental Sation Bhuniky

Figure 3.2: Showing Nili Ravi Buffalo with newly born calf

3.2 Animals and Experimental Design:

The twenty Nili Ravi Buffalos (*Bubalis bubalis*), near to parturition, were selected randomly on the basis of age reared at the Government Livestock Experimental Station Bhuniky Tehsil Pattoki District Kasur. Tags were applied on ears for identification. The trial was started during the months of July to August, which is best calving season for Nili Ravi Buffalos. All the experiments under this study were conducted according to Institutional Ethical Committee and by considering international standards for large animals use and care.

3.3 Feeding of the Dams:

The feeding of the dams was standardized according to energy requirement. The green fodder at rate of 10% of body weight and 1% concentrate were given on daily basis. After parturition, concentrate feeding was fed according to per day milk production of each dam. The health of the animals was supervised by qualified veterinarians present at farm.

3.4 Groups:

After parturition, the newborn calves were randomly distributed into two groups on the basis of birth weight, having 10 calves in each group. The two groups of new born calves were maintained the detail is as follow:

a. Group 1 (Conventional Feeding):

In this group, the colostrum/Milk was fed *ad libitum* as practiced in our local farm practices.

b. Group 2 (Controlled Feeding):
In this group, the colostrum/Milk was fed at 8.5% of body weight in two equal phases, i.e., half in morning and half in evening. The colostrum /milk fed for seven days following parturition.

Parameters Studied
1. Initial weight and Daily weight gain, with Body Condition Score (Wither height, Rump height, Body Length and Chest Circumference)
2. IgG level in Colostrum
3. IgG level in Calf serum
4. Total proteins concentration in colostrum
5. Total proteins concentrations in Calf serum. Concentrations of Serum Glucose

3.5.1 Sample collections:
There were two types of samples which were collected from dams and their newly born calves for this study as mentioned below:

3.5.2 Colostrum Samples;
The 10 mL colostrum/milk was collected from each quarter of every dam. It was pooled and, then, 10mL pooled colostrum/milk sample was collected in falcon tubes and stored at -20 ^{0}C. This routine practice was done on daily basis for each dam separately for seven days in morning time.

3.5.3 Serum Samples:
The blood samples were collected from jugular vein by using 10 mL sterile syringes before colostrum feeding on day-0 (at parturition) and then on days 1, 2, 3, 4, 5, 6 and 7 post- partiturition.as shown in figures 3.3 3.4 below. The whole blood was pooled into vacutainers and allowed to clot by leaving them undisturbed at room temperature for 15 to 30 minutes. The clots were removed by centrifuging at 2,000x g for 10 minutes in centrifugation machine.

The resulting supernatant/serum was collected in 2mL eppendorf tubes and stored at -20 ^{0}C unless analyzed.

3.6 Body Measurements
The Withers height and body length of calves were measured at day zero and then on day seven with girth measuring tape manufactured by Ray-vet International. It is made for body measurements of animals.

3.7 Detection of IgG:
The levels of IgG was detected in colostrum / milk of with the help of Brix Refractometer (Series, M-53; #2353, Atago Co, Ltd., Tokyo, Japan) shown in figure 3.5, before feeding to their calves on day-0 then after colostrum /milk feeding daily for seven days.

Figure 3.3. A Brix Refractometer

Similarly, the level of IgG was detected in serum of the calves. The serum samples were taken before colostrum feeding on day zero then every day in morning after colostrum/milk feeding.

Methodology;
First of all scale was adjusted to zero level, with the help of a adjustable nobe. A single drop of unfrozen colostrum / serum was placed on main prism assembly with the help of a droper, then, it was covered with a cover plate. After spreading sample on all assembly of prism of refractometer, the brix value was checked with eye piece directed towards light. The same method was adapted three times of a single sample to get average Brix value.

3.8 Estimation of Total Proteins (g/dL)

The level of total protein was detected in colostrum/ milk of conventional dams as well as dams of controlled group separately with the help of Brix Refract meter before feeding to their calves at day zero then after colostrum /milk feeding daily for seven days and readings were recorded in excel sheet.

Similarly, the level of Total Protein was also detected in serum of the calves of conventional as well as of controlled group separately. The serum samples were taken before colostrum feeding at day zero then every day in morning after colostrum/milk feeding with the help of Brix Refract meter.

3.9 Estimation of Serum Glucose (mg/dL)

The level of glucose was detected in calves' serum, before colostrum feeding at day zero then after colostrum feeding daily in the morning time for seven days. The reagents and standard solution used were prepared by Biosystem (Reagents & Instruments) IVD (REF 11523) S.A Costa Brava 08030 Barcelona (Spain) EN ISO 13485 and EN ISO 9001 standards. According to the manufacturer's recommendations samples with reagent and standardsolution were placed in 96 wells titration plate. Then absorption of sample and standard solution was monitored with spectrophotometer at 500nm wavelength.

Then glucose was estimated by this formula:

Glucose (mg/dL) = Absorption of Sample / Absorption of standard x Conc. of standard

The estimation of glucose was carried out for both groups of calves' i.e. conventional as well as control group.

Statistical Design:

The Data, expressed as mean±SE was subjected to normal distribution using Kolmogorov Smirnov with the help of software, SPSS (Statistical Package for the Social Sciences, IBM, Inc, USA). The means between two groups across the time were compared with repeated measured ANOVA. The association between various variables was computed using Pearson's Correlation. The significant level was predetermined at < 0.05.

RESULTS

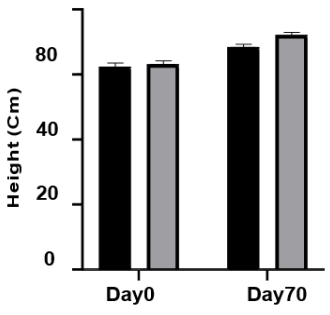
4.1 Body Measurements of Calves

The body measurements of newly born calves at day zero and day 7of both groups of calves of Nili Ravi Buffalos was recorded with the help of Girth Measuring Tape, to check the growth of calves. The data was then analyzed by applying T-test with the help of SPSS Software. The result is shown in tables 4.1 & 4.2 below;

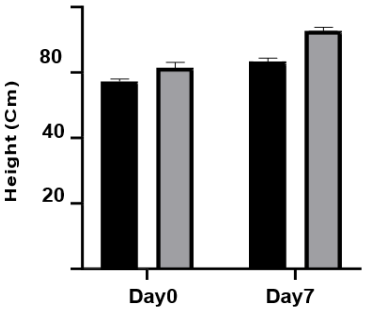
Groups	Height at withers (cm)			Rump height (cm)		
	0 Day	7 day	Change (%)	0 Day	7 day	Change (%)
Standard	63.299±1.037	72.213±0.76	32.135±1.68	61.596±1.61	72.778±1.19	34.813±2.69
Conventional	62.512±1.22	68.52±0.83	29.42±1.10	57.397±0.672	63.393±1.10	30.444±1.55
P value	0.138	0.000	0.000	0.000	0.000	0.000

Table: 4.1 Height at withers and Rump height

Figure 4.1 (A) and (B), showing graphical representation of wither height and rump height in both groups of newly born Nili ravi Buffalo calves at Day zero and seventh day following parturition.



Wither Height
Figure.4.1(A)



Rump Height
Figure.4.1(B)

Groups	Body Length (cm)			Chest Circumference (cm)		
	0 Day	7 day	Change(%)	0 Day	7 day	Change (%)
Standard	91.067 ±0.61	109.368±1.96	26.432±1.76	16.25±0.53	24.013±0.919	21.368±0.884
Conventional	88.045±0.26	99.96±1.74	17.029±2.13	16.535±0.70	20.379±1.062	17.640±0.865
P value	1.00	0.000	0.000	0.324	0.000	0.000

Table 4.2 Body Length and Chest Circumference

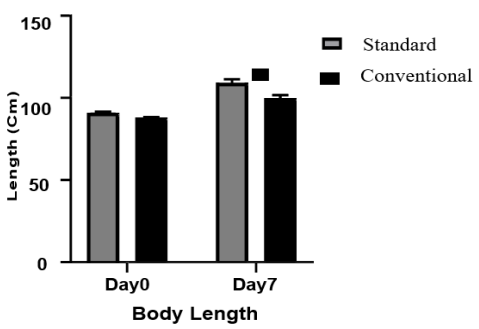


Figure.4.2 (A)

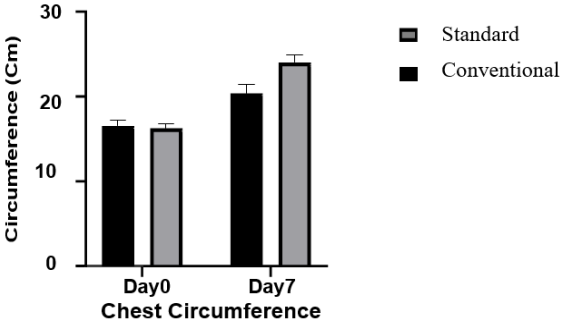


Figure.4.2 (B)

Figure 4.2 A and B, showing graphical representation of body lemgth and chest circumference in both groups of newly born Nili ravi Buffalo calves at Day zero and seventh day following parturition.

The table 4.1 & table 4.2 show that at day zero (at birth) there is no significant difference in withers height, rump height, Body length and chest circumference of calves of both groups. But at seventh day the body measurements of calves in standard group are significantly different with the calves in conventional group. The change percentage of body measurement of standard group calves for wither height, Rump height, Body length and Chest circumference are 13.42, 18.15, 20.10 & 47.74% respectively, which are significantly higher than of conventional group calves. There is significant differences in body length, chest circumference Rump height and wither height in standard group calves means that growth is better than that of conventional group calves having less growth in seven days.

4.2 Weight of Calves (kg)

The absolute weight of Calves was measured with the help of weighing balance from zero day (birth weight) to day seven on daily basis. The data a was then evaluated by applying Repeated Measure ANOVA, the table 4.2 below, shows the weight of calves and interaction between days and groups;

Groups	Days							
	0	1	2	3	4	5	6	7
Standard	35.5±2.01	35.92±2.04	36.34±2.04	36.76±2.05	37.18±2.07	37.77±2.12	37.85±2.03	38.48±2.02
Conventional	32.1±2.2	32.42±2.25	32.76±2.27	33.09±2.25	33.35±2.23	33.68±2.38	34.26±2.28	34.65±2.42

Table; 4.3 (A) Weight of Calves (Kg)

P values		
Group	**Days**	**Group x Days**
0.001	0.000	0.001

Table; 4.3 (B) The statistical data in table 4.2(A) & (B), shows that there is significant difference in days as well as group to days Interaction. According to the above mentioned data the daily mean value of weight gain is high in controlled group as compared to the conventional group as shown in the graph below: The average initial weight of Newly born Nili Ravi Buffalo Calves of Standard group was 35.5±2.01(Kg) on day 0, after seven days the weight gain was 38.48±2.02Kg which is higher than that of Conventional group calves of Nili Ravi Buffalo as shown in figure 4.3,

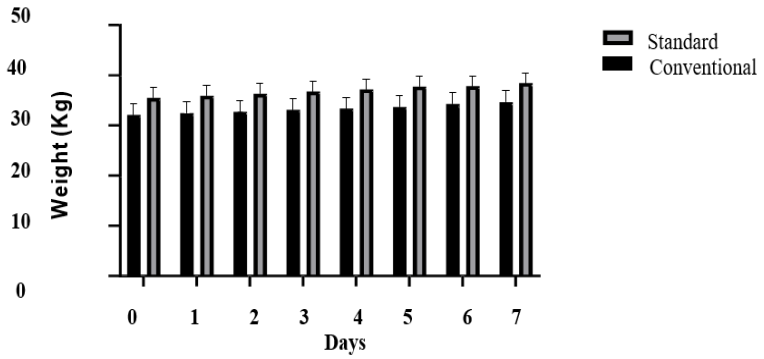


Figure: 4.3. Weight (Kg) of Newly Born Nili Ravi Buffalo Calves

4.3 Detection of IgG in Serum

The IgG Level in serum of calves for both groups was detected by Refractometer on day zero before colostrum feeding then after colostrum feeding upto seven days in morning. The recorded data was then analyzed with Repeated Measure ANOVA by SPSS software, as shown in table 4.4(A) below;

Groups	Days							
	0	1	2	3	4	5	6	7
Standard	1.6±1.2	3.9± 0.87	4.4±0.69	5.1±0.73	5.4±0.67	5.8±0.94	6.1±0.73	6.8±2.3
Conventional	2.1±1.1	3.7±0.94	5.1±1.1	5.1±1.52	5.3±1.56	5.3±2.04	5.8±2.2	6.3±0.82

Table 4.4(A) Level of IgG in Calves Serum

P values		
Group	Days	Group x Days
0.764	0.000	0.026

Table 4.4(B) The statistical data in table 4.4(A) & (B) show that the p value is 0.000 in days which is significant but not days to group interaction is non-significant. The Brix values of IgG in standard group of newly born Nili Ravi Buffalo calves increased from 1.6±1.2 at zero day to 6.8±2.3 at day 7, where as in conventional group the Brix value of IgG at day 7 is 6.3±0.82, which is less than standard group, as shown in figure 4.4 below;

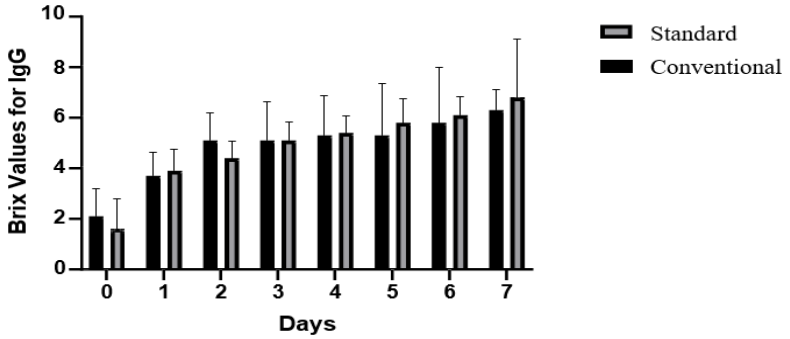


Figure 4.4: Brix values of Serum IgG Level of Newly Born Nili Ravi Buffalo calves

4.4 Detection of IgG in Colostrum/Milk

The IgG level in Colostrum/Milk of Dams of both groups was detected by Refractometer on day zero just after parturition then onward daily in the morning up-to seven days. The recorded data was then analyzed with Repeated Measure ANOVA by SPSS software, as shown in table 4.5, below;

Groups	Days							
	0	1	2	3	4	5	6	7
Standard	24.4±1.42	24±1.24	22.9±1.25	20.9±1.66	18.5±1.58	16.1±2.13	13.5±2.27	11.2±2.14
Conventional	23 ± 2.98	22.7±3.05	20.9±2.99	18.4±3.06	16.5±2.79	14.1±2.76	12.1±2.46	9.5±2.06

Table 4.5(A) Level of IgG in Colostrum/Milk

P values		
Group	Days	Group x Days
0.092	0.000	0.089

Table 4.5(B) The statistical data in table 4.5(A) &(B) shows that P value is 0.000 in days, which is significant but groups into days interaction is not significant. The level of IgG in colostrum of both dams of standard as well as Conventional group of Nili Ravi Buffalos decreases gradually from zero day to day seven, as shown below in figure 4.5;

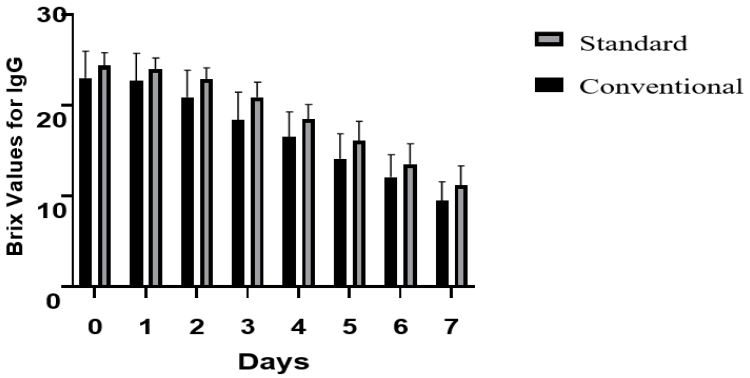


Figure 4.5 Colostrum IgG levels

4.5 Detection of Total Proteins (g/dL) in Calves Serum

The total proteins level in serum of calves for both groups was detected by Refractometer on day zero before colostrum feeding then after colostrum feeding up-to seven days in morning. The recorded data was then analyzed with Repeated Measure ANOVA by SPSS software, as shown in table 4.6,

Groups	Days							
	0	1	2	3	4	5	6	7
Standard	4.5±0.42	4.60±0.34	4.97±0.31	5.25±0.28	5.30±0.24	5.41±0.21	5.55±0.15	5.69±0.12
Conventional	3.7±0.37	4.3±0.21	4.70±0.22	4.95±0.13	5.10±0.10	5.29±0.17	5.49±0.2	5.55±0.17

Table 4.6(A) Level of total proteins (g/dL) in Calves Serum

P values		
Group	**Days**	**Group x Days**
0.026	0.000	0.228

Table 4.6(B) The statistical data in table 4.6(A) & (B) shows that P value is 0.00 in days, which is significant but groups into days interaction is not significant. The level of total proteins in serum of standard group of newly born Nili Ravi Buffalo Calves at zero was 4.5±0.42 (g/dL) and 5.69±0.12 (g/dL) at day seven. In Conventional group of calves the level of total proteins at zero was 3.7±0.379 (g/dL) and at day 7 it was 5.55±0.17 (g/dL), which is less as compared to standard group of calves, as shown in figure 4.6 below:

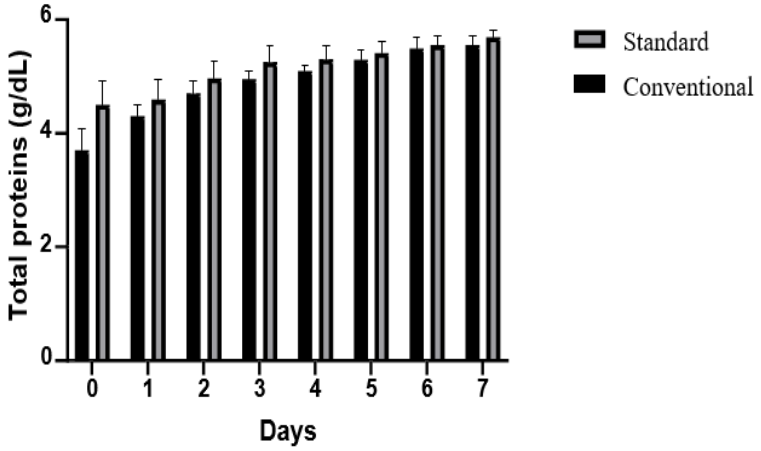


Figure 4.6 Level of Serum Total proteins (g/dL) of Newly Born buffalo calves

4.6 Detection of Total Proteins (g/dL) in Colostrum / Milk

The total proteins level in colostrum/milk of dams for both groups was detected by Refractometer on day zero just after parturition then daily up-to seven days in the morning. The recorded data was then analyzed with Repeated Measure ANOVA by SPSS software, as shown in table 4.7,

Groups	Days							
	0	1	2	3	4	5	6	7
Standard	8.47±0.14	8.44±0.14	8.32±0.18	8.21±0.21	8.01±0.30	7.87±0.37	7.67±0.52	7.52±0.62
Conventional	8.44±0.20	8.41±0.18	8.36±0.21	8.17±0.22	7.97±0.28	7.77±0.22	7.39±0.24	7.13±0.28

Table 4.7(A) Level of total proteins (g/dL) in Colostrum/Milk

P values		
Group	**Days**	**Group x Days**
0.496	0.000	0.57

Table 4.7(B) The statistical data shows that P value is 0.00 in days, which is significant but groups into days interaction is not significant. The level of total proteins in Colostrum of standard group of Nili Ravi Buffalos at zero was 8.47±0.14 (g/dL) and 7.52±0.62 (g/dL). In Conventional group of calves the level of total proteins at zero was 8.63±0.20 (g/dL) and at day 7 it was 7.13±0.28 (g/dL)., as shown in figure 4.6;

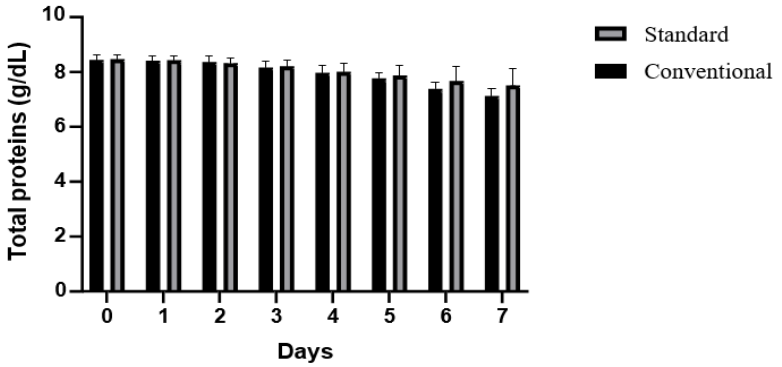


Figure. 4.7 Level of Total proteins (g/dL) in colostrum/milk

4.7 Estimation of Glucose (mg/dL) in Calves Serum

The estimation of Glucose level in calves' serum, of newly born calves of Nili Ravi Buffalos for both group was carried out with Bio system. The values were evaluated by repeated measure ANOVA, by SPSS software, as mentioned below in **Table 4.8;**

Groups	Days							
	0	1	2	3	4	5	6	7
Standard	59.68±5.1	66.48±3.7	70.38±3.65	75.28±3.59	79.71±4.54	84.55±4.58	87.41±4.72	92.43±5.42
Conventional	32.1±2.2	32.42±2.25	32.76±2.27	33.09±2.25	33.35±2.23	33.68±2.38	34.26±2.28	34.65±2.42

Table 4.8(A) Level of Glucose (mg/dL) in Calves serum

P values		
Group	Days	Group x Days
0.394	0.000	0.203

Table 4.8(A) The statistical data shown in table 4.8(A) &(B) There is no significant difference within groups and groups into day's interaction but in day's the p value is 0.000 which is significant.

The level of glucose is higher in standard group than conventional group of Newly Born Nili Ravi Buffalo Calves as shown in figure 4.8 below;

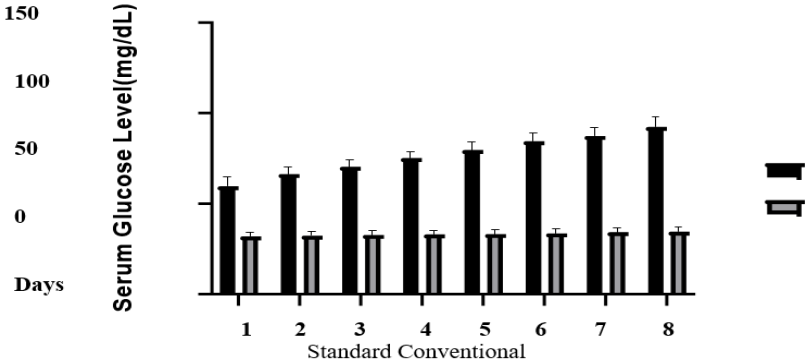


Figure 4.8 Level of Glucose in Calves Serum

4.8 Correlation between IgG levels of Colostrum to Calves Serum IgG

The correlation between colostrum IgG level and Serum IgG level was analyzed with the help of SPSS software. The following results were seen in standard group of calves and dams as shown in Table 4.9 below:

		IgG standard buff	Standard Serum IgG
IgG in Standard Buffalo colostrum	R	1	-.543**
	P value		0.000
	N	80	80
Standard calves Serum IgG	R	-.543**	1
	P value	0.000	
	N	80	80

Table.4.9.

**Correlation is significant at the 0.000 level (2-tailed) between standard colostrum IgG level of dams with standard serum IgG level of Calves, whereas sample size (n) is 80.

As well as the correlation between Colostrum of dams and calves serum of conventional group is shown in Table 4.9 below;

		IgG conventional. Buffalo	Conventional Serum IgG
IgG in colostrum of conv. Buffalo	r	1	-.631**
	P value		0.000
	N	80	80
Conv. Calves Serum IgG	r	-.631**	1
	P value	0.000	
	N	80	80

Table:4.9

**Correlation is significant at the 0.000 level (2-tailed) between conventional colostrum IgG level of dams with standard serum IgG level of Calves, whereas sample size (n) is 80.

DISCUSSIONS

The study was conducted to compare the effects of conventional vs standard colostrum feeding protocol on weight gain, body development, serum glucose, concentration of immunoglobulins and total proteins in newborn Nili Ravi Buffalo calves. As new born calves of bovines have no immunity for the diseases as well as other environmental hazards. The very important and beneficial point in dairy new born calves for their health and survival is the feeding of adequate quantity of high quality colostrum in early life (Phipps *et al*. 2018). The cost of feeding management, replacement of dairy animals increases than normal or sometimes requires high cost treatment protocols against preventive hazards.

At the time of birth the new born calf of buffalo having poorly developed immune system pron to many health issues. The Cotyledonary-Synepitheliochorial placenta in dam does not allow the maternal antibodies especially IgG content to cross the placental barrier to enter into the blood stream of calf (Zakian *et al*. 2018). The true colostrum is the first milk enriched with antibodies that protect the calf from diseases in early life until the development of his own immune system. Colostrum is also a source of energy and nutrients after birth for young one. The antibodies are the proteins that identify and destroy disease causing pathogens in the calf body (Dunn *et al*. 2018).

There are three types of major immunoglobulin present in colostrum i.e. IgG 85%-90%, IgM 5%-10% and IgA is of 5%-10% (Lotito *et al*. 2023). These three immunoglobulin's have their specific roles in blood stream of calf and immune system. The primary role of immunoglobulin A is to identify and destroy invading disease causing pathogens. IgG can move out of the blood stream and invade into the tissue of the body where it identify the disease causing agents. The role of IgM is to identify and destroy the invading bacteria into the blood stream of calves.

IgG attaches with the membranes lining many organs like intestine to prevent the pathogens to attach and cause the diseases (Puppel *et al*. 2019).

The major aim of this study was to determine the better feeding method of colostrum to the new born Nili Ravi Buffalo calves and to detect the level of IgG as well as TP in Calves serum and to find out what is the correlation between maternal and calf IgG.

The results of our study showed beneficial effects of controlled /standard method of colostrum feeding to young ones. The reading of Refractometer for IgG in the controll group of calves was 6.8±2.3 that was equal to the concentration of 15.45 mg/mL, whereas,in conventional group it was 6.3±0.82 that was equal to the concentration of 14.31 mg/mL. The results shows that level of IgG ($p<0.05$) was high in control group in which colostrum was fed at 8.5% of body weight half in morning in half in evening where as in conventional group colostrum was fed *ad libitum*. The quantity of colostrum/milk varies quarter to quarter in our local Nili Ravi buffalo. So when the calves were allowed to take colostrum/ milk *ad libitum* there were chances of low quantity of colostrum to be absorbed in their body. Whereas in the control group the colostrum/milk quantity was equally fed to the calves so there were maximum chances of absorption, hence increased level of IgG and increased passive immunity transfer in-sense of immunoglobulin. The results support the research of (Chigerwe *et al*. 2009) about bottle fed colostrum.

The level of Total proteins (TP) in the standard /control group was 5.69±0.12 g/dL and in the conventional group it were 5.61±0.17g/dL respectively. as observed for IgG results show that TP in control / standard group was higher ($p<0.05$) compared to conventional group of colostrum feeding calves. The serum total proteins concentration is an indicator of successful maternal immunity transfer (PIT) in newly born buffalo calves because there is a positive relationship between TP and immunoglobulin (Soufleri *et al*. 2023). The adequate level of maternal /passive immunity transfer is considered as when Total Proteins concentration at 24_hr post-partum is more than 5.2g /dL. In this study, the control group had a level of Total proteins of about 5.69±0.12 g /dL suggesting there was adequate absorption of immunoglobulin's in calf body. The proteins at early age of life of a calf are mainly antibodies made being absorbed through intestine by colostrum (Godden *et al*. 2019). .

The serum level of glucose in the control /standard group was of 92.43±5.42g /dL where as in the conventional group it is 86.73±7.97g /dL. The result shows that glucose level is higher ($p<0.05$), in the control group when compared group fed on conventional feeding regimine. As the young claves are of mono-gastric (with very less developed rumen, meat for fermentation), they use the glucose as a primary energy source for their body. This glucose comes from colostrum of

dam. The level of glucose depends upon its absorption. After transition of mono-gastric to ruminant, short chain volatile fatty acids (VFA) become the major source of energy.. In this study increased absorption of colostrum increases post fed level of glucose and lowers the fasting glucose concentration coincide with result of López Valiente *et al*. 2021.

The results shows that average weight gain by the calves in the control group is better than the calves in conventional group due to low level of glucose and immunoglobulin, showing reduced development in body growth.

A correlation was also estimated between IgG levels of Dam's colostrum/milk with serum IgG level of new born calves after feeding up-to seven days continuously. The results showed that there was a negative correlation in between them. As there is gradual increase in IgG level in serum of calves, a gradual decrease in IgG level in Dam's milk was seen. Because the colostrum is usually in first milking after parturition to 2-3 days post-partum having high levels of immunoglobulin in its composition, but gradually when colostrum ends and milk starts from teats the level of immunoglobulin decreases. But in case of serum the story is opposite as calves born without immunoglobulin, there is gradual increase in IgG level by intake of colostrum then after by development of immune system in the calf body to be able to produce antibodies against the pathogens (Hue *et al*. 2021b).

The wither height, rump height, body length and chest circumference of calves in both groups were also measured with the help of girth measuring tape. It was observed that calves in the standard group have increased wither height, rump height, body length and chest circumference as compared to the calves in conventional group having less growth (Soufleri *et al*. 2023) (Schäff *et al*. 2016).

Conclusion:

After getting results of all parameters and discussions it is concluded on the basis of whole study that the best method of colostrum feeding in adequate amount in new born calves of Nili-Ravi Buffalo is standard method to attain maximum PIT especially IgG for defense against diseases in early life of calves. There is a need to study more factors to check quality of colostrum for better PIT In New calves of Nili Ravi Buffalo. The enough studies and literature are not available for our local Buffalo, so that researchers can conduct more studies to improve the health and immunity of new born calves for their better production in future without major diseases, in our local environment.

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