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Prebiotic Role of Chitosan Oligosaccharides on Health Status, Blood Chemistry and Diarrhoeal Patterns in Neonatal Calves

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Abstract

TThe aim of this study was to evaluate the prebiotic role of chitosan oligosaccharides on health status, blood chemistry and diarrheal patterns in neonatal calves. A total of 50 local shahiwal crossbreed female calves aged 1 to 5 days whose initial body weight (24 ± 1.0 kg) more or less similar were randomly allocated into two (n=25) experimental groups. The control group (n= 25) were fed milk replacer without no additives and prebiotic group (n= 25) were fed milk replacer with prebiotic (COS) 5 gm per day. Milk replacer were offered twice a day. Normal milk and water were offered adlibitum throughout the trial period of 60 days. Body weight was measured at birth and thereafter at 10 days interval up to 8 weeks of age. Fecal consistency were observed and recorded daily throughout the trial period. Results revealed that calves initial body weight in the control group and in the prebiotic group were 24.30 kg and 24.20 kg from the experiment start reaching 36.70 kg and 42.50 kg respectively at its end. Moreover, Prebiotic (COS) reduced the incidence of diarrhea and was effective after two weeks of application may be as a result of an improved intestinal bacterial flora in calves supplemented with prebiotics. On the other hand, prebiotic (COS) had no significant effect on any of the hematological and biochemical traits measured (P>0.05). These findings provide information that adding prebiotic (COS) to milk replacer can be used to increase the daily weight gain, improved intestinal flora and reduce the incidence of diarrhea.

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Introduction

In modern livestock industry, many different feed additives are routinely used to maintain good health and metabolic status and promote the performance indices of intensively produced farm animals. A prebiotic is a food or dietary supplement product that confers a health benefit on the host associated with modulating the microbiota [1]. A prebiotic is a fiber such as fructose oligosaccharide, galactose oligosaccharide etc. and is consumed that is intended to stimulate the microflora in the large intestine. A common mistake is to require that the prebiotic be shown to increase the population and/or function of the probiotic it is paired with as the probiotic is an external species whereas prebiotics stimulate the flora which is already present. Antibiotics as a growth promoter have been widely used extensively in livestock feed for more than 50 years but it band on in some parts of the world legislations has promoted to the search for alternatives. Therefore, prebiotics are considered to be the alternatives as non-antibiotic growth promoters are being more popular in livestock industry. But recently, the prebiotic such as chitosan oligosaccharides are used in the livestock industry are new concept.

One relatively new and less widely used feed additive is chitosan. It is commercially manufactured from chitin widespread in nature component of external shells of shrimps, crabs and insects [2]. Chitosan Oligosaccharide (COS) has received considerable attention in veterinary applications due to biocompatibility, biodegradability, low cost and non-toxicity. COS can optimize the growth and development of weaned calves [3], reduce intestinal inflammation [4], increase total number of live born calves [5] and improve milk composition of cows [5]. COS has been shown to enhance growth performance, nutrient digestibility, and immune response of weaning calves. It has been shown to reduce the establishment of pathogens in the intestine [6] and improve immune function. COS was successfully used for the clinical management of diarrhoea in Hanwoo calves. It was found to have positive effects on reproductive performance and milk production of cows [5]. In addition, dietary COS was reported to improve nitrogen use and feed conversion in diets for mid-lactation dairy cows [7].

Dairying is one of the major components of animal agriculture in Bangladesh. Neonatal calves' diarrhoea is a major cause of economic loss to the dairy farmers [8]. It is the leading cause of death in dairy heifer and beef calves aged less than four months. Financial losses occur not only from calves' mortality, but also from the cost of medication and labor needed to treat and care for the sick calves [8]. On the other hand, use of antibiotics in animal production is considered as a serious public health issue since it is creating a major reservoir for the development of resistant bacteria. This has required scientists to investigate potential alternative supplements that act like-antibiotics to improve animal production [9]. However, no comprehensive study has yet been undertaken in Bangladesh to evaluate the prebiotic role of chitosan oligosaccharides on health status, blood chemistry and diarrheal patterns in neonatal calves. In this regard, probiotic could be a potential supplement for improving the health status and immunity of newborn calves. The aim of the present study is to assess the prebiotic role of chitosan oligosaccharides on health status, blood chemistry and diarrheal patterns in neonatal calves.

Materials and methods

Experimental statement

The experiment on prebiotic role of chitosan oligosaccharides on health status, blood chemistry and diarrheal patterns in neonatal calves was carried out on savar dairy farm and Physiology laboratory of the Department of Preclinical Courses, Faculty of Veterinary and Animal Sciences at Gono University, Savar, Dhaka. A total of 50 local shahiwal crossbreed female calves aged 1 to 5 days whose initial body weight (24 ± 1.0 kg) more or less similar were randomly allocated into two (n=25) experimental groups. The control group (n=25) were fed milk replacer without no additives and probiotic group (n=25) were fed milk replacer with prebiotic (COS) 5 gm per day. Milk replacer were offered twice a day. Calf Starter and water were offered adlibitum throughout the trial period of 60 days. Body weight was measured at birth and thereafter at 10 days interval up to 8 weeks of age. Fecal consistency were observed and recorded daily throughout the trial period.

Table 1: Chemical composition of Milk replacer and calf starter fed to neonatal calves.

Composition (%)	Milk replacer	Calf Starter
Dry matter	92.40	87.8
Crude protein	20.90	16.40
Crude fiber	1.14	12.10
Ether extract	9.46	3.38
Calcium	0.69	0.74
Phosphorus	0.66	0.57
Metabolic energy (K cal/kg)	3,700	ND**

Sample collection and processing

Five ml of blood samples from the calves of both treated and untreated groups were collected by jugular venipuncture. Immediately after collection 2 ml of the samples were transferred to sterile screw-capped tubes containing EDTA for hematology and immunoglobulin assay. The remaining 3 ml were transferred to the tubes containing lithium heparin for serum biochemistry. The tubes containing blood were placed in slanting position at room temperature for 1 hour. Then the clot were detached from the wall of the test tube carefully and allowed it to settle down and afterward serum were collected. Collected serum were centrifuged at 3000 R.P.M for 15 minutes to obtain clear serum.

Hemato-biochemical analysis

a. Blood analysis

The hematological parameters include i) tRBC (M/µl), ii) tWBC (M/µl), iii) Platelets (10^3/ml), iv) Hb (Gm%), v) PCV (%), vi) MCV (fl), vii) MCH (Pg) and viii) MCHC (g/dl) were observed by analysis of blood in hematological laboratory.

b. Serum analysis

The biochemical parameters include i) ALT (IU/l), ii) AST (IU/l), iii) BUN (mg/dl), iv) Creatinine (mg/dl), v) Glucose (mg/

dl), vi) Total protein (gm/dl), vii) Albumin (gm/dl) viii) Globulin (gm/dl) were observed by analysis of serum in biochemical laboratory.

c. Fecal analysis

Calf diarrhoea were evaluated using the fecal score and recorded according to Larson at el.'s recommendation [10]. For fecal fluidity, scoring were done as: 1= normal, 2= soft, 3= thin, 4= watery during the experimental period.

d. Statistical analysis

Finally, the results will be analyzed for scientific report preparation. The images will be taken by using Nikon photomicroscope and analyzed by Image J software. The data will be analyzed by student's t-test/Anova.

Results and discussion

The present results showed the effective outcome of the prebiotic usage among calves presented with diarrhea.

Table 2: Effects of prebiotic (Chitosan oligosaccharides) on health status (Mean ± SE) of calves.

Experimental groups	Pretreated body weight (kg)	Post treated body weight (kg)			
	Day 1	Day 15	Day 30	Day 45	Day 60
Control group	24.30 ± 1.0	26.38 ± 0.42	30.51 ± 0.80	33.78 ± 0.23	36.90 ± 0.20
Prebiotic group	24.40 ± 1.0	27.10 ± 0.38	32.16 ± 0.32	37.43 ± 0.80	42.28 ± 0.38

The results revealed that calves initial body weight at the initiation of the experiment in the control group and in the prebiotic group were 24.40 ± 1.0 kg and 24.30 ± 1.0 kg respectively. While calves final body weight at the end of the experiment in the control group and in the prebiotic group were 36.90 ± 0.20 and 42.28 ± 0.38 kg respectively. There was proper improvement in the general performance of calves. These positive effects could be due to the decrease in the multiplication of the harmful bacteria in the gut which results from improvement in gut environment and enhanced nutrient utilization by the prebiotic effect [11]. This positive effect was similar to the Abe et al. [12] results as throughout their study, calves to 25 days of age were assessed. Moreover, Hossaini et al. [13] stated that the groups with prebiotic and antibiotic in their study had significantly higher body weight than the control group which is also reliable with Higginbotham and Bath [14] results, who also performed different experiments in the first month of birth and also, Abdala et al. [15] reported a significant difference in the growth of the prebiotic group between 30 and 45 day. The increase in both body weight gain and disease resistance places the young calf in a very favorable situation in which it can continue to gain body weight and be better prepared to resist diarrheal pathogens. Different mechanisms of prebiotic action have been described [16] whish stated that prebiotic compete for different nutrients and produce antibacterial compounds in the intestine that allow them to occupy specific niches of the intestinal mucosa activating the innate immune system. The contribution of both mechanisms is related directly to the prebiotic strain type and the feed consumed by the calves. The improvement in utilization of the feed and consequent improvement in body weight gain is the final consequence of prebiotic action.

Table 3: Effects of prebiotic (Chitosan oligosaccharides) on blood parameters (Mean ± SE) in calves.

Blood parameters	Control group (n=25)	Prebiotic group (n=25)
tRBC (M/µl)	8.31 ± 0.10	8.80 ± 0.24
tWBC (K/µl)	9.38 ± 0.14	9.62 ± 0.13
PLT (K/µl)	460.3 ± 1.18	461.6 ± 1.40
Hb (Gm %)	11.30 ± 0.12	11.80 ± 0.23
PCV (%)	30.12 ± 0.24	30.28 ± 0.32
MCV (fl)	36.08 ± 1.21	37.05 ± 2.01
MCH (pg)	16.02 ± 0.34	16.48 ± 0.80
MCHC (%)	40.26 ± 0.21	41.81 ± 0.36

RBC: Red Blood Cell; WBC: White Blood Cell; PLT: Platelets; PCV: Packed Cell Volume; Hb: Hemoglobin; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration.

Regarding blood parameters in the prebiotic treated calves and their control, mean values of both of them are shown in (Table 3). It was found that the values of all parameters were all in normal physiological range showing that the prebiotic supplementation had no significant effect statistically on any of the hematological traits measured (P>0.05). In the present study, the hematological and biochemical profile showed that the values were all in normal physiological range and the prebiotic had no significant effect on any of the hematological and biochemical traits measured. That was similar to the findings of Adams et al. [17], Moslemipur et al. [18] and Riddell et al. [19] who stated that there were no variations in the hematological and biochemical parameters between prebiotic treated calves and the control group throughout their studies.

Table 4: Effects of prebiotic (Chitosan oligosaccharides) on serum parameters (Mean ± SE) in calves.

Serum parameters	Control group (n=25)	Prebiotic group (n=25)
ALT (IU/l)	70.20 ± 1.12	71.10 ± 0.14
AST (IU/l)	65.28 ± 2.42	65.52 ± 2.46
BUN (mg/dl)	20.42 ± 1.38	20.80 ± 2.26
Creatinine mg/dl)	1.20 ± 0.24	1.28 ± 0.34
Glucose (mg/dl)	58.40 ± 1.81	59.80 ± 1.60
Total protein (gm/dl)	6.50 ± 1.02	6.78 ± 2.10
Albumin (gm/dl)	5.60 ± 2.18	5.91 ± 1.52
Globulin (gm/dl)	3.18 ± 1.04	3.38 ± 1.12

ALT: Alanine Transferase; AST: Aspartate Transferase; BUN: Blood Urea Nitrogen.

Regarding serum parameters in the prebiotic treated calves and their control, mean values of both of them are shown in (Table 4). It was found that the values of all parameters were all in normal physiological range showing that the prebiotic supplementation had no significant effect statistically on any of the biochemical traits measured (P>0.05).

Effects of prebiotic (Chitosan oligosaccharides) on diarrheal frequency:

Calf diarrhea was assessed using the fecal score during the thirty days of the pre-weaning period. Significantly, diarrhea in probiotic group showed no signs of diarrhea after week two which in contrary, diarrhea occurred in calves of un-supplemented group during the entire experiment period. There was significant statistical difference in the fecal score between both groups after two weeks of the experiment where the fecal score became constant in the prebiotic treated group and never exceeded the normal value (Figure 1).

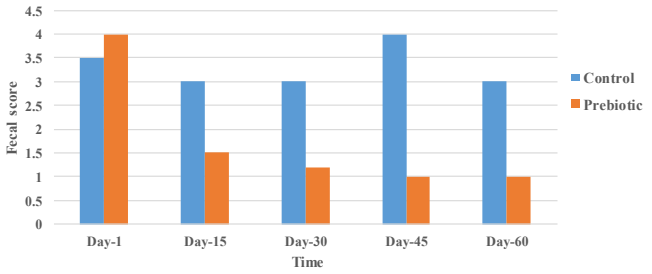

Figure 1: Mean fecal score of neonatal calves supplemented with or without prebiotics. Calve diarrhea was evaluated using the fecal score and recorded according to Larson et al.'s recommendation [10]. For fecal fluidity, scoring was done as follows: 1 = normal, 2 = soft, 3 = runny and 4 = watery during the experimental period.

Though, there was significant difference in the fecal score between the prebiotic group and the control after two weeks of the experiment. Fecal score became constant in the prebiotic treated group and didn't exceed the normal value where the prebiotic reduced the incidence of diarrhea and was effective after two weeks of application. This may be as a result of an improved intestinal bacterial flora in the calves supplemented with prebiotic. This was similar to Abe et al. [12]; Khuntia et al. [20]; Frizzo et al. [15]. On the other hand, previous study by Cruywagen et al. [21] observed that no prebiotic-induced reduction of the occurrence of diarrhea. Kawakami et al. [22] and Gorgulu et al. [23] described and found that, with respect to diarrhea and fecal scoring and similar to the present study, calves fed prebiotic were superior to control group. This may be returned to the fact that lactic acid bacteria can stimulate the development of the immune response against the pathogenic bacteria and counter the negative effects of illnesses [14]. Also, Gorgulu et al. [23] stated that calves supplemented with prebiotic were superior with respect to diarrhea than the control groups and concluded that prebiotic supplementation before weaning could boost calf health and reduce mortality and cost of buying drugs. The same conclusion was reported by Marcin et al. [24] for piglets and calves. Their finding is in agreement with this present study.

Conclusions

In the present study, Adding prebiotic (COS) to milk replacer can be used to increase the daily weight gain, improved intestinal bacterial flora and reduce the incidence of diarrhea. The blood and serum profile showed that the values were all in normal physiological range and the prebiotics had no significant effect on any of the blood and serum traits measured. It is recommended that prebiotic should be used in animal production in order to reduce the use of antibiotics in animal industry which has negative effect on the consumers health. Further studies should be carried out using large number of male calves to assess the prebiotic role of Chitosan Oligosaccharides (COS) on health status, blood chemistry and diarrheal patterns in neonatal calves.

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