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Effect of Probiotic on Growth Performance and Frequency of Diarrhea in Neonatal Buffalo Calves

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Abstract | Neonatal calf diarrhea (NCD) is one of the common causes of illness and death in the pre-weaning calves in farms. Different methods to treat the diarrhea have been used including antibiotics, although it is forbidden now to use them. Probiotics are live microbial feed supplements which helpfully improve intestinal microbial balance in intestine and provide an alternative strategy to the traditional practice of the antibiotic usage. Throughout this study, the beneficial effects of probiotics were observed on animals including growth enhancement and disease prevention. Sixty-five newly born buffalo calves of both sexes aged from 3 days to 1 month, were fed on Milk Replacer in a private farm in Sharkia governorate were assessed to determine the effect of probiotic on growth performance, blood parameters and the prophylaxis of calf diarrhea in them. They were divided to 2 groups; Control group included 30 healthy calves fed on Milk Replacer without addition of probiotic and Probiotic group included 35 apparently healthy calves fed on Milk Replacer with addition of probiotic. Results revealed that calves initial body weight in the control group and in the probiotic group were 40.60 kg and 40.39 kg from the experiment start reaching 49.60 kg and 55.64 kg respectively at its end. Moreover, Probiotic 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 probiotics. Moreover, probiotic had no significant effect on any of the hematological and biochemical traits measured ($P > 0.05$). Usage of probiotics improves health condition of neonatal calves. Adding probiotic to milk replacer can be used to increase the daily weight gain, feed conversion efficiency and reduce the incidence of diarrhea.

Keywords | Probiotic, Growth performance, Diarrhea, Neonatal calves, Milk replacer

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INTRODUCTION

Death incidence of young calves is one of the major problems in dairy farms around the world. Diarrhea is the most common causes of death among calves. In pre-weaning period, young calves are vulnerable to different infectious pathogens that cause the primary damage to the intestine. (Bicknell and Noon 1993). As mentioned, diarrhea is chiefly caused by bacteria called *E. coli*. It remains an overwhelming disease all over the world mainly in calves less than three months of age (Malik et al., 2012). It is concerned that the usage of the antibiotics as feed additive may share in an increase of the bacterial antibiotic resistance, and the usage of some antibiotic types have been restricted by different countries. Additional, European Union has restricted and banned on the application of different antibiotics as feed additives from 2006 onwards. Consequently, this has ensured a search for different and natural strategies to moderate gut development and health away from the usage of antibiotics (Hughes and Heritage 2002). At times of stress such as weaning in calves, digestive upsets are very common. Usage of antibiotics showed that they destroy both desirable and the harmful species, in contrast to probiotics where the inclusion of probiotics in foods is preferable. Probiotics are designed to encourage certain strains of bacteria in the gut at the expense of less

desirable ones. Gut bacteria are supposed to have different requirements for specific nutrients that may not be adequately provided by the animal's diet. So, feeding these nutrients might promote the growth of the gut bacteria, yet, improving the microbial profile in the gut (Quigley, 2011). Probiotics are defined as "live microbial feed supplements" which usefully affect the host by improving its intestinal microbial balance. They provide a proper and alternative strategy to the traditional practice using antibiotic as a treatment. Several studies observed the beneficial effects on animals including growth enhancement and disease prevention FEFANA (2005). The aim of the present study is to assess the effect of probiotics on the growth performance and the frequency of diarrhea in neonatal buffalo calves.

MATERIALS AND METHODS

The present study was carried on 65 newly born buffalo calves of both sexes from 3 days to 1 month of age that were fed on Milk Replacer in a private farm in Sharkia governorate to determine the effect of probiotic on growth performance, blood parameters and the prophylaxis of calf diarrhea in them. they were examined and divided into two groups; Control group (group 1), included 30 apparently healthy calves fed on Milk Replacer without addition of probiotic; Probiotic group (group 2), included 35 apparently healthy calves fed on Milk Replacer with addition of probiotic.

Study Design

Experimental treatments were: (1) control diet without any feed additive and (2) control diet +10 g probiotic per head per day.

Calves were fed 3 L/d for the first 10 days of milk replacer and 4 L/d of milk replacer during the 10 to 30 days. The milk replacer was mixed in hot water to disperse the fat component. Cool water was then added to bring temperature to approximately 38°C. Calves were fed twice daily at 8 AM and 5 PM using a plastic bucket. At each feeding, a bucket containing milk replacer was fitted into the stand and removed after feeding. Nutrient composition of milk replacer and calf starter is listed in (Table 1). Water was provided free choice and changed twice daily. Calves in probiotic group received 10 gm probiotic with the morning milk during the study period the probiotic used in the experiment was a mixure of *lactobacillus acidophilus, lactobacillus blantrum, enterococcus facium, bifidobacterium bifidum, bacillus subtilus extract* and *asperigillus oryzae extract*.

Animal Performance

The body weight of the calves was recorded at the beginning and the end of the experiment. The animals were always weighed at the starting day and once a week during experimental period. The consumption of milk replacer and calf starter was recorded daily.

Records of body weight and feed consumption were used to calculate average daily gain (ADG) and feed conversion ratio (FCR). FCR was calculated by dividing total feed intake per calf by the total body weight gain per the same animal for the study period.

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

Composition (%)	Milk replacer	Calf starter
Dry matter	94.48	86.12
Crude protein	21.79	16.42
Ether extract	9.48	3.35
Crude fiber	1.12	11.02
Ash	7.38	5.25
Ca	0.67	0.73
P	0.64	0.55
ME (k cal/kg)*	3,700	ND**

* ME = Metabolic energy, calculated from NRC (2001). ** ND = Not determined. Ca: Calcium; P: phosphorous.

Fecal Scoring

Fecal scoring for estimation of fecal fluidity was conducted daily in the morning (8 AM) according to the procedure of Larson et al. (1977). Fecal scores based on a four-point scale were recorded. Scoring was as follows: for fecal fluidity, 1 = normal, 2 = soft, 3 = runny, or 4 = watery. A scour day was recorded if fecal fluidity = 3 or 4. The data was averaged per week.

Blood Collection and Analysis

Two blood samples were collected from each calf at the end of the experiment via jugular vein puncture. The first sample (whole blood sample) for hematological examination was collected on evacuated glass tubes "vacutainer" containing anti-coagulant {Ethylene diaminetetraacetic acid (EDTA)} and were examined within an hour of taking the blood sample. This sample was used for evaluation of total erythrocytic count, total leukocytic count, hemoglobin concentration (Hb) and packed cell volume (PCV %). The second sample (coagulated) blood and centrifugation at 3000 rpm for 15 minutes to remove residual red cells, then stored in the deep freezer - 20°C and kept for determination of total protein, albumin, globulin, enzymes (AST and ALT) and electrolytes (Na, Cl , and K).

Statistical Analysis

Data handling and statistical analysis was carried out at the Dept. of internal medicine, Faculty of Vet. Medicine, Zagazig Universiy. Analysis was done using SPSS/PCT,

Table 2: Effect of probiotics supplementation on calves' performance.

Groups (Mean ± SE)	Number of calves	Initial BW (kg)	Finial BW (kg)	DWG (kg/d)	FCR
Control group	30	40.60±40[a]	49.60±40[b]	0.30±003[b]	0.90±0.03[b]
Probiotic group	35	40.39±0.47[a]	55.64±0.47[a]	0.49±0.003[a]	1.40±0.03[a]
P value		0.595	0.001	0.001	0.001

BW: body weight; DWG: daily weight gain; FCR: feed conversion ratio

Table 3: Effects of probiotic supplementation on calves' blood parameters.

Parameters (Mean ± SE)	Un supplemented group (control) n= (30)	Probiotic group n = (35)	P-Value
RBCs (10^6/µI)	8.32±0.04[a]	8.29±0.03[a]	0.641
WBCs (10^3/µl)	9.58±0.03[a]	9.52±0.04[a]	0.310
PCV (%)	30.26±0.03[a]	30.29±0.03[a]	0.604
Hb (g/dl)	10.71±0.03[a]	10.70±0.04[a]	0.904
ALT IU/L	64.58±0.02[a]	64.52±0.03[a]	0.170
AST IU/L	85.71±0.04[a]	85.65±0.03[a]	0.223
Glucose (gm/dL)	77.69±0.04[a]	77.68±0.03[a]	0.855
Total protein (gm/dL)	7.41±0.02[a]	7.37±0.01[a]	0.240
Albumin (gm/dL)	4.20±0.05[a]	4.18±0.01[a]	0.85
Globulin (gm/dL)	3.21±04[a]	3.18±0.01[a]	0.521
Na (mmol/L)	136.71±0.01[a]	136.74±0.02[a]	0.375
Cl (mmol/L)	93.86±02[a]	93.84±0.02[a]	0.696
K (mmol/L)	4.25±0.02[a]	4.28±0.02[a]	0.271

Means carrying different superscripts in the same column are sig. different at (P<0.05). RBCs: Red Blood Cells; WBCs: White Blood Cells; PCV: packed cell volume; Hb: Hemoglobin; ALT: Alanine transferase; AST: Aspartate transferase; Na: sodium; Cl: chloride; K: potassium.

(Statistical Package for Social Sciences version 22.0) (IBM Corp., Armonk, NY, USA) software. Results were reported in means ± SEM (Standard Error of Mean). The value of P < 0.05 was used to indicate statistical significance. The statistical method was ANOVA test (one way analysis of variance) to test the differences in control and probiotics groups. The Duncan multiple range test are also used (Duncan 1955).

RESULTS

Effects of Probiotic on Animal Performance

The effect of Probiotic on body weight (BW), average daily gain (ADG) and Feed conversion ratio (FCR) of calves for the experimental period is shown in (Table 2). The results revealed that calves initial body weight at the initiation of the experiment in the control group and in the probiotic group were 40.60 kg and 40.39 kg respectively. While calves final body weight at the end of the experiment in the control group and in the probiotic group were 49.60 kg and 55.64 kg respectively. The probiotic treated group has higher numerical value (final body weight) than the control at the end of the experiment. Average daily gain was 0.49 kg in the probiotic group and 0.30 kg in the control group. Calves that received probiotic in the milk replacer achieved higher (P < 0.001) average body weight and average daily gain when compared to the calves from the control group. (Table 2) shows that feed conversion ratio was 1.40 in the probiotic group and 0.90 in the control group, a significant (P < 0.001) improvement in feed conversion ratio was occurred in calves received probiotic compared with control calves.

Haemato-Biochemical Analysis

Regarding hematological and biochemical parameters in the probiotic 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 probiotic supplementation had no significant effect statistically on any of the hematological and biochemical traits measured (P>0.05).

Effect of Probiotic on Diarrhea 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 pe-

riod. 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 probiotic treated group and never exceeded the normal value (Figure 1).

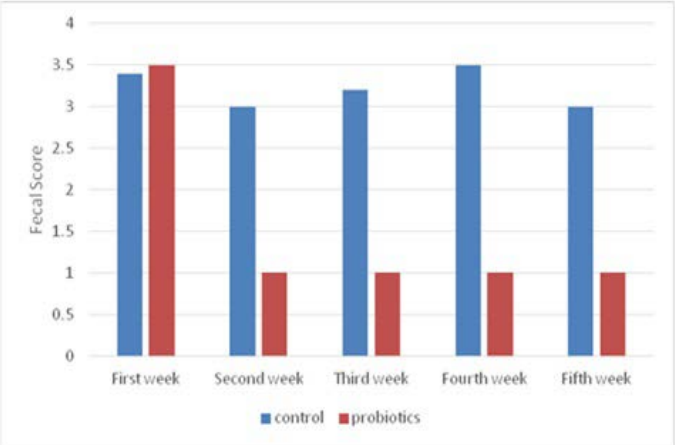

Figure 1: Mean fecal score of neonatal buffalo calves supplemented with or without probiotics. Calve diarrhea was evaluated using the fecal score and recorded according to Larson et al.'s recommendation (1977). For fecal fluidity, scoring was done as follows: 1 = normal, 2 = soft, 3 = runny and 4 = watery during the experimental period.

DISCUSSION

The present results showed the effective outcome of the probiotic usage among calves presented with diarrhea. 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 probiotic effect (Miles, 1993). This positive effect was similar to the Abe et al. (1995) results as throughout their study, calves to 25 days of age were assessed.

Moreover, Hossaini et al. (2010) stated that the groups with probiotic and antibiotic in their study had significantly higher body weight than the control group which is also reliable with Higginbotham and Bath (1993) results, who also performed different experiments in the first month of birth and also, Abdala et al. (2002) reported a significant difference in the growth of the probiotic group between 21st and 42nd day. Against to the present results, Morrill et al. (1995), Kamra et al. (2002) and Gorgulu et al. (2003) found no difference in the daily weight gain in both groups throughout the study.

The present results are supported by those obtained by Mohamadi and Dabiri (2012), who added probiotic to calves diets and observed significant improvement on FCR. These results were disagree with the findings of Riddell et al. (2010) who reported a non-significant effect on FCR in calves fed bacterial probiotic treated diet.

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 probiotics action have been described (Frizzo et al., 2010) whish stated that probiotics 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 probiotic 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 probiotic action.

In the present study, Blood hematological profile showed that the values were all in normal physiological range and the probiotics had no significant effect on any of the hematological and biochemical traits measured. That was similar to the findings of Adams et al. (2008), Moslemipur et al. (2014) and Riddell et al. (2010) who stated that there were no variations in the hematological and biochemical parameters between probiotic treated calves and the control group throughout their studies.

Though, there was significant difference in the fecal score between the probiotic group and the control after two weeks of the experiment. Fecal score became constant in the probiotic treated group and didn't exceed the normal value where the probiotics 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 probiotics. This was similar to Abe et al. (1995); Khuntia et al. (2002); Frizzo et al. (2010). On the other hand, previous study by Cruywagen et al. (1996) observed that no probiotic-induced reduction of the occurrence of diarrhea. Kawakami et al. (2010) and Gorgulu et al. (2003) described and found that, with respect to diarrhea and fecal scoring, and similar to the present study, calves fed probiotics 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 (Frizzo et al., 2010). Moreover, probiotics can cause suppression to the occurrence of diarrhea in calves fed milk replacer (Timmerman et al., 2005). Cruywagen et al. (1996) stated that there was no positive effect of the inclusion of probiotic in milk replacer on diarrhea

incidence.

Also, Gorgulu et al. (2003) stated that calves supplemented with probiotics were superior with respect to diarrhea than the control groups and concluded that probiotics supplementation before weaning could boost calf health and reduce mortality and cost of buying drugs. The same conclusion was reported by Marcin et al. (2003) for piglets and calves. Their finding is in agreement with this present study.

So, probiotics might help in enhancing intestinal health of the calves when experiencing challenges. Transporting of the animals to a long distance can bother and affect their intestinal flora and this cause diarrhea and adding of the probiotics to their diet might help in reducing the incidence of diarrhea through stabilizing their intestinal flora. It is recommended that probiotics 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 animals to assess the effect of probiotic on animal growth performance and health condition of neonatal calves.

CONCLUSIONS

Usage of probiotics improves health condition of neonatal calves. Adding probiotic to milk replacer can be used to increase the daily weight gain, feed conversion efficiency and reduce the incidence of diarrhea.

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CONFLICT OF INTEREST

No conflict of interest.

AUTHORS CONTRIBUTION

Ahmed Shehta collected the data and samples, performed laboratory analysis, analyzed the data and wrote the article. Hassan Omran, Fayez Kiroloss and Mahmoud Azmi have designed the experiment, approved the work protocols and revised the final manuscript. All authors have read and approved the final draft of the manuscript.

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